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HISTOLOGICAL AND IMMUNO-HISTOCHEMICAL STUDY OF THE CYTOTOXIC EFFECTS OF GOLD NANOPARTICLES ON THE PALATE OF ALBINO RATS

Mervat M. Youssef* *and* Ghada M. Saied**

ABSTRACT

Aim: The aim of the present work was to study the possible cytotoxic effect of gold nanoparticles on the palate of albino rats using routine H&E stain, Masson' trichrome and immuno-histochemical detection of any possible changes in NF-κB (nuclear factor kappa).

Materials and methods: Forty-six adult male albino rats with an average of 150-180 gram body weight were used in this investigation. They were housed in rat cages, (five rats per cage), and labeled with numerical numbers and kept in well ventilated animal house at the faculty of dentistry, Suez Canal University, at temperature 27-30°C, 12 hours natural light and 12 hours darkness. The animals were fed with dry pellet and allowed drinking water adlibitum. Animals were divided randomly into three groups as follows: **Group 1:** consisted of 16 rats that received a daily intraperitoneal injection of AuNP solvent (0.5 ml deionized water) for 21 days and served as controls. Half the animals of the control group (sub-group 1.1) were euthanized after 21 days, while the other half (sub-group 1.2) were left untreated for one month, then euthanized. **Group 2:** consisted of 15 rats that received 10mg/kg body weight /day of AuNPs solution dissolved in 0.5 ml deionized water with particle size around 30 nm intraperitoneal for 21 days. **Group 3:** consisted of 15 rats, they were treated as group 2 for 21 days and then left for one month as a recovery period.

The experiment lasted for 21 days for group 2 then the rats were euthanized by cervical dislocation. While the rats of group 3 were euthanized after one month for recovery. The palate of all animals were dissected out, fixed in 10% neutral buffered formalin, processed and embedded in paraffin. Four to five microns thick sections were cut to be stained with; hematoxylin and eosin for histological examination, Masson's trichrome stains for collagen evaluation and immune-histochemical localization of NF-κB (nuclear factor kappa) for detection of any possible cellular changes.

Results: The histological and immune-histochemical results revealed atrophy and degenerative changes in the palatal tissues associated with increased expression of nuclear factor kappa. A recovery period of one month resulted in regeneration and improvement in the histological structure and function of the tissues.

*Assistant Professor of Oral Biology, Oral Biology Department, Faculty of Dental Medicine, Suez Canal University
** Lecturer of Oral Biology, Oral Biology Department, Faculty of Dental Medicine, Suez Canal University

Conclusion: Exposure to over dose of AuNPs causes a degree of cytotoxicity manifested as atrophy and degenerative changes accompanied by increased activation NF-kB. A recovery period of one month resulted in regeneration and improvement in the histological structure and decreased cytotoxicity as detected by low NF-kB expression.

KEYWORDS: AuNPs (Gold nanoparticles), Cytotoxicity, palatal mucosa, NF-κB (nuclear factor kappa)

INTRODUCTION

The focus on applying nano-biomaterials clinically in dentistry has attracted attention of researchers worldwide over the last few years[1]. The basic concept of nanotechnology refers to manipulating matters at the level of molecules or atoms so as to generate new materials and devices having novel extraordinary properties. The nanostructures are considered by the nanotechnology in the approximate range of 1-100 nanometers (10^{-9}m), which results in showing properties and functionalities which are principally different from those of other length scales[2].

Nanodentistry is emerging as a new field as there is an increasing interest in dental application of nanotechnology in the future. Through using nanomaterials and biotechnologies including tissue engineering and nanorobots, the nearly perfect oral health will be possible by developing nanotechnology [3].

Nanotechnology has many applications in dentistry such as curing hypersensitivity permanently, local anesthesia, dentition renaturalization, complete orthodontic realignment during a single office visit, continuous oral health maintenance by using mechanical dentifrobots (nanorobotic dentifrice), and covalently bonded diamondized enamel. Such applications have the ability to destroy caries-causing bacteria and even repair blemishes of decayed teeth[4]. Moreover, there is also a number of synthetic nanoparticles including hydroxyapatite, bioglass, titanium, zirconia, and for dental restoration, silver nanoparticles are proposed[5]. The nanodentistry is expected to eventually lead to the development of highly efficient, effective, and personalized dental treatment[6].

Gold has been used in medicine (chrysotherapy) since antiquity and the purpose of its use was the treatment of diseases in the ancient Egyptian, Chinese and Indian cultures. Diseases including syphilis, small pox, skin ulcers, and measles were treated using gold[7,8]. Several organo-gold compounds with promising antitumor, antimicrobial, antimalarial, and anti-HIV activities have emanated during the last few decades into a set of different sizes and shapes using different strategies[9]. It is possible to synthesize the Gold Nanoparticles (AuNPs). The chemical or electrochemical reduction of a gold (III) precursor is considered the most commonly used method. Shape and size can be controlled under careful experimental conditions including the particular reduction agent, time of reaction, temperature, and using a capping agent that binds for the selection of the growth of nanoparticle faces and leads to blocking growth beyond a particular range of nanometer[10].

The characteristics of gold nanoparticles include high chemical stability, good resistance of oxidation, and biocompatibility. Therefore, they can be employed for diagnostic and therapeutic purposes, or as carriers of drugs. In addition, such characteristics have high dependency on their size and shape. Consequently, the fabrication of gold nanostructures characterized by certain shapes using various methods has received great interest[11].

Despite the fact that metal nanoparticles are innovative in therapy, imaging, and early diagnosis of several diseases, their toxicity has to receive

a special attention as they tend to accumulate in the liver and kidney. As a result, it is necessary to address their removal from the body[12] .

Nanotoxicity is a new study area, which focuses on the toxicological profiles of nanomaterials (NMs) and how different NMs affect living systems. At nanoscale dimensions, the inert elements like gold become active, i.e. there is a belief that AuNPs are more reactive biologically than their bulk material as their size is small and the ratio of surface area to volume is large[13,14].

Several previous studies were carried out on the evaluation of the toxicity of different nanostructured gold compounds. They indicated that the existence of the transition metals core encourages reactive oxygen species (ROS) production, resulting in oxidative stress[15].

