# Histological and Immunohistochemical Alterations Induced by Exposure to Different Doses of Silver Nanoparticles in the Endometrium of Adult Albino Rat

Original Article

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## ABSTRACT

**Introduction:** Silver nanomaterials; the engineered nanomaterials; have many industrial applications. They are used in manufacture of cosmetic and daily used products as food and clothes. Nanoparticles can pass through different biological body barriers, accumulate in the female reproductive organs as uterus and ovaries and exert their toxicity.

**Aim of the Work:** To evaluate the effect of silver nanoparticles on the histological structure of the endometrium using different histological and immunohistochemical techniques.

**Materials and Methods:** Thirty-six adult female albino rats were divided into three equal groups; group I (control group), group II and group III. Silver nanoparticles were administered daily for 2 weeks at dose 30mg /kg and 300mg/kg respectively orally to group II and III. The size of the used nanoparticles is 20 nm. Specimens of uterus were taken to be processed for examination by light microscope (haematoxylin and eosin, Masson trichrome and TNF- alpha immunohistochemical staining) and transmission electron microscope.

**Results:** AgNps-treated animals showed stratification, cytoplasmic vacuolation, discontinuation and desquamation in endometrial epithelial cells. The lamina propria showed cellular infiltration (inflammatory reaction), empty spaces and increase the deposition of collagen fibers (fibrosis). Ultrastructurally, they showed focal loss of the apical microvilli of the endometrial epithelial cells, apical cytoplasmic vacuoles, swollen, distorted and irregularly arranged mitochondria and large amount of secondary lysosomes and autophagic vacuoles. Focal separation of the epithelial cells with destruction of the intercellular junctions was seen. The lamina propria showed accumulation of collagen fibres and eosinophilic infiltration. Immunohistochemical study revealed highly significant increase in the TNF- alpha immunoexpression particularly in group III. All these changes were more severe in animals with high dose than those of low dose.

**Conclusion:** Silver nanoparticles induce structural changes in a dose dependent manner in the endometrium. So, it should be given cautiously to females to avoid uterine damage.

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**Key Words:** Endometrium rat, proestrous phase, silver nanoparticles.

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## INTRODUCTION

Nanotechnology is effectively implemented in diverse fields that employ nanoparticles in many medical devices and food industries. The main characteristic of Nanoparticles (Nps) is their size, which is between 1 and 100 nm in at least one dimension. This can modify the physicochemical properties of the material as well as create the opportunity for increased uptake and interaction with biological tissues[1].

Nowadays, the application of silver nanoparticles in industry and medicine has proved success. For examples , they are used in air sanitizer spray, wet wipes , detergent, shampoo and soap. In medical applications, they are used in manufacture of catheters, orthopaedic bone cement, liquid condoms, contraceptive devices and surgical instruments. They are also used as drug delivery, antiviral, antimicrobial and anticancer agents[2-4].

Silver nanomaterials remain the best widely discovered material, as providing unparalleled physical and biological properties[5,6]. Through advances in nanotechnology, highly stable silver nanomaterials with adjustable dimensions, shape and surface construction are formed and produced. For example, plasmonic silver nano shells existed as innovative investigative tools with numerous indicators[7,8]. Spherical silver nanoparticles (Ag-NPs) as of proteinaceous pigment such as phycocyanin possess anti-cancer action opposed to breast cancer cell lines[9-11]. New developments in nanoscale manufacture systems permitted the integration of silver in many consumer products, such as medical procedures, dyes, foundations,

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toys, cloths, water sanitization technologies and washing materials[12- 14].

Ag-NPs containing products are one of the most quickly developing classes of marketable goods[15]. Previous studies reported that silver nanoparticles (NPs) have strong antimicrobial and antifungal properties and weak cytotoxic effects to mammalian cells. For this reason, it is used as antibacterial and antibiotic for a long time. Ag-NPs also have special physicochemical ocular, electrical, and catalytic possessions. Due to their distinctive properties, they were broadly used in domestic instruments, nutrients packing, fitness care manufacturing, ecological requests, and biomedical applications as wounding bandages, medical devices, in addition antiseptics[16-18].

The wide use of nanomaterials in manufacturing, medication and customer yields elevate alarms around the probable harmfulness. Previous studies revealed that numerous types of NPs have the capacity to pass certain biological barriers and employ toxic effects on vital organs, such as the brain, liver, and kidney. Several studies describe in *vitro* toxicity of silver nanoparticles in different organs, such as lung, liver, skin, and brain. However, lately, care is directed toward the toxic effect of nanomaterials on reproductive system[19,20].

Reproductive toxicity is increasingly becoming recognized as an important part of overall toxicology. However, the reproductive toxicity of NPs has not been studied in depth until very recently. Reproductive physiology involves a series of complex physiological processes that are sensitive to chemical contaminants. NPs can be used as drugs and drug delivery vehicles and for fluorescence imaging in the reproductive system. They used as a drug carrier for targeted therapy directly combine with target organs or target cells, providing an opportunity to enter the reproductive system directly. NPs can enter the female reproductive system and damage the female reproductive organs and cells, thereby compromising their fertility and fetal development[21].

Additionally, previous studies stated that silver nanoparticles have deterioration effect on the ovaries. Other researchers demonstrated that prenatal exposure to silver either in ionic or nanoparticle forms increase their levels in offspring tissues. So, females mainly susceptible and merits a special care, as toxicity can affect their reproductive ability and embryonic growth. It was also proved that silver nanoparticles accumulated in the testicular tissue and leads to deleterious effects on histology of the testis and the sperms morphology[22-24].

Therefore, this research purposed to study the effect of silver nanoparticles doses on the endometrium of female rats by utilizing assorted histological techniques.

## MATERIALS AND METHODS

### *Preparation of silver nanoparticles(AgNPs)*

Preparation of 5x10-3 mol dm-3 of silver nanospheres (puress, Fluka) was done by citrate reduction according to Turkevich protocol. Silver nitrate weight 0.0850 gram were added to 100 ml of double distilled water. Then, 25ml of the stock solution was added to 100ml of double distilled water. The solution was heated until it begins to boil. To the boiling solution, 5ml of 1% sodium citrate was added with vigorous magnetic stirring. Heating was continued until the colour of the gradually changed to yellow. Heating was continued for another 15 minutes after that the solution was removed from the heater and stirred for further 15 minutes. The silver nanoparticles solution was completed to 125 ml by double distilled water and stored at 4°c[25]. To study the particles size, the aqueous dispersion of the nanoparticles was drop cast onto a carbon coated copper grid and the grid was air dried at room temperature before viewing under the microscope, and the diameter was determined from the micrographs. The size of the particles was about 20nm.

### *Animals*

The animal work followed the guidelines for animal use of the Ethics Committee for Scientific Research of the National Research Centre, Egypt. Thirty-six adult female Wister albino rat weighing 140-160g and their ages between 12 and 14 weeks were used in the present study. They were kept under optimum environmental conditions in suitable cages with proper ventilation before and throughout the work. The rats were examined for the regularity of their oestrous cycle, where vaginal smears were collected daily for three consecutive cycles and only rats with consistent 4-day cycles were included in the study.

The rats were randomly divided into 3 equal groups:

1. **Group I ( control group):** animals of this group received physiological saline orally once daily for 2 weeks.
2. **Group II:** animals of this group received low dose of silver nanoparticles (30 mg/ kg) orally once daily for 2 weeks according to Kim *et al*. 2008[26].
3. **Group III:** animals of this group received high dose of silver nanoparticles (300mg /kg) orally once daily for 2 weeks according to Kim *et al*. 2008)[26].

At the end of the experiment, the proestrus phase was resolute according to the cell types detected in the vaginal smear[27]. The animals were sacrificed by cervical dislocation then the uterus of each animal was dissected, and specimens were handled for light and electron microscopy.

### *Histological processing*

The specimens were immersed in 10% neutral-buffered formalin, washed, dehydrated, cleared, and embedded in paraffin. Sections of 5 µm thickness were stained with H&E and Masson trichrome[28].

