Effect of Gold Nanoparticles on The Histology of Accessory Glands of Male Albino Rats Rattus Norvegicus

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Abstract
Background: Gold nanoparticles [AuNPs] were highly used recently in many therapeutic approaches and medical applications, a few is known about the effects of those nanoparticles on prostate and seminal vesicles histology in animal model. Materials and methods: Thirty-five healthy male adult albino rat Rattus norvegicus aged 10–12-week-old with 200-230 body weight were used in the current study. Rats were randomly grouped into five groups: first group given distilled water, second group given 40µg/kg body weight of gold nanoparticles, third group given 80 µg/kg body weight of gold nanoparticles for 30 day; four group give 40µg/kg body weight of gold nanoparticles,five group give 80µg/kg body weight of gold nanoparticles for 60 days for all the administration done peritoneal injection. After the 24 of administration animals were sacrificed and their prostate and seminal vesicles were excluded and fixed in formalin then processed for light microscope examination. Results: Histological investigation of prostate,seminal vesicle of rats from treated group with 40 µg\kg,80µg/kg of body weight for 30 days revealed the presence of The section of the prostate shows an increased in alveoli folding,increased connective tissue(stromal) between the secretory alveoli and atypical hyperplasia,but the seminal vesicle where epithelial cell shrinkage with epithelial necrosis and cytoplasm vacuolation with atypia degeneration,as for injections for 60 day it leads to the expanding of secretory alveoli in prostate and their thin walls, accompanied by cell degeneration, and tissue sections of seminal showed shrinkage of the epithelial cells with atypical degeneration hyperplasia and degeneration of the sub-epithelial stromal. Conclusion: Our present study indicated that a dose of 80µg\kg of body weight of gold nanoparticles could cause a deleterious toxic effects on the prostate,seminal vesicle structure more prevalently than the dose of 40 µg\kg of body weight,referring that low doses could cause less toxic effects and dose related toxicity.
Keywords: Gold nanoparticles, prostate, seminal vesicle,rat, histology

1. Introduction

Nanotechnologies in spite of all their far- reaching benefits has concerns about the health risks of NPs [1]. Application and large production of nanoparticles lead to release of such material ls into the environment [2]. NPs have unique characteristics therefore used industrial and in biomedicall[3,4,5,6]. There are 1,814 products composed from nanoparticles such as food items,electronic materials sport tools,textiles,antibiotics [6, 7] applications of nanotechnology exposed the humans to toxic that enter the body by ingestion, injection,skin uptake, implantation in halation [8].the wide used of nano materials has increase concerns mainly on reproductive system of both women and men small size and bio compatibility of NPs lead to their ease of human caused irreversible testis damage and organ toxicity,renal toxicity, pulmonary injury, hepatotoxicity[9,10,11,12,13,14,6].The male reproductive is more liable from other organ system to different stress [15].One of the most important nano materials is gold (Au NPs) have been widely used in drug delivery [16].Used gold nanoparticles for cancer targeted and photo thermal therapy PTT [17].and imaging diagnosis[18].due relatively easy synthesis,chemical inertness,combine ligand and high stability gold nanoparticles considered higher bioactivity in biomedical fields but the increasing use of Au NPs has raised concerns about in body fat and toxic effects in human [19].more efficiently spherical Au NPs than gold nano rods[20].Oxidative stress is one of reasons for the deterioration human male reproductive system [21,22].NPs increase in production reactive oxygen species lead to inflammation and cancer [23]. Au NPs can penetrate the blood testes barrier (BTB) there is limited evidence that certain enter the testis in animal models [24].Au NPs is a variety of ways may impair testicular function, change in testicular, prostate histology, reduced fertility decreased sperm quality and alteration in testosterone levels[25].The average concentration of Au element in normal people of whole blood was about 0.05ng/g [26],was about 0.23ng/g in platelets[27].

There are some studies use of NPs in targeted therapy associated with reproductive cancer[28].Development reproductive organ function, physiological structure,germ cell and fertility NPs may have negative effects on it, in humans and animals[29].Au NPs have different properties from material which they were formed[30].used Au NPs in bio nanotechnology

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because their unique properties and multiple surface functionalities[31].Au NPs have the physicochemical properties such as redox behaviour, conductivity and surface plasma resonance [32].And high surface area of Au NPs serves as there peutic agents e.g " drugs " and "targeting agents "[34].