Moreover, when such nanoparticles are used into a biological system, they can have a strong relationship with a various range of molecules existing in the extra- and intracellular environment. Low-molecular-weight species, lipids, nucleotides, and proteins in particular, which have ability to build strong interactions with these nanoparticles, are amongst these molecules. In turn, this results in an imbalance in the oxidative status. Consequently, it affects the function of these molecules[16].

The nuclear factor-κB (NF-κB) refers to a dimerictranscription factor, which is in charge of controlling the expressing genes included in several critical physiological responses including proliferation, inflammatory responses, cell adhesion, apoptosis and differentiation[17]. The NF-κB plays a critical role in human health. In addition, the aberrant activation of NF-κB makes a contribution to developing numerous autoimmune, inflammatory and malignant disorders such as atherosclerosis, rheumatoid arthritis, multiple sclerosis, malignant tumors and inflammatory bowel diseases[18,19]. On the other hand, the inducible activation of NF-κB is reliant on the phosphorylation-induced proteosomal degradation of the inhibitor of NF-κB proteins (IκBs). Such proteins are responsible for the retention of inactive NF-κB dimers in the cytosol in unstimulated cells. Most of diverse having the signals of pathways leading to that activation of NF-κBare convergent on the IκB kinase (IKK) complex. This complex is in charge of IκB phosphorylation and it is critical for the transduction of signal to NF-κB[20].

The activation of NF-κB is the result of a wide range of different external stimuli. Further, critical and conserved roles are played by the genes. The regulation of the expression of those genes is by NF-κB, in immune and stress responses. Also, the genes have an effect on processes including proliferation, differentiation, apoptosis, and development. Further, the activation of NF-κB is result of bacterial and viral infections (for example, by recognizing the products of microbes by receptors including the Toll-like receptors), antigen receptor engagement, and inflammatory cytokines. As a result, this is an indication of its crucial role in innate and adaptive immune responses. Moreover, the physical (UV- or g-irradiation), physiological (ischemia and hyperosmotic shock), or oxidative stresses can lead to the activation of NF-κB[21.22].

Despite the usefulness of gold nanoparticles in a large number of medical applications, the AuNPs are relatively biocompatible due to their inert nature. Some scientists perceive NPs as nontoxic, while other studies indicated that the NPs has toxic effects[23,24]. As a result, using routine H&E, Masson's trichrom stains and immune-histochemical detection of any possible changes in NF-κB (nuclear factor kappa) in the surface epithelium, lamina propria, as well as palatal salivary glands, this study aims to examine the histological and immuno-histochemical effect of AuNPs on the palate of rats.

MATERIALS AND METHODS

Forty-six adult male albino rats with an average of 150-180 gram body weight were used in this investigation. They were housed in rat cages (five rats per cage), and labeled with numerical numbers and kept in well ventilated animal house of the faculty of dentistry Suez Canal University, at temperature 27-30°C, 12 hours natural light and 12 hours darkness. The animals were fed with dry rat pellet and allowed drinking water adlibitum.

Animals were divided randomly into three groups as follows:

Group 1: consisted of 16 rats, that received a daily intraperitoneal injection of AuNP solvent (0.5 ml deionized water) for 21 days and served as controls.

Half the animals of the control group (sub-group 1.1) were euthanized after 21 days, while the other half (sub-group 1.2) were left untreated for one month, then euthanized.

Group 2: consisted of 15 rats, that received 10mg/kg body weight /day of AuNPs solution(25) dissolved in 0.5 ml deionized water with particle size around 30 nm(26) intraperitoneal for 21 days.

Group 3: consisted of 15 rats, they were treated as group2 for 21 days and then left for one month as a recovery period.

Preparation and characterization of the Au nanoparticles

Rapid green method was used for preparation and characterization of AuNPs; it was done in the nanotechnology lab in the faculty of science, South Valley University.

1. From the local market, pomegranate fruits were collected, and Chloroauric acid (>99.9%) was obtained from International Company for Scientific and Medical Supply. Using de-ionized water, all glass-wares and pome fruit were properly washed and then dried in oven. Fruit peel extract (FPE) of pome was used as a reducing agent to develop gold nanoparticles.
2. In 250ml ultra-pure water in 500ml Erlenmeyer flask, an amount of 50g of properly washed fresh peels of the fruit were added and boiled for 10-15mins. Using Whatman filter paper (No. 40), the boiled material was filtered to prepare the aqueous fruit peel extract (FPE) in order to be used as such for metal nanoparticle synthesis.
3. After the Aqueous solution (1mm) was prepared of chloroauric acid solution, by the use of 1.8ml of FPE at room temperature for 5 minutes, 50ml of the metal (Au) ion solution was reduced. Lower than this FPE quantity, more than 10 mints were the duration for the solution to reach a significant surface plasmon resonance (SPR) for the metal nanoparticle. Accordingly, pink-colored solution, which is an indication of gold nanoparticles' formation, was accomplished after the addition of the FPE.
4. At different reaction conditions, the spectral analysis was observed using X- Ray diffractometer (XRD) in order to develop nanoparticles. To analyze the size and shape of the developed nanoparticles, Transmission electron microscope (TEM) JEM-1200EX, JEOL 1010 was used.
5. To get the measurements of TEM, an amount of 3μl of the sample solution was added on a grid of copper for forming a thin film of the sample on the grid. Then, it was kept for drying at room temperature for 15mins. Through the use of the cone of a blotting paper, the extra sample was eliminated and kept in a grid box.
6. To measure x-ray powder diffraction, the resultant solutions of the developed AuNPs were dried at 800°C, and using GBC EMMA diffractometer in the Central laboratory at South Valley University, the data of X-ray powder diffraction was obtained.

The experiment lasted for 21 days for group 2 then the rats were euthanized by cervical dislocation.