### *Immunohistochemical tests*

For immunohistochemical staining with Tumour necrosis factor-alpha (TNF-α):

Sections of 5 µm thickness were dewaxed, rehydrated, and washed with phosphate buffered saline (PBS) and then incubated with PBS containing 10% normal goat serum. Sections were incubated with the rabbit polyclonal antibody against TNF-α (ab6671, Abcam, Cambridge, Massachusetts, USA) overnight in a humid chamber at 4°C and then incubated with biotinylated goat anti-rabbit IgG for 60 min at room temperature. Sections were incubated with a streptavidin–biotin–horseradish peroxidase complex for another 60 min. The immunoreactivity was visualized using 3,3′-diaminobenzidine (DAB) hydrogen peroxide as a chromogen, sections were counterstained with Mayer's haematoxylin. The negative control sections were prepared by excluding the primary antibodies[29]. Positive controls for TNF-α were human tonsils (as provided by the manufacturer).

### *Electron microscopy examination*

The specimens were fixed in 2% buffered glutaraldehyde, washed in PBS and fixed in 1% osmium tetroxide, and dehydrated in alcohol and embedded in epoxy resins. Ultrathin sections (50–60 nm) were cut on a Leica Ultramicrotome (Leica Microsystems, Vienna, Austria), mounted on copper grids, and stained with uranyl acetate, followed by lead citrate[30]. The grids were examined using a Jeol JEM- 100 Transmission Electron Microscope (Jeol, Tokyo, Japan) at the Electron Microscopic Unit of the Faculty of Medicine, Tanta University (Tanta , Egypt) and at the Electron Microscopic unit of the Faculty of science, Alexandria University.

### *Morphometric study*

The slides were examined using Leica QWin 500C Image Analyzer Computer System (Leica Imaging System Ltd, Cambridge, UK) at the Central Research Lab, Fac¬ulty of Medicine, Tanta University (Tanta, Egypt). Ten different nonoverlapping randomly selected fields from each slide at a magnification of 400 were quantified for the following:

1. The mean area percentage of the positive histochemical reaction for Masson trichrome stain expressed as a blue colour of the collagen fibres in the endometrium.
2. The mean colour intensity of positive immunohistochemical reaction for tumour necrosis factor alpha expressed as a brown cytoplasmic coloration in the epithelial cells of the endometrium.

### *Statistical analysis*

The data were analysed using one-way analysis of variance followed by Tukey's test for comparison between the groups using the statistical package for the social sciences (version 11.5; SPSS Inc., Chicago, Illinois, USA). All values were expressed as mean ± SD. Differences were regarded as significant if *P values* were less than 0.05 and highly significant if *P values* were less than 0.001[31].

## RESULTS

All animals lived to the end of the experiment and no mortality could be observed between them.

### *Light microscopic examination*

### *H&E results*

The endometrium was formed of a surface epithelium invaginated to form numerous tubular uterine glands and a thick lamina propria (the endometrial stroma)

**Group I (control group):** Examination of H&E stained sections obtained from the control group showed the well known histological structure of endometrium in the proestrus phase. It consisted of surface epithelium invaginated to form numerous tubular uterine glands and a lamina propria. The surface epithelial cells were a mixture of ciliated and secretory simple columnar cells. Each had elongated pale nucleus and regular apical brush border lining uterine lumen. The uterine glands in the lamina propria were lined by simple columnar epithelial cells mainly secretory with fewer ciliated. The connective tissue of the lamina propria was rich in fibroblasts and contained abundant ground substance (Figures 1 A, B, C).

**Group II (animals received low dose of Ag NPs):** Endometrial sections obtained from animals of group II revealed histological changes in both lining and glandular epithelium. The height of the epithelium was apparently increased and the cells showed stratification with cytoplasmic vacuolation. Scattered cells revealed small dark nuclei pushed peripherally by vacuolated cytoplasm. There was also mononuclear inflammatory cellular infiltration particularly with eosinophils in the lamina propria and among uterine glands (Figures 2 A, B, C, D).

**Group III (animals received high dose of Ag NPs):** Examination of sections obtained from this group displayed more severe histological changes than in group III. The lining epithelium revealed discontinuation and partial desquamation with inflammatory cellular infiltration and empty spaces of variable size in its lamina propria. Stratification was also seen with vacuolated cytoplasm in focal areas. The endometrial glands were irregular in shape and surrounded by extensive eosinophilic cellular infiltration and empty spaces. Their epithelium exhibited focal discontinuation, vacuolation and stratification. Their epithelial cells showed more vacuolation and small dark peripheral nuclei (Figures 3 A, B, C, D).

### *Masson trichrome results*

With Masson trichrome stain few blue colour collagen fibres could be detected in the control sections in the lamina propria among the endometrial glands. Sections obtained from group II revealed an obvious increase in the collagen fibres in the lamina propria in-between and around the endometrial glands. Marked increase in the amount of collagen fibres could be detected in sections obtained from group III (Figures 4 A, B, C).

### *Tumour necrosis factor alpha (TNF- alpha) Immunohistochemical results*

Examination of endometrial sections obtained from group I showed negative TNF-alpha immune reaction in the cytoplasm of the lining and glandular epithelium (Figure 5). While sections obtained from animals of group II which exposed to low dose of AG-NPs exhibited positive cytoplasmic immunoreaction in the form of brown cytoplasmic coloration in both lining and glandular epithelium in focal areas (Figures 6 A, B). Strong positive cytoplasmic immunoreaction could be detected in the majority of lining and glandular epithelium in sections obtained from group III (Figures 6 C, D).

### *Electron microscopic results*

Examination of ultrathin sections of group I showed closely packed surface epithelium appeared as a simple columnar epithelium that possessed scattered groups of ciliated and nonciliated secretory cells. The ciliated cells had numerous of cilia and contained large euchromatic nuclei with prominent nucleoli. Their cytoplasm contained many apical located mitochondria and multiple organized parallel Golgi profiles. The secretory cells contained widely dilated profiles of endoplasmic reticulum and showed numerous regular microvilli at the luminal surface. The epithelium of the uterine glands was similar to the surface epithelium, but the ciliated cells were fewer. The epithelial cells appeared closely packed with narrow intercellular space and multiple cell junctions in-between (Figures 7 A, B, C, D).

Ultrathin sections obtained from group II displayed focal loss of surface epithelial cells apical microvilli. The cytoplasm of some cells had different sizes vacuoles and multiple prominent Golgi apparatus. Most of the mitochondria appeared swollen but few of them showed disrupted cristae. Many cells contained multiple secondary lysosomes and autophagic vacuoles. Focal separation of the epithelial cells with destruction of the intercellular junctions was also seen. The lamina propria showed accumulation of excess collagen fibres with eosinophilic infiltration, focal disruption of cell junction and widening of the intercellular space were observed (Figures 8 A, B, C, D, E, F).

Regarding group III, it showed marked and extensive ultrastructural changes more than group II. The microvilli of many epithelial cells were distorted and destructed. The cytoplasm contained large vacuoles, many lysosomes, autophagosomes and irregular shaped nuclei. The mitochondria were disarranged, varying in size and form. Some of them appeared swollen with disrupted cristae. Shedding of the apical part of some cells was observed. Wide intercellular spaces and destruction of cell junctions were also detected. The lamina propria showed multiple eosinophilic infiltrations and deposition of large amount of collagen fibres (Figures 9 A, B, C, D, E, F, G, H).

### *Morphometric and statistical analysis (Tables 1& 2) and Histograms (1&2)*

The mean area percentage (%) of collagen fiber content in the low dose group (Group II) showed a highly significant increase compared to the control group . Moreover, the high dose group (Group III) showed a highly significant increase compared to the control and low dose group (Group II) ( Table 1, Histogram 1)

The color intensity of TNF-α positive immunoreaction in the low dose group (Group II) showed a significant increase compared to the control group), while high dose group (Group III) showed significant increase compared to the control and low dose group (Group II) (Table 2, Histogram 2).

