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EFFECTS OF SILVER NANOPARTICLES ON THE HISTOLOGY OF TESTIS AND SOME ACCESSORY SEX GLANDS OF MALE ALBINO MICE

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ABSTRACT : Although, several benefits of nanoparticles prompted, their increasing usage rises to worry round the consequences and the health threats that it potency brings to humans, so, this research aims to explain histological effects of silver nanoparticles on testis and some accessory sex glands of male albino mice, therefore, we used 30 healthy male mice, weighing 20-25g, and age (12-15week) were a randomly dispersed into five groups with a respect to concentration of nanopartic1es (l00, 200, 300 and 400mg/kg) and control group receives distal water, the animals were injected intraperitoneally and that sacrificed after 4 weeks. Results obtained a significant decreased in testosterone hormone level and FSH, LH hormones, add to histological changes in testis, prostate, seminal vesicl, and preputial gland as compared with control, histological results showed decreased and destruction in epithelial cell of seminiferous tubules and atrophy in organ add to many changes in organ tissue explained in the result.

Key words : Silver nanoparticle, sex hormones, testis, prostate, seminal vesicle, preputial gland.

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INTRODUCTION

Nanoparticles are the simply defined as the particles less than 100 nm in size (Aitker, 2014). Pure silver has distinguished characteristics as highest electrical, the thermal conductivity and low contact resistance. AgNPs are considered the best-known nano products (Chen, 2008), because AgNPs have unique properties physical and chemical (Thomask, 2005), therefor used in very various of applications (Drakel, 2005). AgNPs participation in the number of the products involving: a catheter, the clothing and the electrica1 home appliances because their a high precise surface area and high characteristics of the surface atoms (Tavares, 2012). In addition to, AgNPs were used in many consumer products involving food products, industrial and utilities in medical applications (Maneewattanaping, 2011; Khodadadi, 2014). Because of the increasing their uses in various fields in recent years, in addition, *in vitro* and *in vivo* studies shown to have harmful effects on human health, and lacking sufficient information on AgNPs. Therefore, its effects on organs and tissue of the human body must be studied. Previous research has revea1ed the reproductive toxicity of the nanomateria1 (Yang *et al*, 201) observed testicu1ar harm in mice on repeated exposure to carbon nanoparticles without the affecting their ferti1ity, then Ema *et al* (2010) shown the potentia1 of nanoparticles to cross the biological barrier of reproductive tract and the tissue harm less of the numbers and viabi1ity of the sperms. The sex glands are the important part of the reproductive system so the possible toxic effects on glands and hormone must be studies (Iavicoli *et al*, 2013).

MATERIALS AND METHODS

Silver nanoparticles

Silver nanoparticles were bought from sigma alderich, based on the provisions provided by the manufacturer, AG 50 nm, spherical, 99.9% metal basis, Appearance: grey black solid powder, Apparent Density: 0.92 g/mL, Tap Density 2.16g/mL COA of 50 nm sized silver nanoparticles have been checked silver nanoparticles by Scanning Electron microscopy (SEM) Quanta 450 FEI USA sure from a nanoparticle and at that time dilute to concentrations to 100, 200, 300, 400mg/kg at the time of injection.

Experimental animals

Thirty healthy male albino mice the weight between (20-25g), age (12-15) week bought from [the house animals] the Faculty of Science, University of AL Kufa. The mice stay for 2 weeks under control condition (12-hour light, 12-hour dark at 22-24°C with enough food and water) to acclimatization. The animals were weighed and randomly divided into five groups (n=6 per group) G1 control group receiving distill water and four experimental groups (G2, G3, G4, G5) were receiving dose of AgNPs at (100, 200, 300 and 400mg/kg) concentrations, respectively. The animals were injected intraperitoneally and then sacrificed after 4 weeks. blood Directly have been collected from heart, and underwent to the centrifuged at (1500 rpm) f0r three minute, then a result serum was kept in tips in frozen at (-20^{0}C) unti1 the hormone ana1ysis by special kit for it. For histopathological studies the testicular tissue samples and some accessory sex glands were co11ected just after the sacrifice, stable in (10%) formalin, a dehydrated, the embedded in paraffin wax conferring to the dull processing procedure, the sections of (5 µm) thickness were stained by the hematoxylin and the eosin (Bancroft. and Gamble, 2007) examined and photographed by the light microscopy.