While the rats of group 3 were euthanized after one month for recovery by the same way; their palate were dissected out, fixed in 10% neutral buffered formalin, processed and embedded in paraffin. Four to five microns thick sections were cut to be stained with:

001. Hematoxylin and eosin for histological examination.

002. Masson's trichrome stains for collagen evaluation.

003. Immuno-histochemical localization of NF-κB (nuclear factor kappa) for detection of any possible cellular changes.

Immuno-histochemical staining procedure used for NF-κB detection[27]:

1. From the palatal tissues, 4-μm-thick paraffin-embedded sections were deparaffinized and rehydrated. Then, they were immersed in target retrieval solution, and blocked to prevent the activity of endogenous peroxidase.
2. Then, in TNB-BB (100 mMTris, pH 7.5, 150 mMNaCl, 0.5% blocking agent 0.3% Triton-X, and 0.2% saponin), those sections were permeabilized. Then, they were incubated in primary monoclonal antibodies of NF-κB/p50, NF-κB/p65, and IκBα (Santa Cruz Biotechnology) at a dilution of 1:200, respectively, overnight at 4°C.
3. After washing sections for three times in TBS, at room temperature, they were incubated for 2 hours with HRP-labeled polymer conjugated with secondary antibody.
4. Through the use of AEC$^+$ substrate chromagen, which consists of 3-amino-9-ethylcarbazole, immunoreactive complexes were detected. Then, slides were counterstained in Mayer's hematoxylin. Afterwards, they were mounted in crystal mount media. Finally, they were dried overnight on a level surface.
5. Positive staining section appeared brown in color. The intensity of the immunohistochemical staining results was assessed as follows: negative, weakly positive, moderately positive and strong positive staining reactions.

RESULTS

I. Histological results:

Hematoxylin& eosin and Masson's trichrome stains

1-Group 1: control group (sub- group 1.1, sub-group 1.2)**:**

Normal histological features of the surface epithelium, lamina propria, submucosa and underlying palatal bone were revealed by the histological examination of the palate obtained from control groups. The keratinized stratified squamous epithelium formed the surface epithelium. In addition, the surface epithelium was characterized by folding toward the underlying lamina propria forming regular, broad and short epithelial ridges.

The epithelium was formed of four layers; the basal cell layer formed of a single row of low columnar cells resting on the basement membrane, prickle cell layer formed of several rows of polyhydral cells. Then the granular cell layer formed of 2-3 rows of large flattened granular cells and then the most superficial hornified layer with its eosinophilic amorphous appearance.

The connective tissue of the lamina propria showed regular arrangement of collagen fiberswhich revealed normal distribution and strongly positive staining reactivity to Masson's trichrome stain (Figure 2, group 1), fibroblasts, progenitor cells and small sized blood vessels.

There is a submucous layer connecting the lamina propria to the underlying palatal bone. This submucosa was formed of densely packed collagen fibers, in between them there was loose connective tissue containing fat cells and palatal salivary glands which were pure mucous. The underlying mature lamellar bone were composed of bone trabeculae and intervening bone marrow spaces (Fig. 1, A).

2- Group 2: The palate obtained from rats received 10mg/kg b.w/day of AuNPs solution for 21 days showed variable histopathological changes in their structures manifested as atrophic and degenerative changes. The surface epithelium showed marked decrease in its thickness, the rete ridges were too short, scanty, and in most cases lose their normal pattern with possible total absence of multiple areas.

The lamina propria showed degeneration of the collagen fibers with weakly positive reactivity to the Masson's trichrome stain (Fig.2, group 2). The palatal salivary gland showed cystic transformation of some of the mucous acinar cells and others showed degeneration and necrosis as well as dissociation of the collagen septa between them. Extreme widening of the marrow cavities in the underlying palatal bone and massive fatty infiltration. Inflammatory cell infiltrate was a common feature together with osteoclastic bone resorption resulting in marked rarefaction of bone trabeculae (Fig, 1; B, C, D, E).