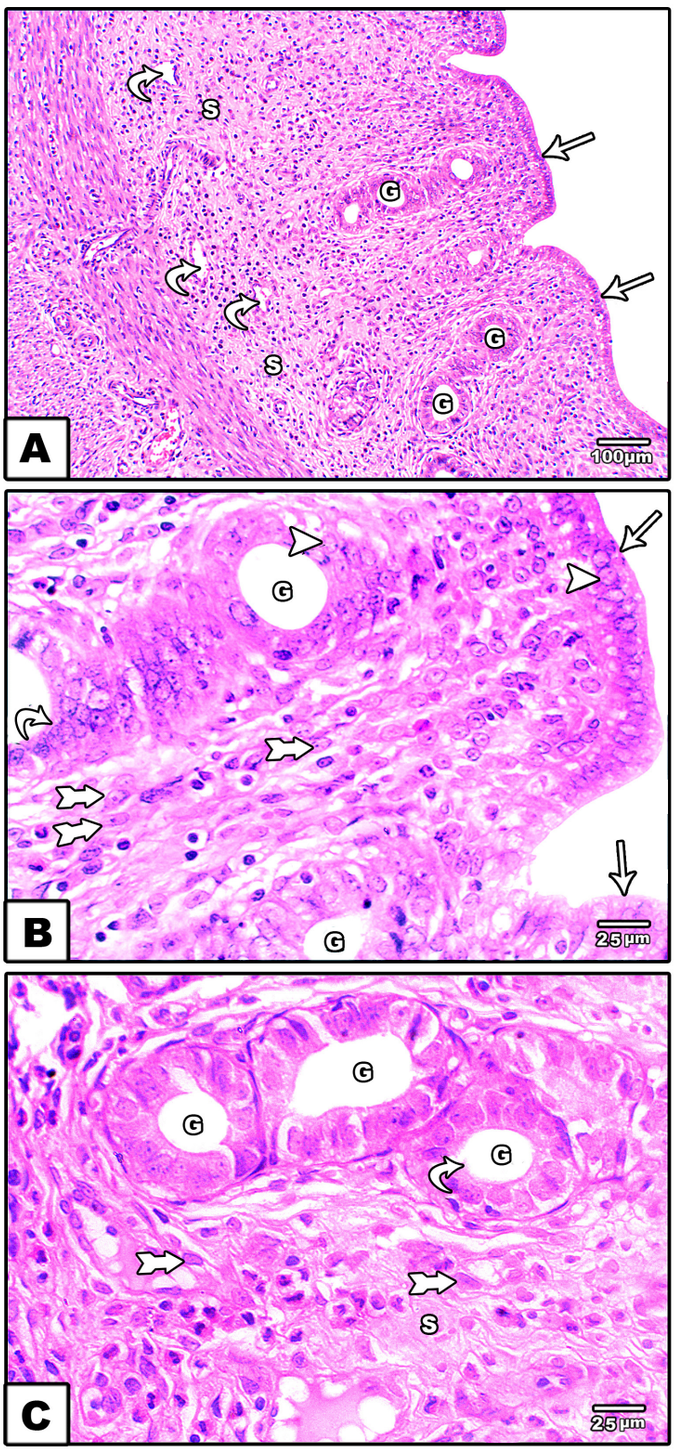



**Fig. 1:** Photomicrographs of the endometrium during proestrous phase obtained from the control group showing the lining epithelium (arrow) and a lamina propria (S) containing uterine glands (G). A: blood capillaries are seen (curved arrows) (H&Ex 100). B: Higher magnification showing simple columnar epithelial cells (arrow) lining the uterine lumen with intact nucleus ( arrow head) and the glands with brush border (curved arrow). The underlying lamina propria contains stroma cells (bifid arrow) and uterine glands (G) (H&Ex 400). C: uterine glands (G) lined by simple columnar epithelial cells with brush border (curved arrow) and the stromal cells in between (bifid arrow) (H&Ex 400).

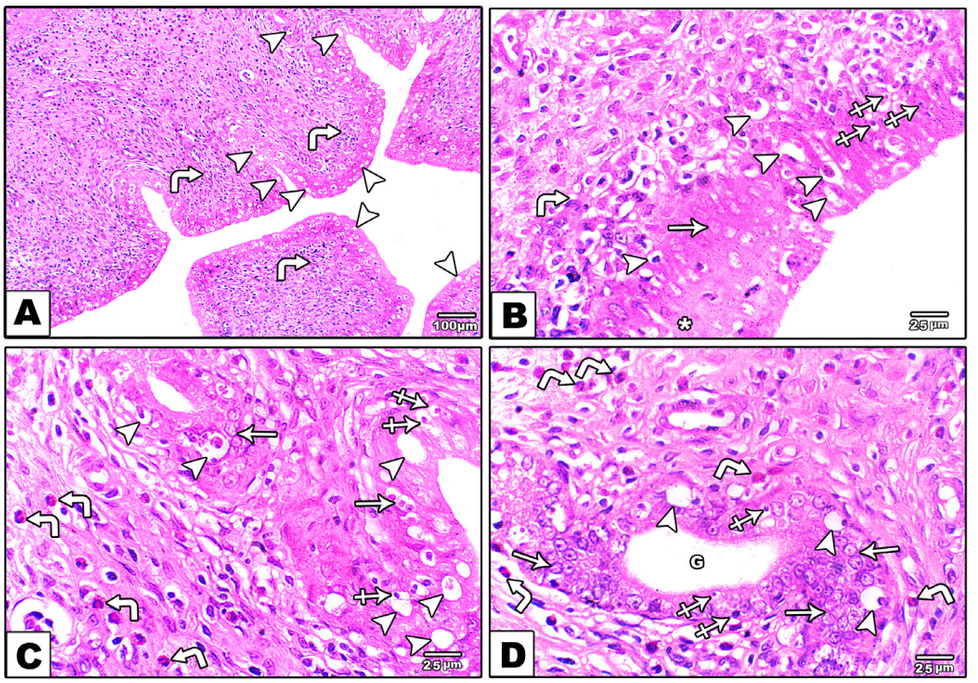



**Fig. 2:** Photomicrographs of the endometrium obtained from group II showing stratification (arrow) and cytoplasmic vacuolation (arrow head) of both lining and glandular epithelial cells and mononuclear cellular infiltrations in the lamina propria (angular arrows) (H&Ex100). B: showing apparent increase in the height of the lining epithelium (star), some cells contain small dark nuclei (crossed arrow) (H&Ex 400).C &D : showing eosinophilic infiltration in the lamina propria (angular arrow) and small darkly stained nuclei (crossed arrow) in both lining and glandular epithelium (H&Ex 400).