Statistical analysis

Data was analyzed using SPSS (version 20, SPSS Inc. Chicago, Illinois, USA). Descriptive statistics (mean, standard Error) and differences were compared by One-way ANOVA at $p \leq 0.05$.

RESULTS AND DISCUSSION

Toxicological valuation of Nanosilver

In the time of treating 28 days, totally the experimenta1 mice were look over for the clinica1 marks of the toxicity, which were involved: a sensation (excitability and aggressiveness) and independent functions (diarrhea, dieresis and salivation), and morta1ity.

Nanoparticles were used in widely fields and nanomaterials has developed quickly in diverse fields recently due to their possessing developed properties that depend on size and shape (Korbekandi *et al*, 2012; Kaviya *et al*, 2011) and nanoparticles are capable to penetrate through biological barriers into reproductive tissues (Taylor *et al*, 2012). Too, AgNPs is known to prompt the oxidative stress and rise the freeing of ROS, which increase the oxidation of cellular macromolecules for example proteins (Car1son *et al*, 2008).

The result in our study indicated to decrease leve1 of the serum testosterone, may possib1y be because the possible hurt of 1eydigs cells when, AgNPs penetrated cells this led to decreased secretion and synthesis of testosterone. This agrees with Yoshida *et al* (l999), described that the nanopartic1es reason damage of Leydig's cell. Too, agree with Tsukuee *et al* (2004) and Koomatsu *et al* (2008) are reported similar observation.

Table 1 : Level of sex hormones (testosterone, FSH, LH)of male mice in control and group were treated.

Hormone levels	Testosterone (nmol/L)	LH (Mlu/ml)	FSH (Mlu/ml)
Groups	Mean ±S.E		
G1	3.09±0.34	2.77±0.46	3.16±0.31
G2	2.08±0.19	2.51±0.25	2.02±0.33
G3	1.44±0.22	2.68±0.42	1.58±0.24
G4	0.87±0.05	2.13±0.43	1.30±0.07
G5	0.75±0.19	1.16±0.27	0.92±0.48
LSD	0.542	0.125	0.773

Level of significance values $p<0.05$.

Too, the serum levels of FSH and LH levels in the time related orders. That reduction in the serume FSH, and LH potency be due to AgNPs prompted damage of interstitia1 Leydig cells that products testosterone. AgNPs intakes affects the releasing of LH as of the pituitary gland, which advance reducing the levele of the testosterone.

The earlier *in vitro* the study described the contact of AgNPs mammalian cells cause of change membrane, hence the permeable to flow calcium. and induce increase the calcium inside cells and may cause apoptosis cells (Cheng *et al*, 2013) and decreased of thie GnRH secretions, which is added to responsib1e for the decreases in the, FSH and LH levels in the pituitary as GnRH creation that decrease disorders the spermatogenesis, affect the production of androgens and the secretion of testosterone hormone via Leydig's cells (Lee *et al*, 2011; Shahraki *et al*, 2004).

Reza *et al* (2013) shown that dealing of wasters rats by high amount (40mg/kg), of ZnONPs intraperitonially made significant reduction ($P<0.05$), in FSH but no alteration observed in LH serum levels too described that it is potential NPs inhibited functions of the endocrine systems through blocking of a pituitary-hypotha1amus alliance and it is may be due to decrease in GnRH levels Histological results in our study were discovered a testicular damage at the level of Leydig cells and the interstitial (Mayyas *et al*, 2005; Guo *et al*, 2005) because AgNPs can arrive in cells through diffusion or endocytosis to effect mitochondria1 dysfunction, generation of the reactive oxygen speciose (ROS), leading to damage to for proteins and the nucleic acids inside cell and lastly