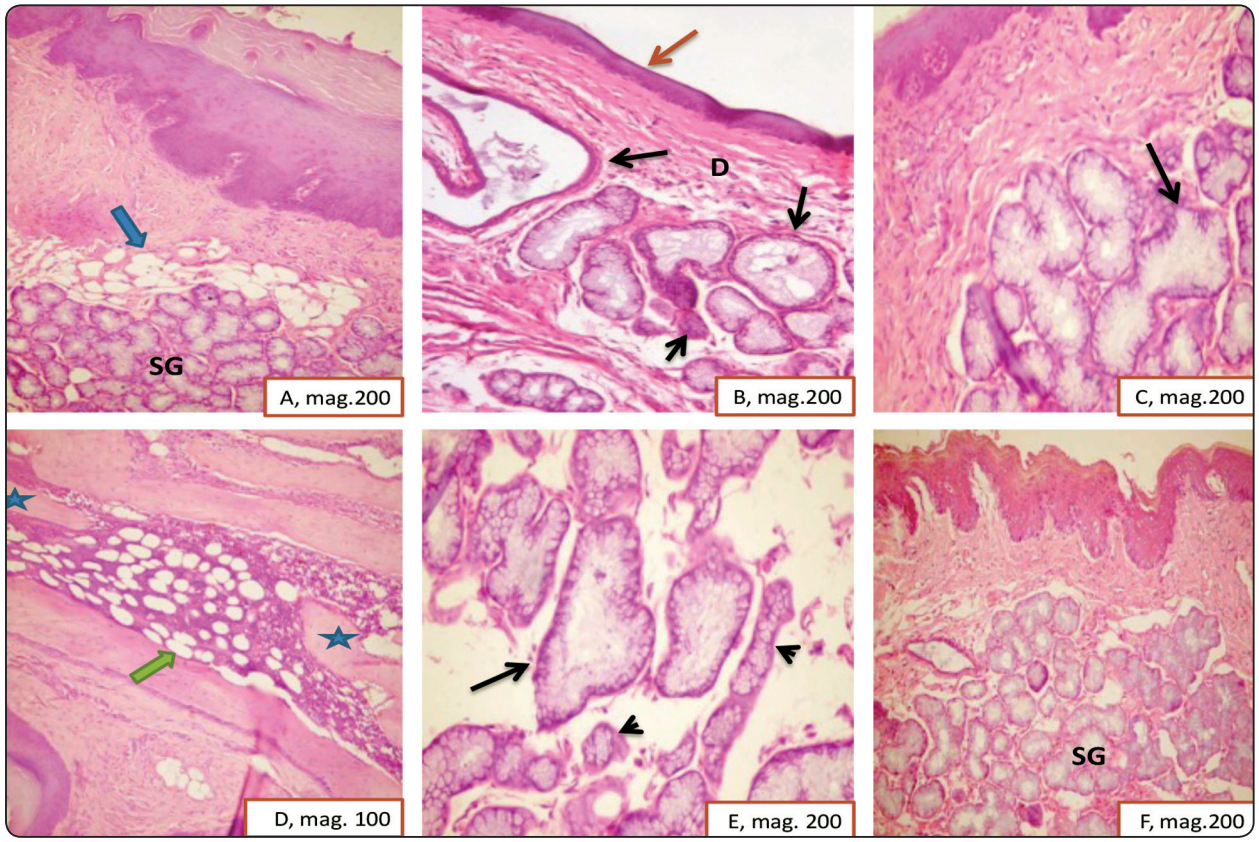

Fig. (1): Photomicrographs of palate of different groups. A-Showing the palatal mucosa of control group with normal histological structure. The surface epithelium of the keratinized stratified squamous type, regular, broad, few and short epithelium ridges . The lamina propria and submucosa formed of densely packed collagen fibers, fat cells (blue arrow) and palatine pure mucous salivary glands (SG) (H&E, ori. Mag.200). B, C, D, E-Photomicrographs of the palate of rats received 10mg/kg b.w/day of AuNPs solution for 21 days showing attrophy of the surface epithelium with rete ridges loss of their characteristic pattern (red arrow). Degeneration of collagen fibers in the underlying lamina propria (D) with mild inflammatory cell infiltration. Cystic transformation (black arrows), degeneration and necrosis (arrow heads) of some of the mucous acini. Underlying bone showing resorption, thinning of bone traceculae (star) and Extreme widening of marrow cavities with massive fatty and inflammatory cell infiltration (green arrow)(H&E, ori. Mag.100,200). F- Photomicrograph of the palate of rats that were left for one- month as recovery period showing improvement in the condition of the palatal mucosa, salivary glands (SG) and bone, nearly normal (H&E, ori. Mag.200).

3-Group 3: The examined palate of group3 rats that were left for one month as a recovery periods showed an improvement in the condition of the palatal mucosa, salivary glands and bone. The surface epithelium had normal thickness, normal pattern of epithelial ridges with normal density of collagen fibers that showed moderately to strongly positive reactivity to the Masson's trichrome stain (Fig, 2, group 3). Although areas of degeneration of fibers and cells were still existing, dissociation of the collagen fibers was limited to focal areas. Decrease in the tendency of bone resorption was observed clearly and the widening of marrow cavities of the alveolar bone was limited compared to those of group 2 (Fig. 1; F)

II-Immuno-histochemical results:

Immuno-histochemical localization of NF-κB (nuclear factor kappa)

1. **Group 1 (Control group):** Nuclear factor (NF)-κB expression in palatal surface epithelium and salivary glands of control rats using primary monoclonal antibodies of NF-κB/p65, NF-κB/p50, and IκBα showed negative to weakly positive staining reactivity (Fig.3,A).
2. **Group 2:** Examination of sections taken from the palate of group 2 rats incubated with subunits of NF-κB primary monoclonal antibodies revealed moderately to strongly staining reactivity of their epithelium, lamina propria and cells of the duct and mucous acini of the palatal salivary glands (Fig.3, B, C, D, E).
3. **Group 3:** Nuclear factor (NF)-κB expression in the palate of rats left for one -month as recovery period using subunits of NF-κB primary monoclonal antibodies showed weakly to moderately staining reactivity of surface epithelium and the palatal salivary glands (Fig.3, F).

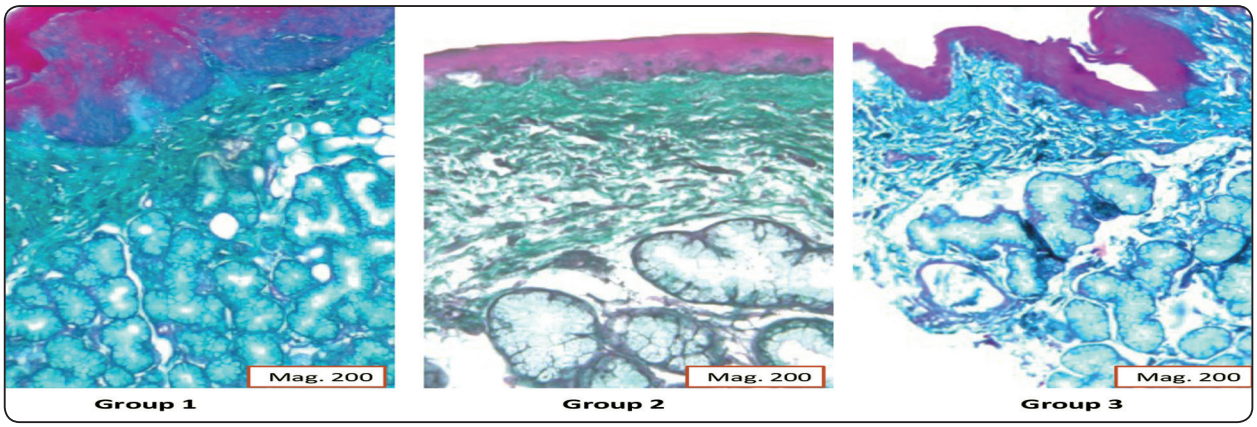

Fig. (2) A photomicrograph of the palate of different groups stained with the Masson's trichrome stain. Group 1 showing strongly positive reactivity of the collagen fibers with Masson's trichrome stain. Group 2 animals received 10mg/kg b.w/day of AuNPs solution for 21 days showing weakly to moderately positive reactivity to the Masson's trichrome stain. Group 3 animals showing moderately to strongly positive reactivity to the Masson's trichrome stain (Masson's trichrome, origin mag.200)