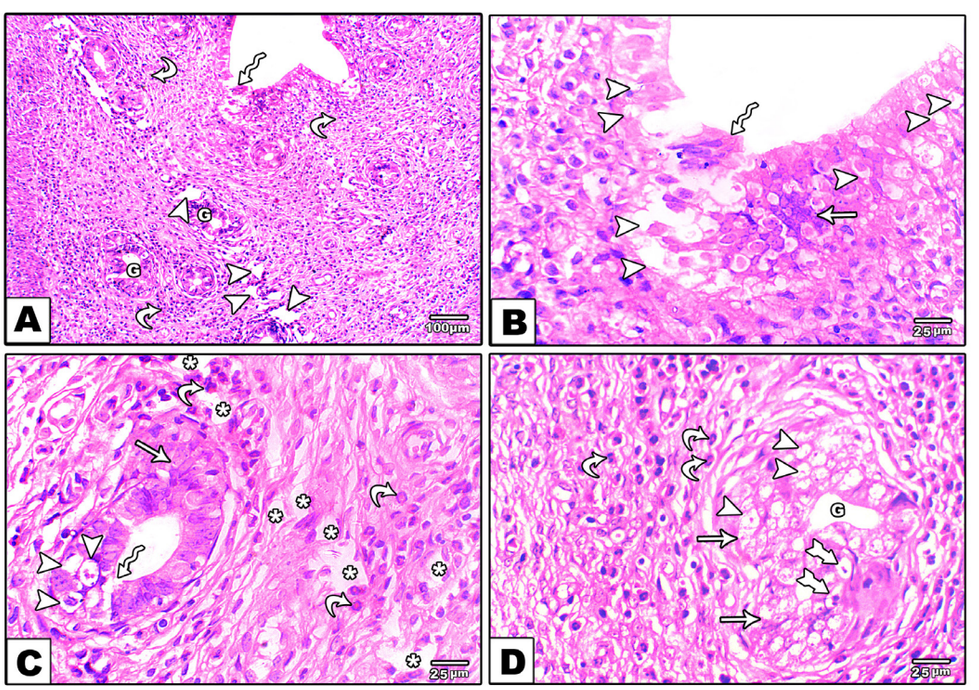



**Fig. 3:** Photomicrographs of the endometrium obtained from group III showing: A: discontinuation and desquamation of the lining epithelium (wavy arrow) with inflammatory cellular infiltration (curved arrows), irregular shape uterine gland(G) and variable size empty spaces (arrow heads) in the lamina propria (H&Ex100). B: stratification (arrow), discontinuation of the lining epithelium with desquamation of some cells (wavy arrow) and vacuolated cytoplasm of surrounding cells (arrow head)( H&E x400). C: discontinuation of the glandular epithelium (wavy arrow), stratification (arrow) and cytoplasmic vacuolation (arrow head) of some cells. The lamina propria shows wide empty spaces (star) and eosinophilic infiltration (curved arrows) (H&Ex400). D: irregular shape glands (G), stratification (arrow) and vacuolation (arrow head) of their epithelial lining with eosinophilic cellular infiltration (curved arrows). Notice the presence of small dark nuclei (bifid arrow) (H&Ex400).

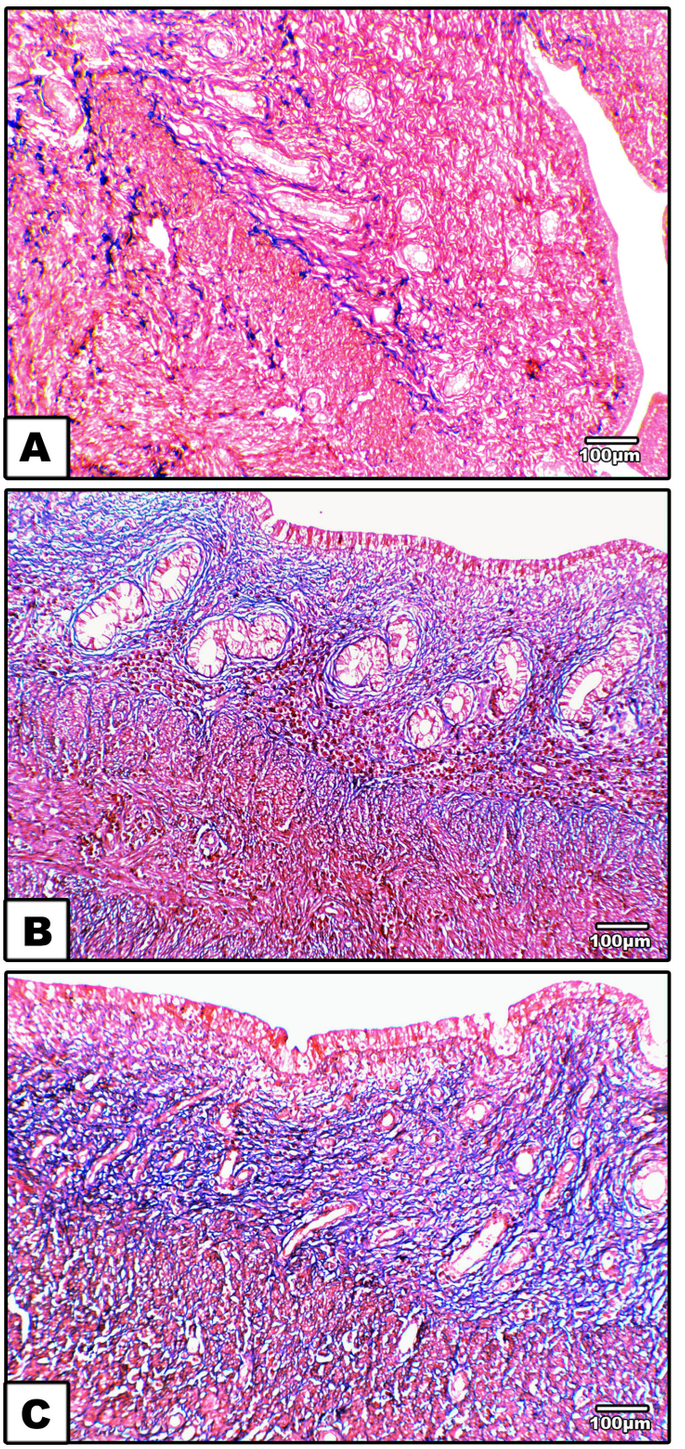



**Fig. 4:** Photomicrograph of the endometrium stained with Masson trichrome. A: control group showing few blue- stained collagen fibres between the uterine glands ( Masson trichrome x100). B: group II showing apparently increase the amount of collagen fibres around and among the uterine glands (Masson trichrome x100). C: group III showing excess amount collagen fibres around and among the uterine glands( Masson trichrome x100).

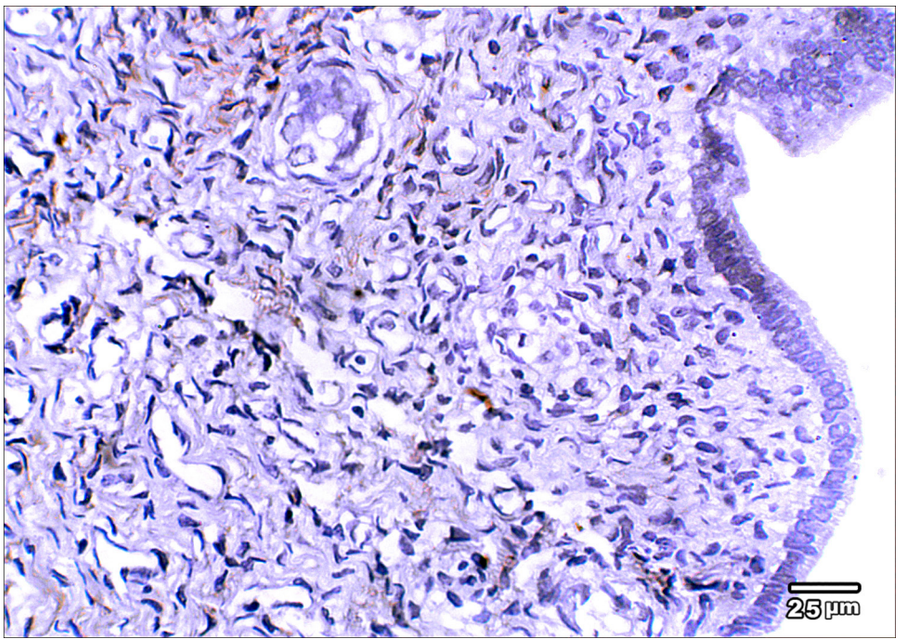



**Fig. 5:** A photomicrograph of the endometrium obtained from control group showing negative immune reaction in both lining and glandular epithelium. TNF-alpha immunostainingX400