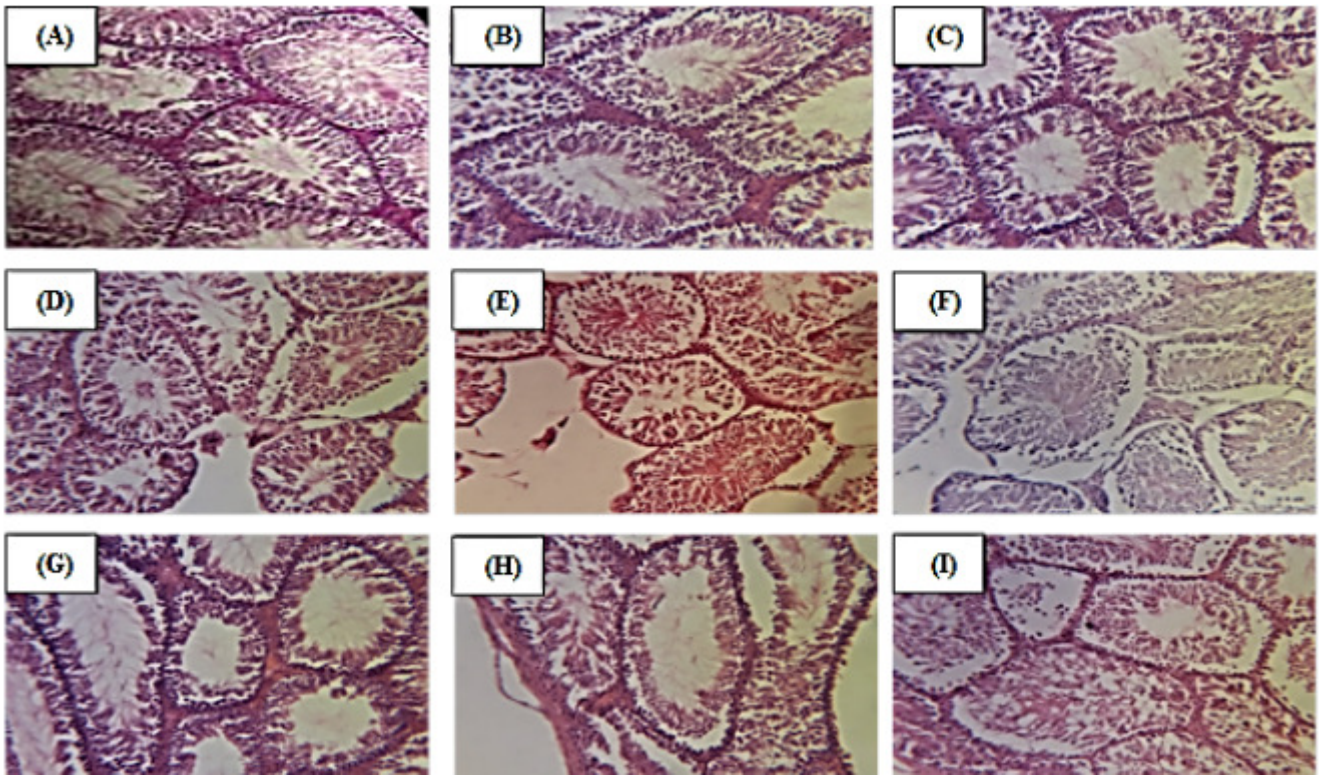

Fig. 1 : Mice testis A Figure 1 : Cross section of control mice testis show intact seminiferous tubule architecture with intact spermatogenesis. (H & E Stain, 100X), B, C Cross section of 100 mg SNps/kg of body weight mice testis show intact seminiferous tubule architecture with some reduction in the number of the spermatogenic cell lines and vacuolation. (H & E Stain, 100X), D,E Cross section of 200 mg SNps/kg of body weight micr testis show intact seminiferous tubule architecture with some reduction in the number of the spermatogenic cell lines and separated germinal epithelium from their basement membrances. (H & E Stain, 100X), F, G Cross section of 300mg SNps/kg of body weight mice testis show intact seminiferous tubules, other have exfoliated germ cells. Acidophilic vacuolation appears in the interstitium. (H & E Stain, 100X), H,I Cross section of 400mg SNps/kg of body weight mice testis show some seminiferous tubules are mardebly distorted, others have germ cells separated away from their basal lamina. Acidophilic vacuolated hyaline material appears in the interstitium. Congested blood vessel between is also observed. Some sections of the same group have disorganized and shrunken seminiferous tubules, sloughed germinal epithelium and germinal epithelium hypocellularity some seminiferous tubules with marked cellular loss and absence of sperms in most of them are detected. Mutlinucleated giant cells are also detected in some (H & E Stain, 100X).

inhibitions cell proliferations. Takeda *et al* (2009) explicated that is damaged seminiferous tubules in great doses treated AgNPs can be correlated to the inhibitory ro1e of a particles in cel1 multiplying, nanoparticles effects in a cell cycles and significant reduction of sperm precursor cells or releases of them to the mid duct of seminiferous tubu1es. A lot of investigators (Hess *et al*, 2000; Yavasoglua *et al*, 2008) suggested that s1oughing is affected thru the effects of the chemica1 on microtubules and intermediary filaments of the Sertoli ce1ls, then this belongings spread to dividing germ cells and natura11y clue to tubu1ar atrophy. The noticeable reduction in a number of germina1 epithe1ial cells potency reason a reduction in a number of spermatocytes and spermatids, which wou1d eventua11y result in the diminution of spermatozoa. This indicates incomplete spermatogenesis and the degeneration of germ cells.