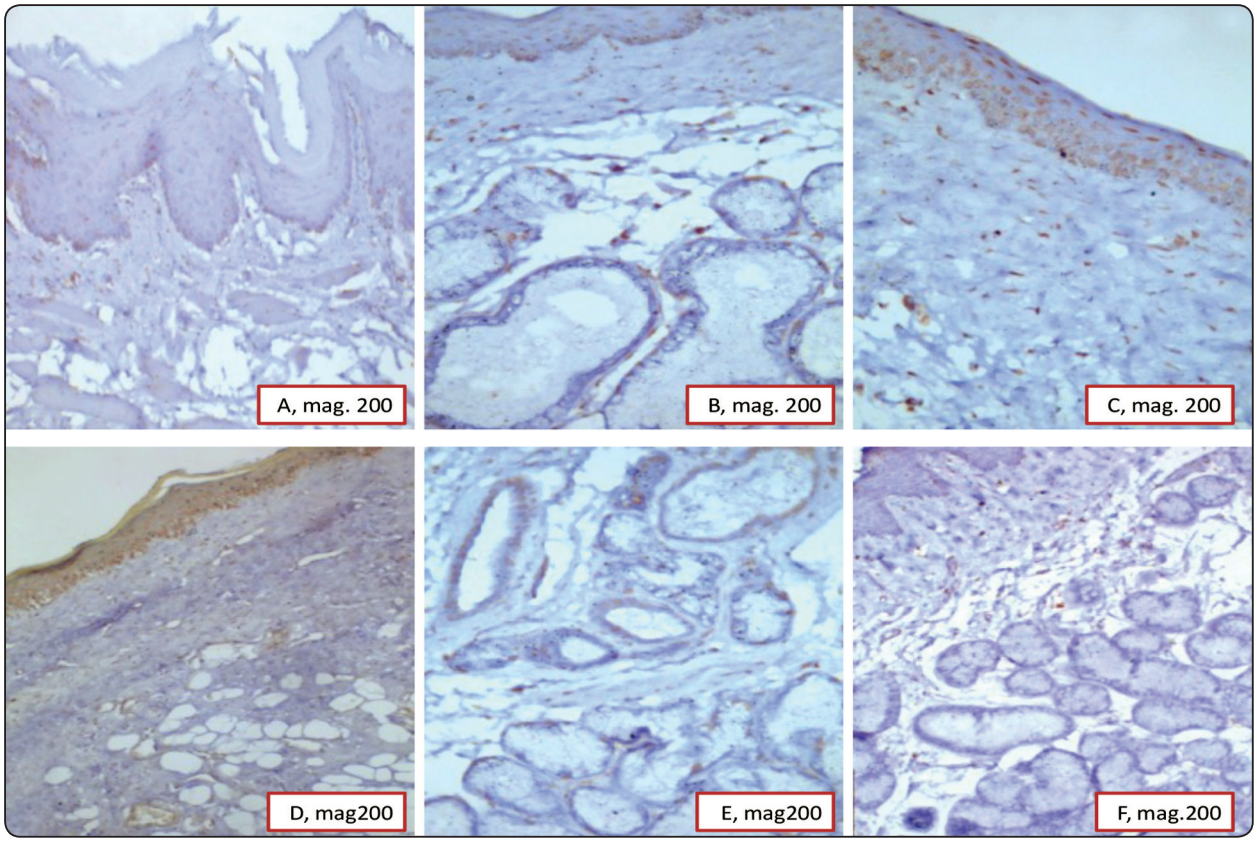

Fig. (3): Photomicrographs of the palate of all groups incubated with subunits of NF-κB primary monoclonal antibodies. A- showing negative to weakly positive staining reactivity. B,C, D, E- showing moderately to strongly staining reactivity of epithelium, underlying lamina propria and cells of the duct and mucous acini of palatal salivary glands. F-showing weakly to moderately staining reactivity to the primary monoclonal antibodies of NF-κB/p65, NF-κB/p50, and IκBα subunits of NF-κB (Orig. mag. 200).

DISSCUSSION

Traditionally, Gold has been characterized by being inert and biocompatible. In addition, due to its physicochemical properties and high surface area, it is considered a crucial factor which should be taken into consideration prior to clinical application. In biomedical research, gold nanoparticles (AuNPs) are commonly used[28]. This is because of the easiness of their synthesis and characterization. Also, it possible to conjugated them with multiple chemicals and biological molecules to create biocompatible, targeted, and controlled diagnostic, treatment, and delivery systems[29].

A plenty of experimental work was carried out to confirm the non-toxicity of AuNPs[30]. However, on the contrary, there are several conflicting research findings indicating the toxicity of gold NPs[31]. As the cytotoxicity of the nanoparticles are reliant more or less on their surface properties, shape, size, and chemical composition, it should be assessed due to the large gap existing between the data which is available on the production of nanomaterials and the assessments of toxicity[32].

In the present study, the rats received 10mg/kg b.w/day of AuNPs solution with particle size around 30 nm intraperitoneally as a toxic dose coincided with that presented by Katsenelson et al[25], Chaves et al[26] and Zhang et al[33].

The results of current study revealed noticeable histopathological changes displayed as atrophic and

degenerative changes in the palatal tissues of rats of group 2, which received AuNPs. In turn, this shows the cytotoxic effect of gold nanoparticles on tissues under examination. A large number of previous studies carried out by[34-36] examined whether there are several different mechanisms which cause the toxicity of nanoparticles (NP)in body. However, most of intracellular and in-vitro toxicities extracted from NPs are the result of the production of excess reactive oxygen species (ROS). Such ROS are physiologically important, however, they are potentially destructive. At moderate levels, the ROS have certain roles in the modulation of a lot of cellular events such the transduction of signals, gene expression, proliferative response, and protein redox regulation[37,38]. However, at higher levels, the ROS represent an indication of oxidative stress and can result in the damage of cells through the peroxidization of lipids, the alternation of proteins, the disruption of DNA, the interference with the functions of signaling, and gene transcription modulation and finally ending up in cancer, cardiovascular, renal disease, neuro-degeneration, or pulmonary disease[39]. Moreover, the ROS have the ability to steal electrons from lipids in cell membrane, leading to decreasing physiological function and cell death[40]. However, excess amounts of ROS can result in damaging the mitochondrial DNA (mtDNA) and accordingly provoking mutations, which alter mitochondrial function and shut down mitochondria, causing cell death[41].