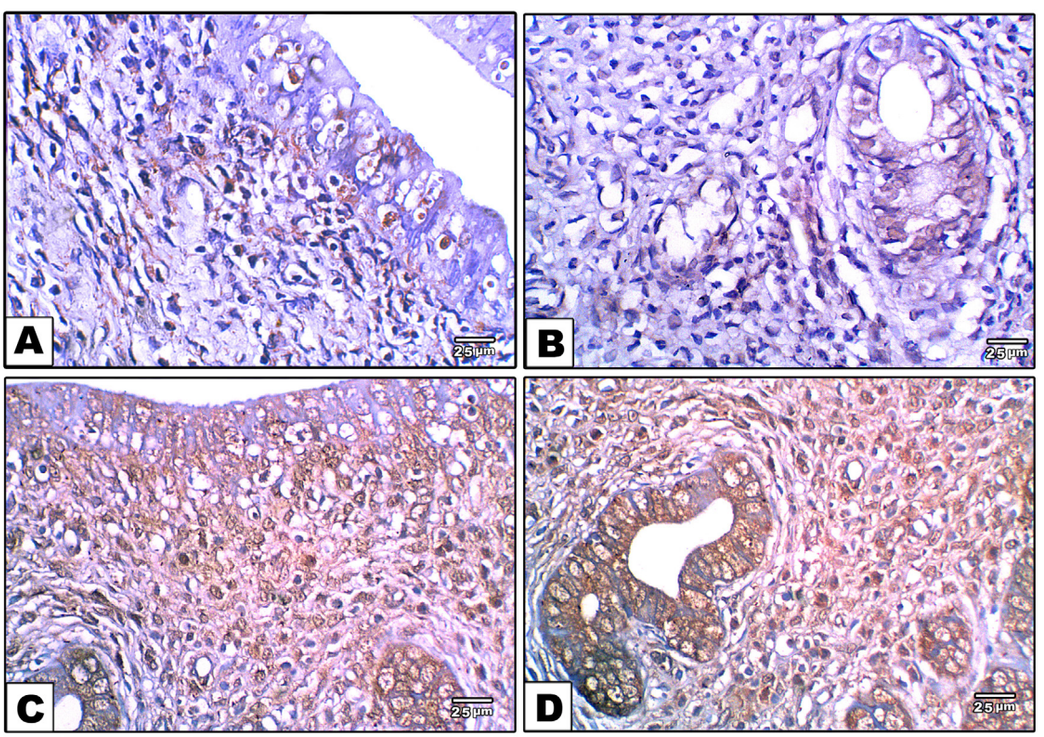



**Fig. 6:** Photomicrographs of the endometrium stained with TNF-alpha immunostaining A&B: group II showing positive cytoplasmic immune reaction in both lining (A) and glandular epithelium(B) x400. (C&D): group III showing strong positive cytoplasmic immune reaction to in both lining (C) and glandular epithelium and the stroma cells(D) x400.

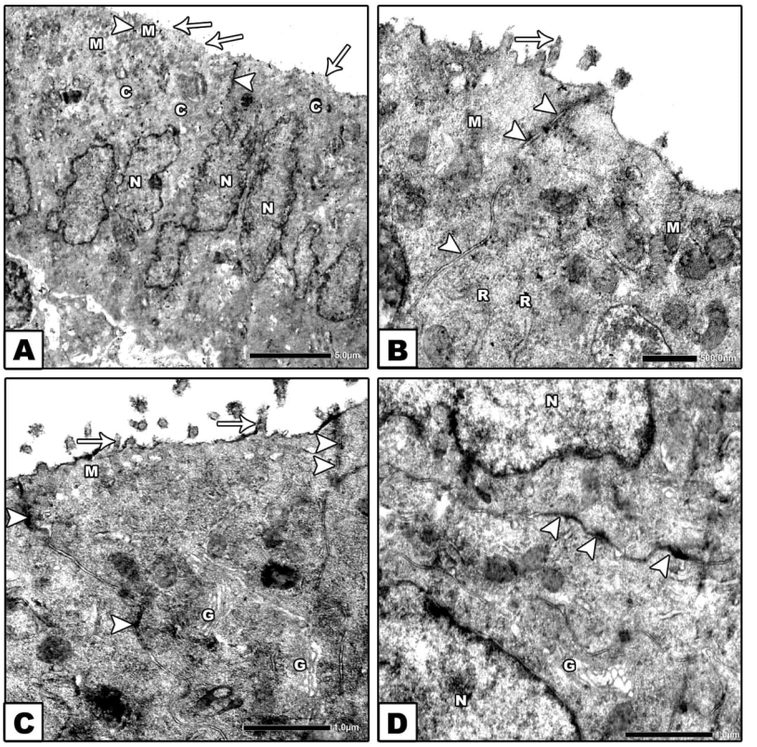



**Fig. 7:** Transmission electron micrographs of rat endometrium during prosperous phase, obtained from a control group showing: A: densely crowded columnar cells (C) with numerous cilia (arrow) and intact cell junctions (arrow head). Their cytoplasm contains euchromatic nuclei (N) with prominent nucleoli and apical mitochondria(M). x 1500 B: two secretory cells with intact intercellular junctions (arrows head), apical microvilli (arrows), apical mitochondria(M) and rough endoplasmic reticulum (R) x10000. C: endometrial cells with intact intercellular junctions (arrow head) covered with microvilli (arrow) and contain apical mitochondria(M) and well organized parallel Golgi profiles (G) x 8000. D: two endometrial cells with intact intercellular junctions (arrow heads) and contain euchromatic nuclei (N) and well-organized parallel Golgi profiles (G) x 8000