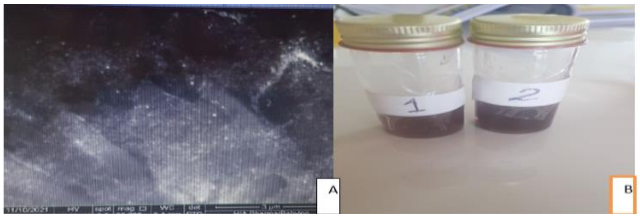
The male reproductive system in human subject for exogenous material causes for its deterioration [21].gold nanoparticles induced ROS can cause damage in sperm cell and impaired fertility may result in congenital defects in offspring[35].Au NPs (10-30nm) repeated intra peritoneal injection result increased number of abnormal spermatozoa and Au NPs penetrate the BTB accumulation in testicular tissue[36] in study Au NPs may impair testicular function [25].Potential health and Environmental risk due Au NPs present with the widespread use in biomedical fields necessary known their fat in vivo and toxicology effects especially on male reproductive system[19].Nanoparticles can enter into the reproductive system in different paths: delivery drugs, penetration when used in many medicine applications detrimental effects on morphology, motility of sperm and DNA damage[37],dermatological therapy and skin care[38], when exposed for a long time it causes abnormal tissue changes and reduce the motility and number of sperm in the testes. The nanoparticles traveling end up in the reproductive system throughout the body leak into the circulation systemic prompt inflammation [39]. Au NPs pass through the outer membranes of gonads stimulate the damage antioxidant result generation of free radicals and disrupting cellular metabolism[40].

2. Materials and Methods

Nanoparticles characterization

The materials used in this study consisted of gold nanoparticles (Au NP). Au NPs used in current study was obtained from (Iran). they were examined under transmission electron microscope,have characteristic

- Weight: concentration 100ppm
- Appearance: color red solution
- Additives:Au
- Morphology:spherical
- Size range:5-20 nm
- Product number: VCN 4021w.



Picture 1: A, showing the colour of gold nanoparticles suspension. B showing gold nanoparticles under electron microscope

Animals

thirty-five healthy male adult albino rat Rattus norvegicus aged 10-12 week old with 200-230 body weight were used in the current study.

Animals were housed in the animal house of Babylon university and left a week for acclimatization with giving food and water ad libitum with half to half night and light period. Rats were randomly grouped into five groups: first group given distilled water, second group given 40µg/kg body weight of gold nanoparticles, third group given 80 µg/kg body weight of gold nanoparticles for 30 days; four group give 40µg/kg body weight of gold nanoparticles,five group give 80µg/kg body weight of gold nanoparticles for 60 days for all the administration done peritoneal injection. After the 24 of administration animals were sacrificed and their prostate, seminal vesicle was excluded and fixed in formalin then processed for light microscope examination.

3. Data Analysis

The data was analysed using SPSS (version 23,SPSS Inc.Chicago, Illinois, USA). Descriptive statistics (mean,standard deviation),and differences were compared by one-way ANOVA,by using Duncan's test.As well as,it was carried out using student's-t test, followed by chi-square. A statistically significant result was one with a($p<0.05$). The relationship between studied parameters was determined by person's correlation coefficient (r). Analyses of statistical according (Duncan,2010)

4. Results

Macroscopic observations showed that there were not apparent signs of toxicity or deaths among all the treated rats with gold nanoparticles for 30, 60 days intraperitoneal injection among with no behavioral signs' differences were seen. Body and prostate seminal weights were measured weekly, results explained that there were non-significant differences in weights in comparison with weights of control group rats.

Histological results of the current study of all treated groups exhibited a pathological change in all rats of the treated groups in comparison with control group. Figure 1 picture 1 explains a prostate for rat of the control group it explains the intact normal histologic architecture of prostate. whereas pictures2,3 showed stromal connective tissue was increased between the secretory alveoli, atypical hyperplasia.

Whereas pictures 4,5of figure 1 explained the acinar dilation, epithelial degeneration is characterized by granular to foamy cytoplasm alteration of enlarged acinar epithelial cells.