In our current study, the presence of tissue damage i.n the seminal vesicles and prostate in animals treated with different level of AgNps concentration compared with the control group showed that the groups treated with AgNps showed changes that included the effect of silver nanoparticle on the prostate gland of mice led to dilation of prostatic alveoli and flattened of the cuboidal epithelial cells linings so, effect AgNPs in seminal vesicles included epithelial destruction and increased fibro-muscular stroma, reduction in fluid and vesicular volume in the AgNPs group designates that epithelial cell secretions are affected by the treatment with these nanoparticles.

According to the results in our study and other studies, AgNPs may have caused histological change by production of free radicals and the formation of oxidative stress. AgNPs have been revealed to induce ROS production, thereby inhibiting regenerated glutathione (GSH), DNA damage, protein carbonylation, and membrane oxidation (McShan *et al*, 2014; Ahmed *et al*, 2017).

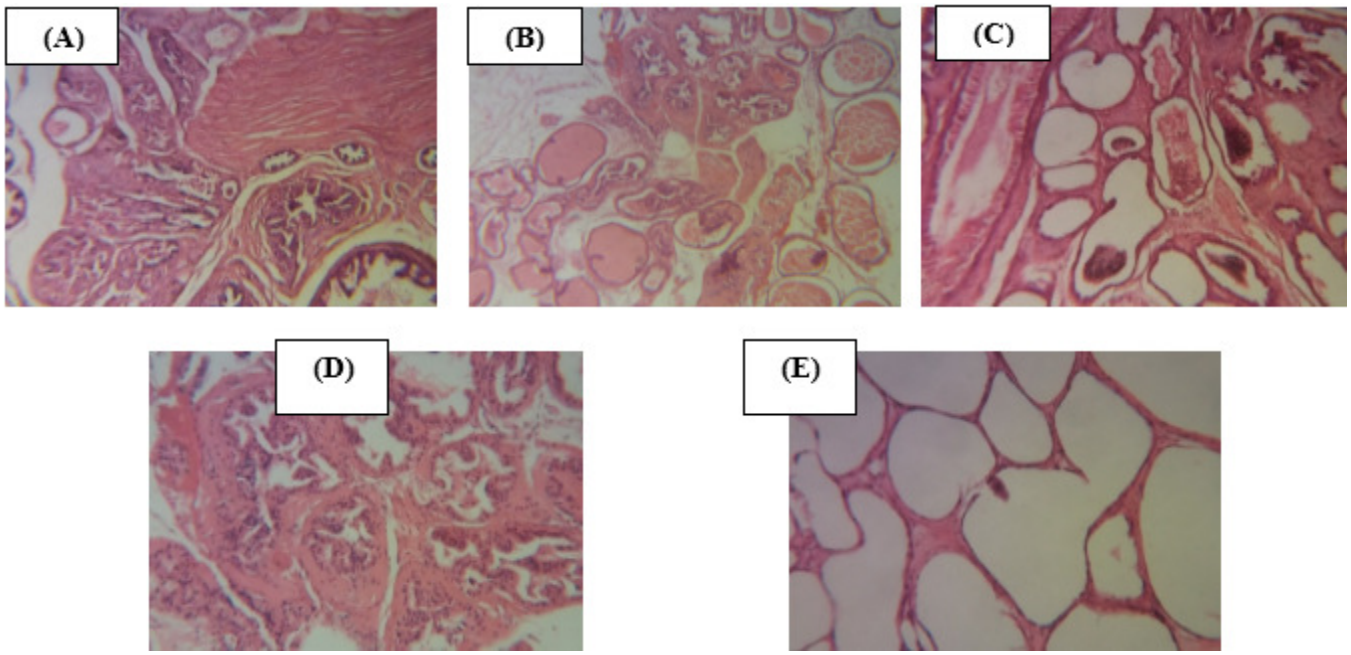

Fig. 2 : Mice prostate gland A, Cross section of control mice prostate gland show normal tubuloacinar and fibromuscular stomal components. (H & E Stain, 100X), B Cross section of 100mg SNps/kg of body weight micr prostate gland show intact tubuloacinar and fibromuscular stomal components with mild stromal congestion. (H & E Stain, 