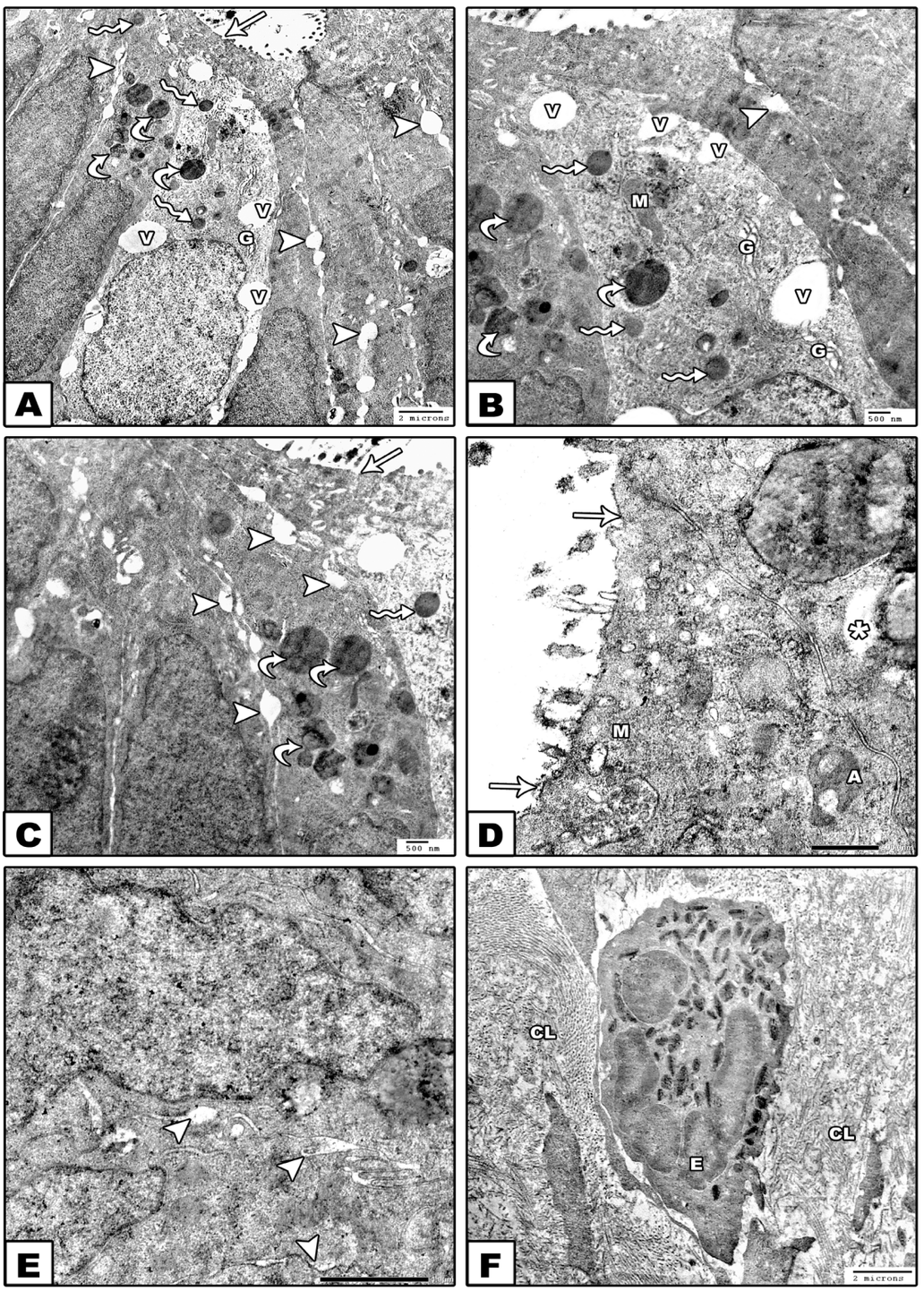



**Fig. 8:** Transmission Electron micrographs of rat endometrium obtained from group II showing, A: focal loss of the apical microvilli(arrow), cytoplasmic vacuolation (v), secondary lysosomes(curved arrows), electron dense bodies(wavy arrows), well organized parallel Golgi profiles (G)and focal destruction of cell junction associated with focal increased intercellular space ( arrow heads) x1500. B: higher magnification of A, showing cytoplasmic vacuolation (v), secondary lysosomes (curved arrow), megamitochondria (M), electron dense bodies ( wavy arrow), well organized parallel Golgi profiles (G ) with focal disruption of intercellular junction( arrow head) x3000. C: showing secondary lysosomes (curved arrow), electron dense bodies (wavy arrow). Notice focal destruction of cell junction (arrow head) x3000. D: two cells with focal loss of apical microvilli (arrow), their cytoplasm contain swollen mitochondria with destructed cristae(M) and autophagosomes (A) and rarefied areas of cytoplasm (star)x10000.E: two cells with focal widening of the intercellular space and destruction of the cell junctions(arrow head)x8000. F: lamina propria with eosinophilic ( E ) infiltration and deposition of excess collagen fibres( CL) x2000.

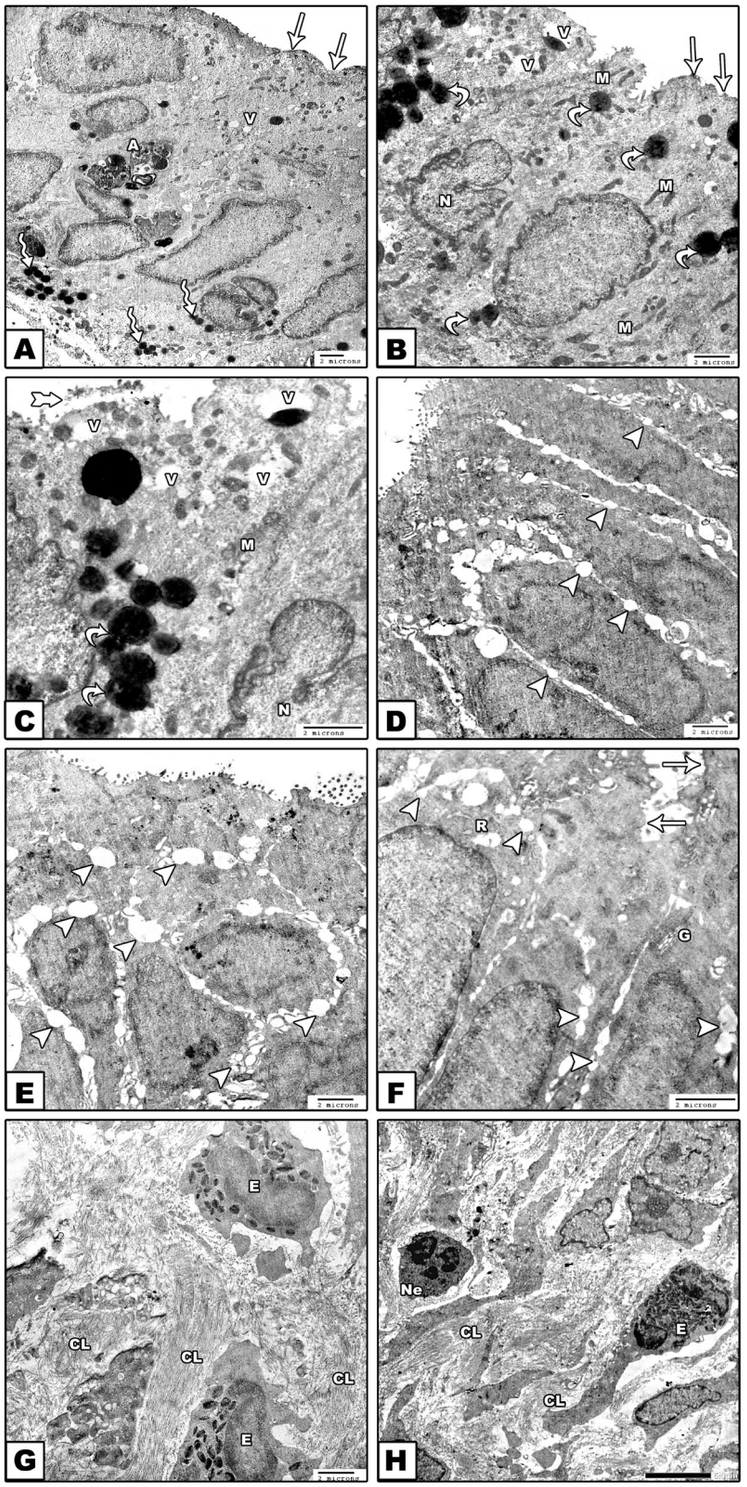


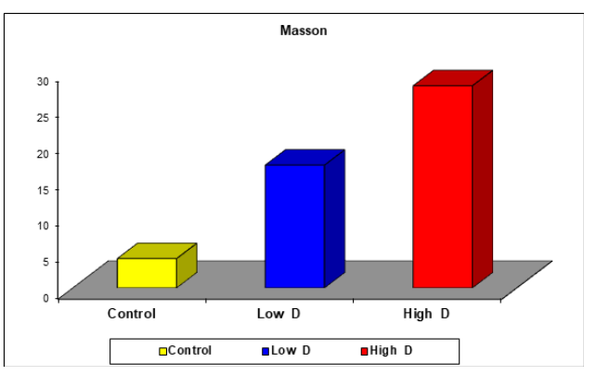

**Fig. 9:** Transmission electron micrographs of rat endometrium obtained from group III showing, A: stratification of endometrial cells and loss apical microvilli (arrow). The cytoplasm contains variable sizes vacuoles(v), electron dense bodies (wavy arrow) and autophagosomes (A) x1000. B :abnormal distribution of variable sizes mitochondria (M), variable sizes vacuoles( v), secondary lysosomes( curved arrow) and irregular shaped nucleus (N)x1500. C: shedding of the apical part of the cells (bifid arrow). The cytoplasm contains secondary lysosomes (curved arrows), variable sizes vacuoles (v) and vacuolated mitochondria (M). Notice, the nucleus has irregular outlines (N) x2500 . D &E: widening of the intercellular spaces (arrow heads) with destruction of intercellular junctions x1500. F: widening of the intercellular spaces( arrow heads) with destruction of intercellular junctions. The cytoplasm of some cells contains dilated rER (R) and parallel Golgi profiles (G) x2500. G&H: lamina propria with with eosinophilic (E) and neutrophilic (Ne) infiltration and marked deposition of collagen fibres (CL) x1500,1200.