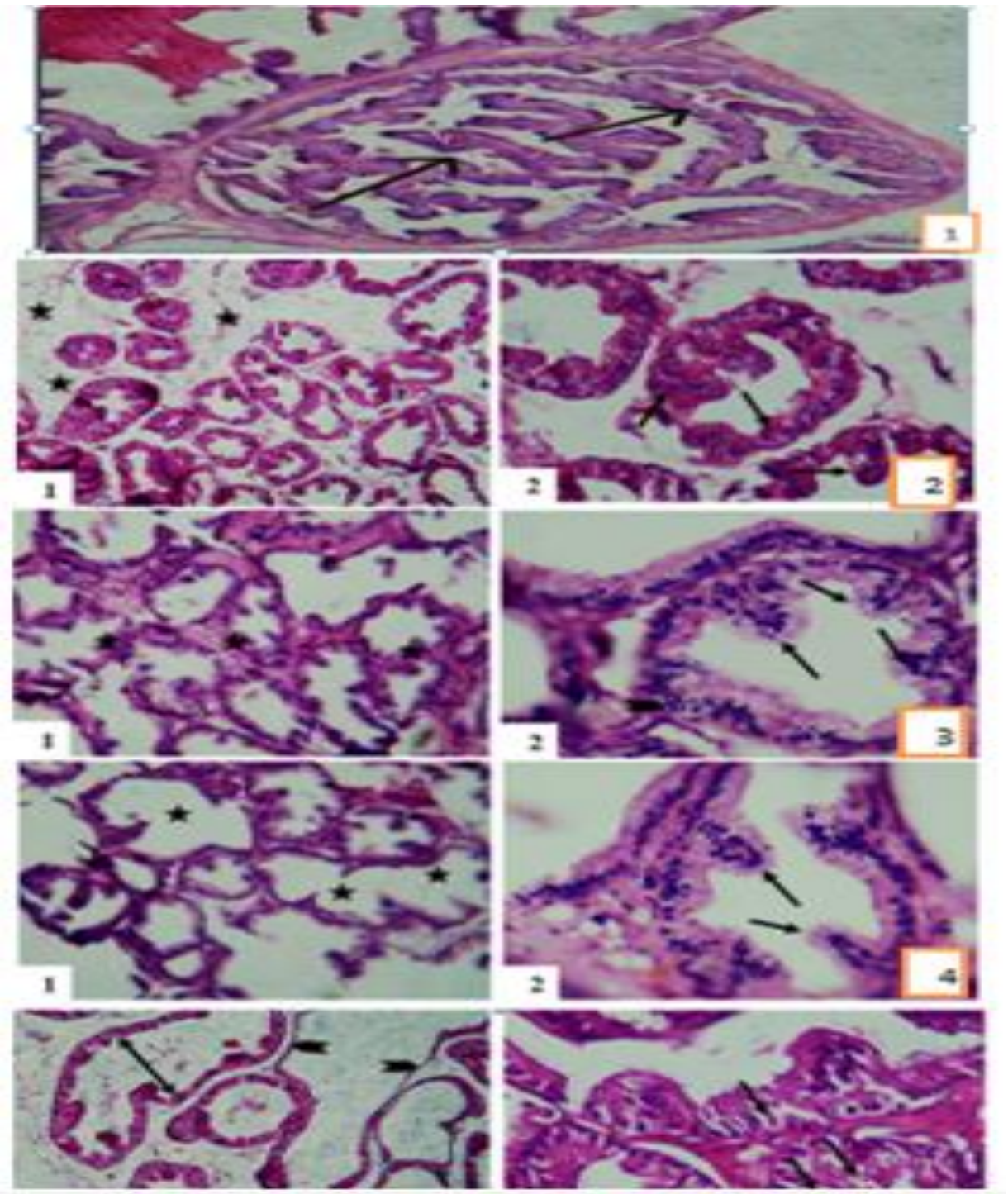
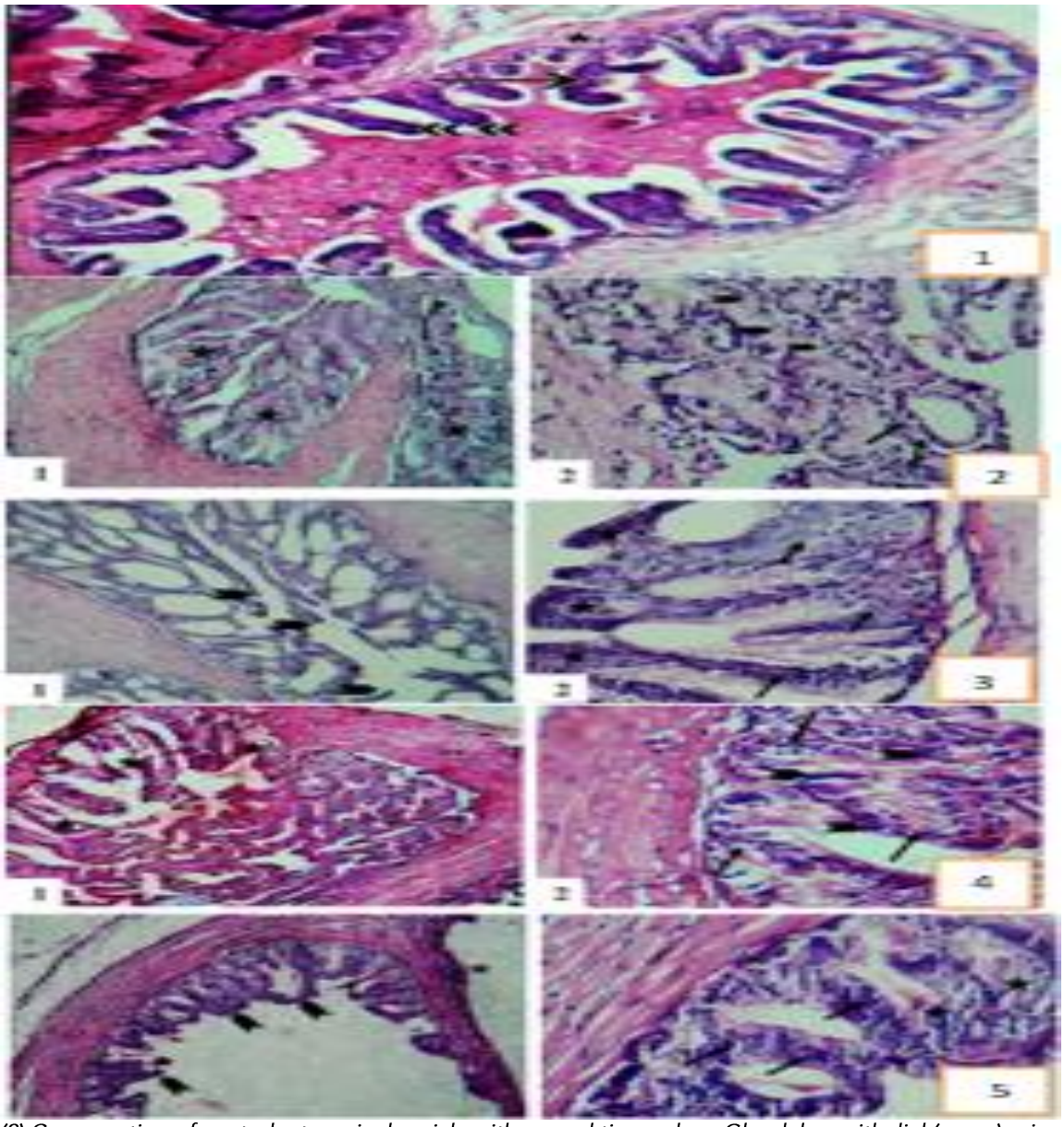


