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HISTOLOGICAL EVALUATION OF THE ROLE OF ZINC OXIDE NANOPARTICLES ON SUBMANDIBULAR SALIVARY GLANDS IN RATS AND THE PROPHYLACTIC EFFECT OF QUERCETIN

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ABSTRACT

Introduction: Nanoparticles have different effects on humans as they have specialized characteristics from the bulk materials. Zinc oxide nanoparticles are most commonly utilized nowadays due to their unique characteristics. On the other hand Zinc oxide nanoparticles have been found to have adverse effects on humans as cytotoxicity so it is a double-edged sword. Quercetin is a flavonol with beneficial anti-oxidant and antiinflammatory effect. **Aim :**The present work aimed to investigate the effects of Zinc oxide nanoparticles on rat submandibular glands and to evaluate the prophylactic effect of Quercetin . **Materials and Methods**: Twenty four male rats were equally divided into three groups: group A control, group B treated with Zinc oxide nanoparticles and group C treated with Zinc oxide nanoparticles and Quercetin for twenty eight days. **Results:** At the end of the study, rats were sacrificed and the effects of Zinc oxide nanoparticles as well as the prophylactic role of Quercetin on the submandibular glands were evaluated by light microscopy. The cells of the acini in group B revealed signs of cytotoxicity as loss of the acinar architecture, apoptotic nuclei, and cytoplasmic vacuolation while, in group C submandibular glands showed accentuated preservation in the cells of the acini and ducts that was comparable to that of the controls. **Conclusion:** This experiment clarified that Zinc oxide nanoparticles has a cytotoxic effect and illustrated that prophylactic administration of Quercetin efficiently counteracted the toxic effect of Zinc oxide nanoparticles administration in rat models. Therefore, Quercetin can be prophylactically used to prevent Zinc oxide nanoparticles cytotoxicity.

KEYWORDS: Zinc Oxide Nanoparticles, Quercetin, Histology.

INTRODUCTION

Nanotechnology and nanoparticles(NPs) are extensively used in various fields including physics, chemistry and biochemistry, and molecular biology. This is due to their special properties compared macro-sized ones[1]. Zinc is considered an essential element in our body and it was approved as a safe material by the FDA . Zinc Oxide has found to be one of the most widely used materials in various fields as medical diagnosis due to its unique optical and magnetic properties [2].

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Zinc oxide nanoparticles (ZnO NPs) are one of the most commonly used nanoparticles as it has multiple uses. Sources of Zinc oxide nanoparticles that are subjected to oral exposure are: sunscreens, lipstick, filling material, toothpaste and food preservatives [3] .

It was documented that ZnO NPs has an antimicrobial effect, thus they were used in food preservatives [4,5].

Although the wide implications of these NPs, few studies were done to investigate their toxicological effect. Some studies revealed that the side effects of Zinc oxide nanoparticles are more in comparison with bulk materials. However, some studies revealed that ZnO-NPs were non-toxic for cultured human cells. while other studies also suggested that they were toxic for different types of cells. Thus, several studies should be done to understand the potential cytotoxicity of these nanoparticels [6,7]. Relatively few reports have investigated the toxic adverse effects of ZnO NPs usage. It was found that the usage of food supplements including ZnO NPs by humans, even at very low concentrations, may have hazardous consequences on human cells such as cytotoxicity and genotoxicity [8,9]. Therefore, Nanotechnology is controversial [10].

Among the wide use of Zinc oxide NPs in the industrial field, it is suggested that the humans may be subjected to these nanoparticles either intentionally or un-intentionally through different ways as, ingestion, inhalation and dermal penetration, but gastrointestinal tract is the preliminary route. [11,12] When the NPs enter the circulatory system they accumulate in specific organs and are taken by the cells by phagocytosis[13].

The toxic effects of ZnONPs on the humans have been attributed to various mechanisms that are not well investigated. However, some investigations assumed that the toxicity of these particles was due to the free Zn2+ released in the culture media together with the intracellular uptake of these nanoparticles. Also the oxidative stress is considered to be one of the important mechanism responsible for zinc oxide nanoparticles side effects, as it promotes the release of the reactive oxygen species (ROS) and other oxidative agents [14]. When ROS level exceeds the anti-oxidative defense mechanism of the cells, it leads to oxidative damage that results in apoptosis of the cells [15,16] .

Flavonoids are considered one of the most powerful antioxidants that is owing to their intra-cellular free radical scavenging capability. They are found mainly in plants as apples and berries. Quercetin (Qc) is considered to be the most effective antioxidant among the different flavonoids. The antioxidant activity of Qc was assumed to be due to its phenolic hydroxyl groups [17,18].