**Table 1:** Morphometric analysis of the mean area percentage of collagen fiber content

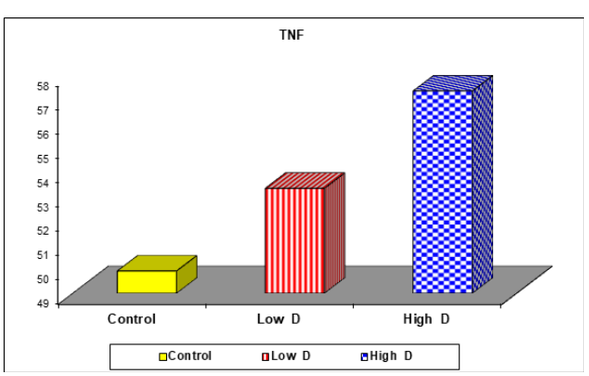
| Masson | Control | Low D | High D |
|---|---|---|---|
| Range | 3.49 – 4.47 | 16.25 – 17.79 | 27.07 – 28.27 |
| Mean ± SD | 4.0337±0.49909 | 16.8967±0.79908 | 27.825±0.65732 |
| f. test | | 967.168 | |
| *p. value* | | 0.001* | |
| Control & Low D | Control & High D | | Low D & High D |
| 0.001* | 0.001* | | 0.001* |

**Table 2:** Morphometric analysis of the mean colour intensity of TNF-α positive immunoreaction

| TNF | Control | Low D | High D |
|---|---|---|---|
| Range | 49.6 – 50.11 | 52.59 – 54.05 | 55.93 – 58.8 |
| Mean ± SD | 49.914±0.27383 | 53.317±0.72904 | 57.327±1.43915 |
| f. test | | 46.279 | |
| *p. value* | | 0.001* | |
| Control & Low D | Control & High D | | Low D & High D |
| 0.005* | 0.001* | | 0.002* |




**Histogram 1:** Showing the mean value of collagen fiber content of different groups




**Histogram 2:** Showing the mean value of TNF- alpha immunostaining of different groups

## DISCUSSION

Healthy female reproductive system is essential for fetal development and continuity of life. The number of ovarian follicles which reach the maturity during woman lifetime is only 400, so she has a limited opportunity for reproduction. Environmental pollution with inhaled nanoparticles together with their wide application in cosmetic and textiles making the reproductive toxicity and exchangeable harmful effects of next generation is necessary to be understood[32,33].

Nowadays, silver nanomaterials take a great interest due to their antibacterial properties. These properties are well known since the ancient times and recorded in the early civilization. Recently, silver is incorporated widely in many products such as medical instruments, paints, foundations, toys, cloths, water sanitization technologies and cleaning agents. The broad-spectrum usage of silver nanomaterials can result in discharge of them in the surroundings and reason wellbeing hazards out of skin touching, eating, or else breathing. So, silver nanomaterials can go in our body through numerous ways, as respiration, gastrointestinal tract, dermal contact, otherwise crossing the placenta[34,35].

Many studies have been done in *vivo* and in *vitro*, however, the research on the harm of NPs is much less than that on its progressive application. NPs enter the body easily due to their small size through different structures as digestive tract, skin, eyes, and nose. Then, they pass in systemic circulation and cross tissues, cells and organelles in addition to organs of the reproductive system[36,37].

Additionally, some authors investigated the maternal and developmental toxicity of the high oral dose Ag-NPs and its distribution in maternal and fetal organs. They found that the maternal spleen and kidney presented the highest Ag accumulation, followed by uterus, liver, and heart. It also found much lower Ag content in maternal blood compared to tissues which indicated that Ag was sequestered from blood to organs. Moreover, they reported that Ag can cross the placenta as it is accumulated in offspring tissues[38].

Oestrus cycle in rats represents the menstrual cycle in human. It takes about 4 to 5 days and consists of four stages; that is proestrus, oestrus, metestrus and di-oestrus[39]. Proestrus phase coincides with the oestrogenic proliferative phase, which is accompanying with increase the level of oestradiol in blood and low level of prolactin, resulting from an increase in both luteinizing (LH) and Follicle Stimulating Hormones (FSH). Surge of FSH is accompanied with rapid decline of oestradiol associates to ovulation and oestrus phase. Metestrus and di-oestrus correspond to initial and end of progesterone phase of menstrual cycle in human, correspondingly, by increase in progesterone level. Endometrium provide an important guide to receptivity, as the blastocyst has to come into physical contact with its epithelium before implantation. Healthy development of the endometrium during the proliferative phase is essential for successful implantation[40].

In this work, different structural changes were detected in the endometrium during treatment by silver nanoparticles, these changes were dose dependant in the form of disorganization of endometrium, stratification and cytoplasmic vacuolation. The lamina propria was also affected showing mononuclear inflammatory cellular infiltration and empty spaces. Also, a significant rise in collagen fibres deposition and tumour necrosis factor alpha cytoplasmic expression in lining epithelium were found and were more prominent with high dose. In addition, the ultrastructural changes in this study confirmed the light microscopic findings. These changes included focal loss of the apical microvilli, and presence of apical cytoplasmic vacuoles, swollen mitochondria with disrupted cristae, numerous secondary lysosomes and autophagic vacuoles. Shedding of the apical part of some cells and focal separation of the epithelial cells with destruction of the junctions between the cells were also seen.

These changes can be attributed to the oxidative and inflammatory effects of Ag-NPs. It was found that Ag-NPs increase ROS generation in rats after Exposure to Ag-NPs[41]. The cytoplasmic vacuolation which is observed in the lining and glandular epithelium was previously recorded by other authors in hepatic cells after exposure to silver nanoparticle. They attributed it to cellular necrosis leading to impaired transport membrane activity, influx of sodium and water into the cell with resultant swelling of the cells and organelles[42].

The previous changes were more severe in animals of group III that received high dose of Ag-NPs. This was in parallel to[43] who mentioned that the toxicity of Ag-NPs occurs on cells according to the given doses and their cellular toxicity was also size dependence. They explained that the transfer of nanoparticles across cellular membranes depends on their sizes. Other authors elucidated that the size of protein channels and nuclear membrane pores was about 9 nm., so, if the Ag-NPs had the same size, it can cross and react to cellular organelles and contents and stayed inside the membranes. The depositions of Ag-NPs through the membranes affect solutes movement, interchange of proteins and cell recognition[44].

Noteworthily, many researchers illustrated that after exposure to NPs, they overwhelmed the traditional barriers the human body, reaching blood stream in which they meet of the immune system the 'guard cells'. Silver can react with these cells and provoke motivation or regression, causing numerous pathological situations. A little interaction of Ag-NPs might elicit inflammatory reactions including stimulation of neutrophils, macrophages, and T helper, subsequently resulted in cytokines appearance as Tumour Necrosis factor, Interleukins[14]. Park ,s *et al*[45] explained that engulfing of Ag-NPs excite inflammatory signalling done by release of ROS, from the triggered cells followed by increase discharge of TNF-α causing cell membrane destruction of and apoptosis. Thiago *et al*[46] explained that silver nanoparticles exposure is accompanied with "inflammatory, oxidative, genotoxic, and cytotoxic consequences". They also found that application Ag-NPs to tissue-cultured cells causes free radicals' creation, raising anxieties of possible health risks.

The cellular infiltration which found in this work, was previously documented by other researchers who supposed that exposure to nanoparticles may affect cell membrane permeability leading to sequestration of inflammatory cells. Also, the vascular endothelium cadherin (VE-cadherin); which keeps the endothelial cell to cell integrity; was lost after exposure to Ag-NPs, leading to leaking out of Nps and endothelial damage. They also added that the nanoparticles attracted immune cell in the peripheral tissue causing inflammatory reactions[47]. Furthermore, several studies were conducted and showed a precedence for allergenicity of Ag-NPs[48]. This goes in line with the current study and explained the presence of eosinophilic infiltration.

Empty spaces observed in the lamina propria was suggested to be oedema that signifies an increase in fluid amount in the extravascular spaces and it is a characteristic sign of inflammation[49]. This is in consistence with Kang *et al*.[50] who stated that the Ag-NPs toxicity is elicited by inflammation and caused by oxidative stress. In addition, the cytotoxic effect of Ag-NPs was suggested to be due to their attachment to cell membrane leading to change its permeability with accumulation of intracellular reactive oxygen species (ROS) and oxidative stress[51]. As a result of ROS generation, an order of pathological proceedings in the form of inflammation followed by fibrosis, genotoxicity, and even carcinogenesis will appear. This goes in line with the current work and can explain the significant increase in the deposition of the collagen fibers in the lamina propria.