Figure 2: A cross section of seminal vesicle picture1 of rats from control group revealed a normal intact architecture of seminal;

Figures (1) Cross section of the rat prostate(Picture2)represent 40µg/kg for30 day,(1)showed stromal connective tissue was increased between the secretory alveoli. (2)Arrows indicate multiple areas of hyperplasia.Cross section of the rat prostate (Picture3)represent 80µg/kg for 30day,(1) showed increase in alveoli folding is considerable(star)(2)atypical hyperplasia (arrow) and showing focus crowded with epithelial cells(arrow head).Cross section of the rat prostate(Picture4) 40µg/kg for 60 day,(1)showed the acinar dilation (star) (2)atypical hyperplasia(arrow).cross section of the rat prostate(Picture5) 80µg/kg for 60 day(1) showed the expanding of secretory alveoli (arrow double head) and thin-walled considerable.(2)epithelial degeneration is characterized by granular to foamy cytoplasm alteration of enlarged acinar epithelial cells that from a single lining layer (arrow)[Haematoxylen & Eosin stain, 100X and 400X].

whereas picture2,3 showed the shrinking of the epithelial cells, some acini, lined by simple cuboidal,point epithelial necrosis,degenerative atypia and epithelial hypeplasia,note papillary growth.whereas picture 4,5 showed hyperplasia of seminal vesicle epithelium with degenerative atypia and vacuolation, tightly clustered glands with plump, hyperchromatic nuclei.