Higher levels of oxidative stress leads to the activation of inflammation signaling through NFκB, and very high levels are associated with activating apoptotic pathway sand necrosis[42]. This agrees with our immuno-histochemical study. The findings of current study are consistent with those of Sonis[43] who showed that the activation of NF-κB leads to the upregulation of expressing pro-inflammatory cytokines such as interleukin (IL)-1β, tumor necrosis factor (TNF) α, and IL-6. Possibly, the higher levels of such cytokines results in the induction of inflammatory reactions in oral mucosa, the promotion of damaging the underlying connective tissues, the reduction of the epithelial oxygenation, and ultimately the death and injury of epithelial basal cell. Due to the efficiency of TNFα and IL-1β as activators of NF-κB, when they repeatedly lead to the activation of NF-κB, they can result in the amplification of the mucosal damage in a vicious circle. Deng et al[44] revealed that unfolding fibrinogen is caused by some NPs. Therefore, this can promote its interaction with the integrin receptor, Mac-1. In case of activating this receptor, the result is upregulating the NFκB signaling pathway and releasing inflammatory cytokines.

The characteristics of gold nanoparticles (including surface chemistry, shape, and size) play an important role in the determination of the interactions between inorganic nanoparticles and biological membranes[45]. Yao et al[46] revealed that if the particle size is small, the total accumulation of gold within the epithelial cells is greater. Further, their TEM images indicated that the gold nanoparticles spread gradually all over the model epithelium cells after absorption. Then, eventually, the accumulation of these nanoparticiples takes place within numerous cellular organelles including as lysosomes, endosomes, mitochondria, Golgi apparatus, and nucleus. In addition, the accumulation of AuNPs within these cellular substructures promotes cytotoxicity. The results of previous studies have shown that the AuNP shave the ability to depolarize mitochondria membranes in mammalian cells[47]. Any decrease in the mitochondrial membrane potential is considered an early indication of cell death[48]. From our point of view, the impaired mitochondrial function can lead to elevating the levels of DNA damage and cellular toxicity as show by our results.

In a similar manner, our findings are consisted with those of Lu et al[50] who examined the impact

of 34 nm AuNPs in different concentrations on keratinocyte proliferation. They found that low concentrations (5 ppm) can induce keratinocyte proliferation, however higher concentration (>10 ppm) can be toxic to keratinocytes.

Furthermore, our results agree with previous studies suggesting that the viability of human bone marrow mesenchymal stem cells (**hBMSCs)** relies on the size of AuNPs. At the smallest size, the AuNPs not only exhibited significant cytotoxicity but also substantially suppressed the clonogenic ability of hBMSCs. When the AuNPs are used at medium size, the study of cell death pathway revealed that AuNPs triggered hBMSCs' necrosis, possibly elicited from excessive ROS formation. Despite the fact that at medium size, the AuNPs had minor cytotoxicity to cells, they suppressed osteogenic and adipogenic differentiation[51].

The histological observations of our study are consistent with those of Pernodet et al[52] who investigated the adverse impacts of citrate coated AuNPs on human dermal fibroblast cells and showed that when AuNPs are existing, they result in major changes in cell growth, cell shape and alteration of actin fibres. Furthermore, they revealed that 14 nm AuNPs has the ability to easily pass through the cell membrane and accumulate into vacuoles. In case AuNPs are existent, this causes the constructs of abnormal actin filaments and extra cellular matrix in dermal fibroblasts. Further, their existence reduces cell adhesion, proliferation, and motility. On the contrary**,** the study carried out by[53] found that with negatively charged surface (-23.4 mV), the AuNPs (average diameter 25-50nm) were not toxic to the dermal fibroblasts and epidermal keratinocytes of normal human. Also, they exerted anti-inflammatory activity by inhibiting anti-angiogenic activity and IL-6, IL-12 and TNF-α level, resulting in reducing the expression of vascular endothelial growth factor (VEGF) and basic fibroblast growth factor (bFGF). Such characteristics are significant clinically and make a contribution to the application of AuNPs in biomedicine.

In our study group 3, rats that were left for one month as a recovery period revealed an improvement in the condition of the palatal mucosa, salivary glands and bone. The observations of our study are consistent with those of Miranava et al[54] who showed that the damage of AuNPs to cells is not permanent and cells can totally recover as a function of the size, concentration and exposure time of AuNPs. Taken together, their findings suggested that AuNPs have detrimental impacts on cell function, which can reverse after the removal of AuNPs.

Finally, it could be postulated that the exposure to over dose of AuNPs causes a degree of toxicity on the tissue under investigation but also recovery take place after a period of time.

CONCLUSION

Despite all the benefit of GNPs and biocompatibility, over dose of AuNPs causes a degree of cytotoxicity manifested as atrophy and degenerative changes accompanied by increased activation of NF-kB, the factor of transcription, which is included in regulating a large number of genes controlling various aspects of the immune and inflammatory response.

A recovery period of one month can lead to regeneration and improvement in the histological structure and function of the tissues.

REFERENCE

1. Padovani, G. C.; Feitosa, V. P.; Sauro, S.; Tay, F. R.; Duran, G. and Paula, A. J.: Advances in dental materials through nanotechnology: facts, perspectives and toxicological aspects. Trends Biotechnol.2015; 33: 621-636.
2- Warren, H. and Hunt, J.: Nanomaterials: Nomenclature, novelty, and necessity. JOM.2004; 56: 13-18.
3- Shetty, N. J.; Swati, P. and David, K.: Nanorobots: Future in dentistry. Saudi Dent J.2013 ; 25: 49–52.
4- Rybachuk, A.V.; Chema, I. S. and Nebesna, T. Y.: Nanotechnology and nanoparticles in dentistry. J Pharmacol Pharm. 2008;1:18–20.

5- Shashirekha, G.; Jena, A. and Mohapatra, S.: Nanotechnology in Dentistry: Clinical Applications, Benefits, and Hazards. CompendContinEduc Dent. 2017; 38: 1-4.