In the present work, stratification of the luminal epithelium could be observed. This explained of follow; with silver NPs and isoniazid drug, the height of the luminal epithelium increases due to physiological effect of oestrogen on uterine tissue leading to its hyperplasia and hypertrophy, inducing uterine growth[52]. However, (Hou and Zhu, 2017) established that treatment with silver NPs caused reduction in number of antral follicles and accumulated in theca and granulosa cells and affect steroidogenesis[53].

Kong *et al* (2016)[54] studied the Ni NPS impact on ovaries and observed reproductive toxicity in the form of enlarged mitochondria with cristolysis, and dilatation of endoplasmic reticulum in ovaries. They attributed these changes to inhibition of superoxide dismutase (SOD) and catalase (CAT) and accumulation of ROS, malondialdehyde (MDA) and NO, in addition to activation of pro-apoptotic proteins. They also suggested that the oxidative stress and apoptosis were responsible of damaging effects of the Ni NPs. This goes in line with Ebabe *et al* (2013)[51] and Kohen *et al* (2002)[55] who found that exposure to Ag-NPs was associated with increased generation of ROS and inhibition of SOD. So, mitochondrial structural alterations observed in our study may be also attributed to increase ROS and inhibition of SOD.

Tumour necrosis factor- alpha is a cytokine produced in the endometrium, expressed in glandular epithelial cells and present in uterine secretion relative to the menstrual cycle, that proposes a control of its secretion by steroid hormones of the ovaries. It helps in maintaining tissue homeostasis. It was expressed in the ovaries, the oviduct, and the endometrium[56]. TNF-alpha has a vital role in the periodic alterations of the endometrium, controlled by various cell types modification. It enhances DNA synthesis at the beginning of proliferative phase and initiates the menstrual shedding by facilitating apoptosis. However, at high concentrations it has pathological and physiological effects revealed by its participation in abortion due to implantation failure, immunologically induced and endometriosis[57].

Expression of TNF-alpha depends on the phase of the reproductive cycle, proposing that ovarian steroid hormones regulate its production and release. This goes in line with Laird *et al*. (1996)[58] who discovered that maximal TNF-alpha secretion occurred at end proliferative and middle secretory phase during cell culture experiments. Previous studies clarified that oestradiol and progesterone stimulate uterine TNF-alpha mRNA expression during their researches on mice after removal of the ovaries and hormonal replacement. However, additional studies in mice recognised resistance of TNF-alpha genes to steroid hormones[59].

Likewise, Goldblum *et al* 1993[60] elucidated that increase the amount of TNF-alpha leads to proliferation inhibition and induction of apoptosis in the endometrial epithelial and endothelial cells. They added that it makes endometrial glands like that in the menstrual phase by induction of apoptosis, cell to cell dissociation, aberrant expression of adhesion molecules, in addition change filamentous actin from F to G. Moreover, they also revealed that TNF- alpha damages bound between endothelial cells and increase the permeability of the lining endothelium to macromolecules and lower molecular weight solutes resulting in tissue oedema. These are accompanied with variations in cell shape, cytoskeleton increase in intercellular gaps, moreover, change actin from F to G actin. Concurrently, Tabibzadeh *et al*., 1995[61] explained that loss of expression of the adhesion molecules at the cell-cell border of the endometrial epithelial cell, dissociation of the epithelial cells leading to glandular fragmentation, loss of filamentous actin and development of epithelial apoptotic cells. However, Tabibzadeh *et al*., 1999[62] found that continuous release of TNF- alpha is not accompanying with tissue destruction and endometrial shedding, so its effects on the endometrium may occur in dose dependant manner. Therefore, TNF – alpha may have a dual effect on endometrium in low dose, it supports the growth of endometrium and in large amount it inhibits the proliferation of these cells. This is in consistent with our findings and explained the wide spaces between the endometrial cells and destruction of intercellular junctions.

It was suggested that different NPs alter the expression of genes encoding proteins including ovarian genes critical to the production of oestrogen and/or progesterone. They also alter the genes related to apoptosis and acute inflammatory response[63]. Moreover, Han *et al*., 2016[64] elucidated that in *vitro* exposure of granulosa cells of mice` ovarian follicle to high level silver NPs lead to increase of mitochondrial-mediated apoptosis. However, in *vivo* exposure is associated with loss of germ cells and increase in pro-inflammatory cytokines. So, NPs has harmful consequences for apoptosis induction and/or acute inflammatory responses in the ovaries.

## CONCLUSION

The findings of the current research revealed that, silver nanoparticles have harmful effects on the endometrium in a dose dependant manner. So, Ag-NPs should be given cautiously to females as they affect on the reproductive function, and the high dose should be avoided. Further studies are required using different doses of Ag-nanoparticles in different periods of the cycle, and further clinical studies on human are also needed to confirm the results of the animal studies.

## CONFLICT OF INTERESTS

There are no conflicts of interest.

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**الملخص العربى**

# التغيرات الهستولوجية والهستوكيميائية المناعية الناجمة عن التعرض لجرعات مختلفة من جسيمات الفضة النانوية على بطانة الرحم في الجرذ الأبيض البالغ

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**المقدمة:** إن المواد النانوية الفضية وهي مواد نانوية تم اعدادها هندسيا لها الكثير من التطبيقات الصناعية. فهي تستخدم في صناعة مستحضرات التجميل والمنتجات ذات الاستخدام اليومي كالاقمشة والمنتجات الغذائية.بامكان الجزيئات النانوية عبور الحواجز الطبيعية الموجودة في الجسم والتراكم في الأعضاء التناسلية الأنثوية كالرحم والمبايض واحداث تأثيرها السمى عليها.

**الهدف من البحث:** تقييم تأثير جسيمات الفضة النانوية على التركيب الهستولوجي لبطانة الرحم باستخدام تقنيات هستولوجية وهستوكيميائية مناعية مختلفة.

**مواد وطرق البحث:** تم تقسيم ستة وثلاثون من أناث الجرذان البيضاء البالغة الى ثلاثة مجموعات متساوية: المجموعة الأولى (الضابطة) والمجموعتين الثانية والثالثة، وقد عولجت المجموعتين الثانية والثالثه بجسيمات الفضة النانوية ٣٠ و٣٠٠مجم /كم يوميا بحجم ٢٠ نانوميتر عن طريق الفم لمدة أسبوعين على التوالي. تم أخذ عينات الرحم وتجهيزها للفحص بالميكروسكوب الضوئي (صبغه الهيماتوكسيلين والأيوسين، وماسون ثلاثي الألوان والهستوكيميائية المناعية TNF-alpha) والالكتروني النافذ.

**النتائج:** أظهرت خلايا بطانة الرحم للحيوانات التي عولجت بجسيمات الفضة النانوية تعدد في طبقات الخلايا وفجوات سيتوبلازمية. كما أظهرت الصفيحة المخاطية ارتشاح خلوي بخلايا وحيدة النواة (رد فعل التهابي) وفراغات واسعة وازدياد ترسب الياف الكولاجين (تليفات). وعلى مستوى التركيب الدقيق أظهرت فقدان بؤرى لزغيبات خلايا بطانة الرحم و فجوات سيتوبلازمية في قمة الخلايا، تورم وتشوة في الميتوكوندريا وعدم انتظام في مكانها وزيادة في كمية الأجسام المحلله الثانوية والبلعمة الذاتية. كما وجد تمزق في قمة الخلايا وتحطم في الروابط الموجودة بين الخلايا ووجد ارتشاح خلوي بخلايا الاوزينوفيل وقد أظهرت الدراسة الهستوكيميائية المناعية زيادة ذو دلالة إحصائية عالية في التفاعل المناعي، وكانت كل هذه التغييرات أكثر حدة في الحيوانات ذات الجرعات العالية عن تلك ذات الجرعات المنخفضة.

**الخلاصة:** ونستنتج من ذلك أن جسيمات الفضة النانوية تسببت في حدوث تغيرات تركيبية في بطانة الرحم معتمدة على الجرعة، لذلك يجب أن يعطى بحذر للإناث لتجنب تلف الرحم.