Figure(2):Cross section of control rat seminal vesicle with normal tissue show Glandular epithelial (arrow) primary fold in the mucosa(arrow head) lamina propria (star)

Figure(2)Cross section of the rat seminal vesicle (picture2) 40µg/kg for30 day (1) showed the shrinking of the epithelial cells(stars).(2) some acini, lined by simple cuboidal (arrow)and(arrow head) point epithelial necrosis but the larger cells with degenerative atypia and vacuolation observed in the cytoplasm.Cross section of the rat seminal vesicle (picture3) 80µg/kg for 30 day,(1)showed bleb-like apical projections(arrow head).(2)epithelial hypeplasia,note papillary growth(stars) and epithelial necrosis but the larger cells with degenerative atypia occasional nuclei are hypertrophied (arrow) Cross section of the rat seminal vesicle (picture 4) 40µg/kg for 60 day, (1)showed the shrinking of the epithelial cells(s. (2)hyperplasia of seminal vesicle epithelium with degenerative atypia and vacuolation (arrow) and arrow heads indicate typical hyperchromatic nuclei. Cross section of the rat seminal vesicle (picture5) 80µg/kgfor 60 day, (1) showed the enlargement and shorting acini (arrow head). (2) tightly clustered glands with plump, hyperchromatic nuclei(arrow) and sub-epithelial stromal degeneration(star)[Haematoxylen & Eosin stain, 100X and 400X].

5. Discussion

The high quality of nanoparticles engineering gave it a wonderful many characteristic that made them able to penetrate and biologically distributed within animal bodies a case that made nanoparticles be highly used in medicine [42]. The results showed a clear tissue effect in the prostate section that were treated40µg/kg,80µg /kg for 30 days, and compared it with the control group that appears normal section a columnar epithelium aligned in one layer with epithelial fold in acinus. The section of the prostate shows an increased in alveoli folding,increased connective tissue(stromal) between the secretory alveoli and atypical hyperplasia. It is known that the effects Au NPs depend on the dose given, duration of exposure and the size of surface area of gold particles and this corresponds with (43) where morphological

abnormalities were found with more spread in the glandular epithelium area and abundant folds with simultaneous administration of Au NPs led to exacerbation and development of BPH and promotion of the inflammatory process. It was found that when the level of the transformed growth factor TGF-β1 causes the proliferation of the appearance cells, and this corresponds with (44, 45).

Also, increased expression of vascular endothelial growth factor (VEGF), an angiogenesis process was also observed in BPH,as it is the main driver of its formation increased VEGF expression is implicated in the formation of BPH(46). Research has shown that excessive TGF leads to fibrosis, which contributes to the formation of tumours while it is considered as cytokine that has a major role in limiting cellular proliferation and thus leads to apoptosis, but it may act as a traitorous friend by increasing cellular transformation and low apoptosis this agree with(47,48).The elevated expression of the interleukins IL-17, IL-6 and IL-8 the two major pathways in stromal epithelial growth in BPH, stimulate an inflammatory response that can exacerbate BPH development this agree with (49,50,51). But, when dosed for 60 days, it leads to the expanding of secretory vesicles and their thin walls, accompanied by cell degeneration due to the decrease in testosterone which appeared in the current results resulting from oxidative stress in the production of ROS in ledyig cells accompanied by decrease in DHT which is one of the by-products of testosterone, where10% of testosterone is converted to DHT Dihydrotestosterone by enzyme Alpha-reductase, which it produced in the testes, prostate and ovaries and plays a role in the development of prostate this agree with (52).

The result show the effect of Au NPs on seminal vesicle where epithelial cell shrinkage with epithelial necrosis and cytoplasm vacuolation with atypia degeneration at a concentration of 40µg /kg,80µg /kg for 30 days comparative with the control group it was observed that normal structures of muscle tissue surrounding the folds lined with columnar epithelial tissue or columnar psudo stratified. When the dosing for 60 days, tissue sections showed shrinkage of the epithelial cells with atypical degeneration hyperplasia and degeneration of the sub-epithelial stromal this agree with (53) using Cu NPs at concentration of 100ppm. It is found that NPs easily pass through the testicular and brain barriers. The seminal vesicle gland is important in male animals as it is one of the accessory glands that secretes about 60 % of seminal plasma rich in proteins, complex carbohydrates and fructose (54). The seminal vesicle is highly dependent on androgenic hormones, including testosterone to maintain its structure and function,which is very sensitive to blood levels of androgens this agree with(55). The researchers confirmed the role of testosterone inhibiting the action of substances that affect the activity of the seminal vesicle agree with (56). The results showed a decrease in seminal vesicle weight hypertrophy, necrosis and atrophy,the reason is due to the decrease in the secretory activity of the leydig cells of this hormone (Testosterone).This result is in agreement with (57) which observed a significant decrease in the level of T hormone when the rat was treated with 100mg of Au NPs.

6. Conclusion

Our present study indicated that a dose of 80µg\kg of body weight of gold nanoparticles could cause a deleterious toxic effect on the prostate. seminal vesicle structure more prevalently than the dose of 40 µg\kg of body weight.

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