100X), C Cross section of 200 mg SNps/kg of body weight of mice Prostate gland with fibrosis and hypertrophy, cell infilteration and disassembled stromal H & E. x100. D Cross section of 300mg SNps/kg of body weight of mice Prostate gland with some fibrosis and cell infilteration with increase in the glandular size and disassembled stroma H&E, x100, E Cross section of 400mg SNps/kg of body weight of mice Prostate gland with thin secondary ducts lined by attenuated simple cuboidal epithelium.

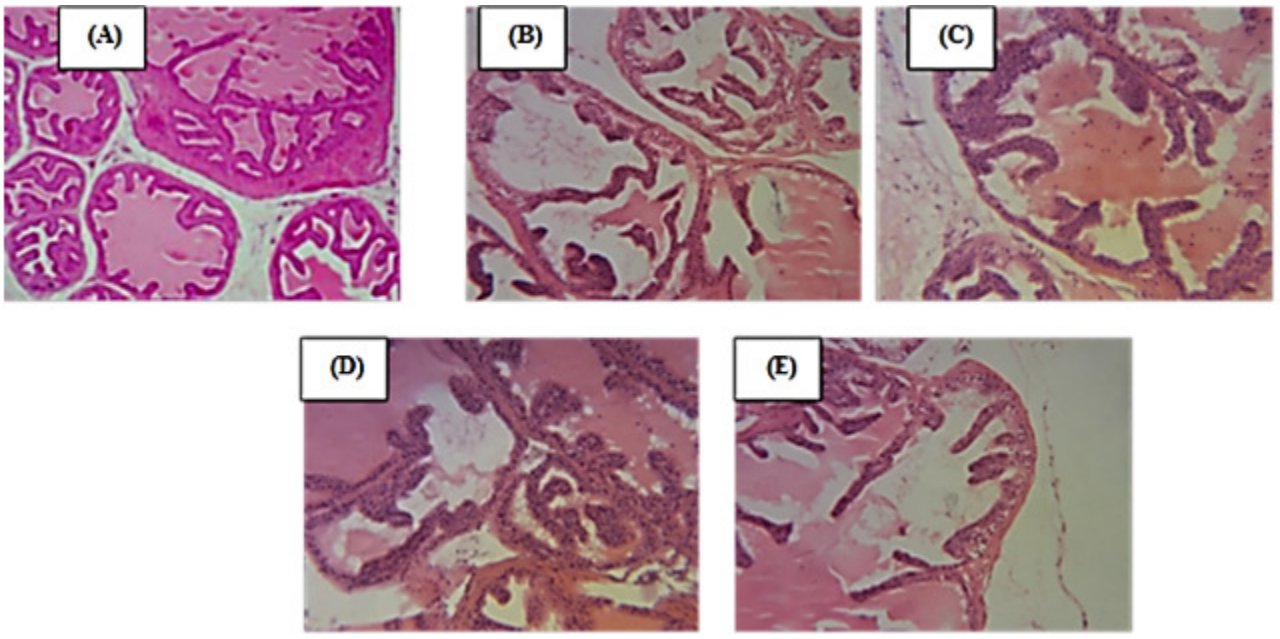

Fig. 3 : Mice Seminal vesicle A Cross section of control mice Seminal vesicle with normal structure. H&E. x100X. B Cross section of 100mg SNps/kg of body weight of mice Seminal vesicle with a focal area of apocrine metaplasia (lining epithelial cells have abundant eosinophilic cytoplasm. H&E. x100), C Cross section of 200mg SNps/kg of body weight of mice Prostate gland with fibrosis and hypertrophy, cell infiltration and siassembled stroma H&E. x100, D Cross section of 300mg SNps/kg of body weight of mice Seminal vesicle with a focal area of apocrine metaplasia (lining epithelial cells have abundant eosinophilic cytoplasm). H&E. x100, E Cross section of 400 mg SNps/klg of body weight of mice Seminal vesicle with a metaplasia (lining epithelial cells have abudant eosinophilic cytoplasm) the epithelium folds and height became reduced in size with reduced secrets. H&E. x100.