6- Besinis, A.; Peralta, T. D.;Tredwin, C. J. and Handy, R. D: Interactions with the Oral Microenvironment, Clinical Applications, Hazards, and Benefits. ACS Nano. 2015; 9: 2255–2289.

7- Huaizhi, Z. and Yuantao, N.: China's ancient gold drugs. Gold Bull.2001; 34: 24-29.

8- Richards, D.G.; McMillan, D.L. and Mein, E. A.: Gold and its relationship to neurological/glandular conditions. Int J Neurosci. 2002; 112: 31-53.

9- Sun, R. W.; Ma, D. L and Wong, E. L.: Some uses of transition metal complexes as anti-cancer and anti-HIV agents. Dalton Trans. 2007; 43: 4884-4892.

10- Baptista, P.; Pereira, E.; Eaton, P.; Doria, G.; Miranda, A.; Gomes I.; Quaresma, P. andFranco, R.: Gold nanoparticles for the development of clinical diagnosis methods. Anal Bioanal Chem. 2008 , 391: 943-950.

11- Parveen, S.; Misra, R. and Sahoo, S. K.: Nanoparticles: a boon to drug delivery, therapeutics, diagnostics and imaging. Nanomed.Nanotechnol. Biol. Med. 2012, 8: 147-166 .

12- Mohamed A. K. and Sherif A. A.: The gold nanoparticle size and exposure duration effect on the liver and kidney function of rats: In vivoSaudi J Biol Sci. 2013; 20: 177–181.

13- Lanone, S. and Boczkowski, J.: Biomedical applications and potential health risks of nanomaterials: molecular mechanisms. Current Molecular Medicine. 2006; 6: 651–663.

14- Yu, L. E.; Yung, L.-Y..; Ong, C.-N., Tan, Y.-L.; Balasubramaniam, K.S.; Hartono, D. and Et al.: Translocation and effects of gold nanoparticles after inhalation exposure in rats. Nanotoxicology. 2007;1:235–242.

15- Thakor, A. S.; Paulmurugan, R.; Kempen, P.; Zavaleta, C.; Sinclair, R. and Massoud, T.F.: Oxidative stress mediates the effects of Raman-active gold nanoparticles in human cells. Small.2011; 7: 126-136.

16- Li, J. J.; Hartono, D.; Ong, C. N.; Bay, B. H. and Yung, L..L.: Autophagy and oxidative stress associated with gold nanoparticles. Biomaterials. 2010; 31: 5996-6003.

17- Karin, M.; Cao, Y.; Greten, F.R.; Li, Z.W.: NF-κB in cancer: From innocent by stander to major culprit. Nat. Rev. Cancer 2002; 2: 301–310.

18- Westbrook, A. M.; Szakmary, A. and chiestl, R. H.: Mechanisms of intestinal inflammation and development of associated cancers: Lessons learned from mouse models. Mutat.Res. 2010; 705: 40–59.

19- Tilstra, J. S.; Clauson, C. L.; Niedernhofer, L. J.; Robbins, P. D.: NF-κB in aging and disease. Aging Dis. 2011; 2: 449–465.

20- Oeckinghaus, A. and Ghosh, S.: The NF-kB Family of Transcription Factors and Its Regulation .Cold Spring HarbPerspectBiol 2009;1:a000034

21- Baeuerle, P. A. and Henkel, T.: Function and activation of NF-k B in the immune system. Annu Rev Immunol.1994; 12: 141 –179.

22- Hayden, M. S.; West, A. P. and Ghosh, S.: NF-kB and the immune response. Oncogene.2006; 25: 6758–6780.

23- Chithrani, B.D. and Chan, W.C.: Elucidating the mechanism of cellular uptake and removal of protein-coated gold nanoparticles of different sizes and shapes. Nano Letters. 2007; 7: 1542–1550.

24- Pan, Y.; Neuss, S.; Leifert, A.; Fischler, M.; Wen, F.; Simon, U.; Schmid, G.; Brandau, W. and Jahnen-Dechent, W.: Size-dependent cytotoxicity of gold nanoparticles. Small. 2007; 3: 1941–1949.

25- Katsenelson, B. A.; Privalova, L. I.; Guvrich, V. B.; Makeyev, O. H. ; Shur, V. Y. and Brikin, Y. B.: Comparative in vivo assessment of some adverse bioeffects of equidimensional gold and silver nanoparticles and attenuation of nanosilver's effects with a complex of innocuous bioprotectors. Molecular Sceince.2013; 4: 2465-2483.

26- Chaves, C.L.; Alvaredo, J. S.; Bayon, M. M.; Bettmer, J.; Llopis, J. and Gonzalez, C. S. : Gold nanoparticles: distribution, bioaccumulation and toxicity. In vitro and in vivo studies.Nanomedicine: Nanotechnology, Biology and Medicine. 2018;14: 1-12.

27- Sanjeev, S.; Gregory, T. M.; Pingfu, F.; Jigar, P.; Susan, R. M. and et al :Nuclear Factor-κB/p65 (Rel A) Is Constitutively Activated in Human Prostate Adenocarcinoma and Correlates with Disease Progression. Neoplasia.2004;6:390–400.

28- Liu, A. and Ye, B.: Application of gold nanoparticles in biomedical researches and diagnosis. Clin Lab. 2013; 59: 23–36.

29- Kumar, A.; Zhang, X. and Liang, X. J.: Gold nanoparticles: Emerging paradigm for targeted drug delivery system. Biotechnol Adv. 2013; 31: 593–606.

30- Peng, G.; Tisch, U.; Adams, O.; Hakim, M.; Shehada, N.; Broza, Y. Y.; Billan, S.; Abdah-Bortnyad, R.; Kuten,R. and Haick, H.: Diagnosing lung cancer in exhaled breath using gold nanoparticles. Nat. Nanotechnol. 2009; 4: 669–673.