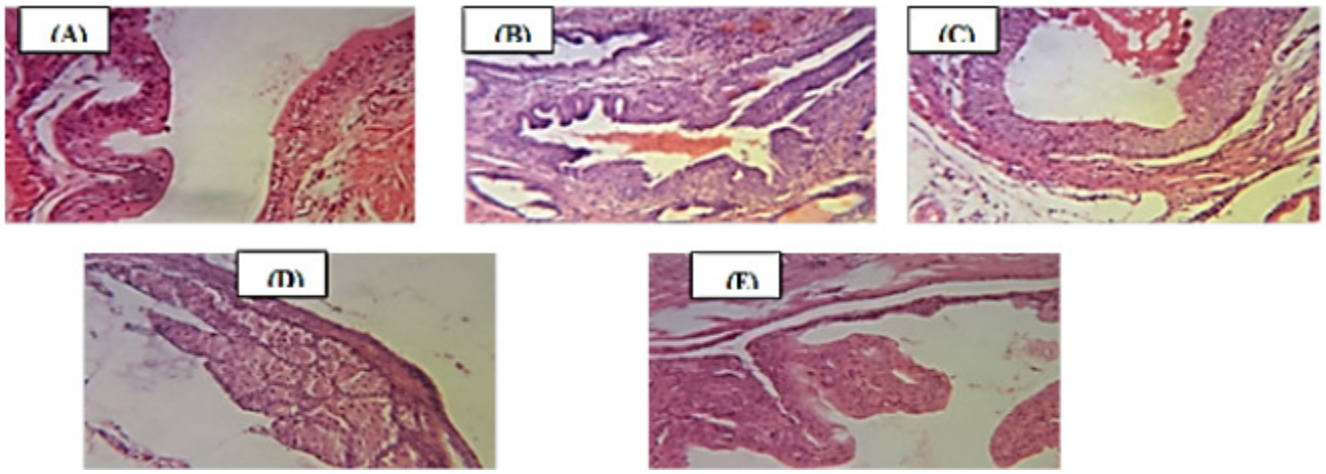

Fig. 4 : Mice Preputial gland A Cross section of control mice Preputial gland duct lined by stratified squamous epithelium. Number of cell layers is 5 in maximum thickness. H&E. x100, B Cross section of 100mg SNps/kg of body weight of mice Preputial gland duct lined by stratified squamous. Number of cell layers is 7 in maximum thickness. H&E. x100, C Cross section of 200mg SNps/kg of body weight of mice Preputial gland lined by stratified columnar epithelium, with apical keratinization. Number of cell layers is 9 in maximum thickness. H&E. x100, D Cross section of 300mg SNps/kg of body weight of mice Preputial gland lined by glands with sebaceous hyperplasia and increased lobular structures. H&E. x100, E Cross section of 400mg SNps/kg of body weight of mice Preputial gland duct lined by stratified squamous epithelium, with an area of epithelial cell atrophy. H&E. x100.

In current study, preputial gland revealed clear histological changes included atrophy in area of epithelial cell, hyperplasia, increased lobular structures, this change because decreased in testosterone hormone level as proven by the results of our study, preputial glands are androgen-dependent on the organ, Androgen, also named androgenic hormone or tested, is a general term for any natural or synthetic compound, generally the steroid hormone that stimu1ates or controls the growth and maintenance of male features in a vertebrates by binding to androgen receptors. This contains the action of the accessory male sex organs, and the development of male minor sex characteristics. The major and best famous androgen is testosterone which is the produce of Dihydrotestosterone (DHT). The action of preputia1 glands a depended on the Melanocortin 5- Receptor (MC5R), which is one of the Melanocortin Receptors (MCRs) are the family of the five G protein- coupled receptors (GPCRs; MC1R–MC5R), expressed in various tissues, which assist distinct physiological functions. A melanocortin 5-receptors (MCRs), that are stimulated via me1anocortin ligands resultant as of the pro protein (Merza, 2018).

CONCLUSION

We conclude from results were obtained in our studies, silver nanoparticles have harmful effect on reproductive system when increasing the concentration and duration of exposure to animals as the concentration of hormones decreases and reproductive system dysfunction occurs.

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