31-Sung, J. H.; Ji, J. H.; Park, J. D.; Song, M. Y.; Song, K. S.; Ryu, H. R.; Yoon, J. U.; Jeon, K. S.; Jeong, J.; Han, B. S.; Chung, Y. H.; Chang, H. K.; Lee, J.H.; Kim, D.W.; Kelman, B. J. and Yu, I. J.: Subchronic inhalation toxicity of gold nanoparticles. Part. FibreToxicol., 2011; 8: 16–18.

32-Sharifia, S.; Behzadib, S.; Laurentc, S.; Forrestd, M.L.; Stroevee, P.; and Mahmoudib, M.: Toxicity of nanomaterials. ChemSoc Rev. 2012; 41: 2323–2343.

33- Zhang, X.D.; Wu, H.Y.; Wu, D.; Wang, Y.Y.; Chang, J.H.; Zhai, Z. B; Meng, A. M.; Liu, P. X; Zhang, L. A. and Fan, F.Y.: Toxicologic effects of gold nanoparticles in vivo by different administration routes. Int J Nanomedicine. 2010; 5: 771–781.

34- Nel, A.; Xia, T.; Madler, L.; and Li, N.: Toxic potential of materials at the nanolevel.Science.2006;311:622–627.

35- Moller, P.; Jacobsen, N. R.; Folkmann, J. K.; Danielsen, P. H.; Mikkelsen, L.; Hemmingsen, J. G.;Vesterdal, L. K.; Forchhammer, L.; Wallin, H. and Loft, S.: Role of oxidative damage in toxicity of particulates. Free Radical Res. 2010; 44:1–46.

36- Unfired, K.; Albrecht, C.; Klotz, L.O.; Von Mikecz, A.; Grether-Beck, S. and Schins, R. P. F.: Cellular responses to nanoparticles: Target structures and mechanisms. Nanotoxicology.2007; 1:52–71.

37- Halliwell, B. and Gutteridge, J. M.: Free Radicals in Biology and Medicine. 4th.Oxford University Press, Inc; New York: 2007.

38-Dalle-Donne, I.; Rossi, R.; Giustarini, D.; Colombo, R. and Milzani, A. S.: Glutathionylation in protein redox regulation. Free RadicBiol Med. 2007; 43:883–898.

39- Oberdorster, G.; Oberdorster, E. and Oberdorster, J.: Nanotoxicology: an emerging discipline evolving from studies of ultrafine particles. Environ Health Perspect. 2005;113:823–839.

40- Sayes, C. M.; Gobin, A. M.; Ausman, K. D; Mendez, J.; West, J.L. and Colvin,V. L.: Nano-C-60 cytotoxicity is due to lipid peroxidation. Biomaterials.2005; 26:7587–7595.

41-Sanz, A. and Stefanatos, R.K.: The mitochondrial free radical theory of aging: a critical view. Curr. Aging Sci. 2008;1:10-21.

42-Chen, L.; Deng,H.; Cui,H.; Fang, J.; Zuo, Z.; Deng,J.; Li, Y.; Wang, X. and Zhao, L.: Inflammatory responses and inflammation-associated diseases in organsOncotarget. 2018; 9: 7204–7218.

43- Sonis, S.T.: The biologic role for nuclear factor-kappaB in disease and its potential involvement in mucosal injury associated with anti-neoplastic therapy. Crit. Rev. Oral Biol. Med. 2002;13:380–389.

44- Deng, Z.J.; Liang, M.T.; Monteiro, M.; Toth, I. and Minchin, R.F.: Nanoparticle-induced unfolding of fibrinogen promotes Mac-1 receptor activation and inflammation. Nat Nanotechnol. 2011; 6:39–44.

45- Nel, A. E.; Madler, L.; Velegol, D.; Xia, T.; Hoek, E. M. V.; Somasundaran, P.; Klaessig, F.; Castranova, V. and Thompson, M.: Understanding biophysicochemical interactions at the nano-bio interface. Nat Mater. 2009, 8, 543-557.

46- Yao, M.; He, H.; McClements, D. J.; and Hang, X.: Uptake of Gold Nanoparticles by Intestinal Epithelial Cells: Impact of Particle Size on their Absorption, Accumulation, and Toxicity Journal of Agricultural and Food Chemistry. 2015, 63:8044-9

47- Dykman, L.A. and Khlebtsov, N.G.: Uptake of Engineered Gold Nanoparticles into Mammalian Cells. Chem Rev. 2014; 114:1258-1288.

48- Thubagere, A. and Reinhard, B. M.: Nanoparticle-Induced Apoptosis Propagates through Hydrogen-Peroxide-Mediated Bystander Killing: Insights from a Human Intestinal Epithelium In Vitro Model. Acs Nano. 2010; 4: 3611-3622.

49- Taggart, L.E.; McMahon, S. J.; Currell, F. J.; Prise, K. M.; Butterworth, K. T., The role of mitochondrial function in gold nanoparticle mediated radiosensitisation. CancerNanotechnol. 2014, 5: 5.

50- Lu, S.; Xia, D.; Huang, G., Jing, H.; Wang, Y. and Gu, H.: Concentration effect of gold nanoparticles on proliferation of keratinocytes. Colloids and Surfaces B, Biointerfaces 2010; 81: 406-411.

51- Fan, J. H.; Li, W. T.; Hung, W. I.; Chen, C.P.; and Yeh, J.M.: Cytotoxicity and differentiation effects of gold nanoparticles to human bone marrow mesenchymal stem cells. Biomedical Engineering: Applications, Basis and Communications. 2011; 23: 141–152.

52- Pernodet, N.; Fang, X.; Sun, Y.; Bakhtina, A.; Ramakrishnan, A.; Sokolov, J.; Ulman, A. and Rafailovich, M.: Adverse effects of citrate/GNPs on human dermal fibroblasts. Small.2006; 2:766–773.

53- Veronika Pivodová, V.; Franková, J. ; Galandáková, A. and Ulrichová1, J. In Vitro AuNPs' Cytotoxicity and Their Effect on Wound Healing. Nanobiomedicine. 2015, 2:7

54- Mironava, T.; Hadjiargyrou, M.; Simon, M.; Jurukovski, V. and Rafailovich, M. H.: Gold nanoparticles cellular toxicity and recovery: Effect of size, concentration and exposure time. Nanotoxicology, March 2010; 4:120–137.