Thus, the aim of our work was to investigate the effect of Zinc oxide nanopatricles on the submandibular salivary glands of rats and to evaluate the prophylactic role of Quercetin .

MATERIALS AND METHODS

The experiment was carried out on 24 male albino rats (aged 3:6 months) with initial body weight 150:200 g. Rats were housed in clean and ventilated cages with constant controlled climate (at Institute of Medical Research, Alexandria University, Egypt). All groups, received filtered tap water ad libitum and standard rodents diet. Rats were divided equally into three groups.

- **Group A [controls, (n=8)]:** received 10 ml/kg distilled water [19] daily by gavage for twenty eight days [20].
- **Group B (n=8):** received 300 mg/kg body weight ZnO NPs (Nanotech - Egypt Chemical Company) daily by gavage for twenty eight days [20].
- **Group C (n=8):** revived 300 mg/kg body weight ZnO NPs together with 200 mg/kg/day Qc (Faddah et al[21]) daily by gavage for twenty eight days[20].

Treated groups received ZnO NPs and Qc in aqueous solutions with dose volume10 ml/kg/day,[18,19] by gavage. Rats were weighted every week throughout the experimental period. At the end of the study, the rats were sacrificed, and the submandibular salivary glands were harvested.. Histological investigations occurred as samples (2-3 cm3) were fixed in 10% formalin neutral buffer, embedded in paraffin blocks according to the standard procedure, sectioned into 5 µm sections and stained by hematoxylin and eosin stain (H&E) [22] for light microscopic examinations.

The shape and size of Zinc oxide nanoparticles was characterized by transmission electron microscopy (TEM)[20, 23] and it revealed that each particle had a diameter <30 nm and were almost spherical in shape and formed aggregates (Fig.1).

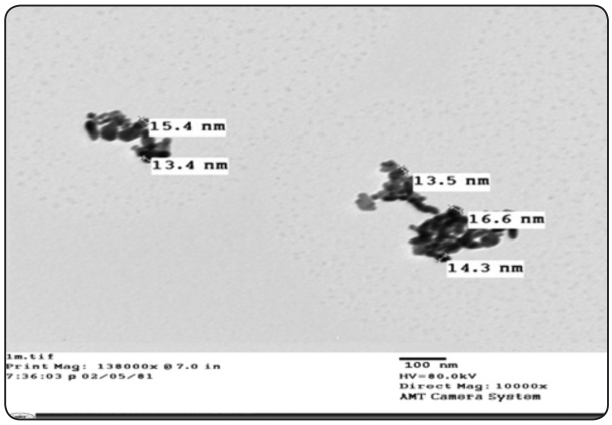

Fig (1) TEM of ZnO NPs' suspension illustrating that the NPs are relatively spherical at a diameter < 30 nm. (X1000).

The body weight was statistically analyzed using IBM SPSS software with version 20.0. ANOVA test in order to compare the 3 study groups for normally distributed quantitative variables [24].

Table (1) Comparison of the mean body weight changes among animals in the different groups at the successive four weeks of observation.

Reading/ week	**Body weight in grams (Mean SD)**			**ANOVA P1 value**
	Group A	**Group B**	**Group C**	
1st reading (at the start of experiment)	169-173 170.9±1.25	168-179 171.6±3.50	169-178 171.8±3.37	0.78 0.68
2nd reading (at the end of the 1st week)	173-179 177.1±1.81	177-185 181.4±2.72	172-180 174.3±2.60	0.85 0.55
3rd reading (at the end of the 2nd week)	184-193 186±3.07	189-204 193±5.57	177-200 185±7.95	1.04 0.22
4th reading (at the end of the 3rd week)	195-205 200±3.251	193-210 199±6.534	182-205 192±7.818	0.41 0.82
5th reading (at the end of the 4rth week)	205-227 215±8.120	200-227 212±9.996	190-219 200±9.223	1.36 0.71
ANOVA P2	12.05 0.001*	15.65 0.002*	10.3 0.006*	

: Statistically significant at P ≤ 0.05.

P1 comparison between the 3 study groups at the same time.

P2 comparison between 5 time intervals of the same group.

RESULTS

Body weight changes:

The rats were weighted weekly throughout the experimental period and the readings of the mean body weights in grams was compared. The readings demonstrated that there was no significant statistical difference in the mean body weight among group A and group B nor group C (Table 1).

Histological results:

Group A (controls):

H & E sections from control group submandibular glands showed the gland acini were formed of pyramidal shaped cells that posses secretory granules in cytoplasm apical part as nucleus was pushed to cell base. The secretory striated intralobular ductal cells were columnar with rounded central nucleus with basal striation **(Fig.2)**.

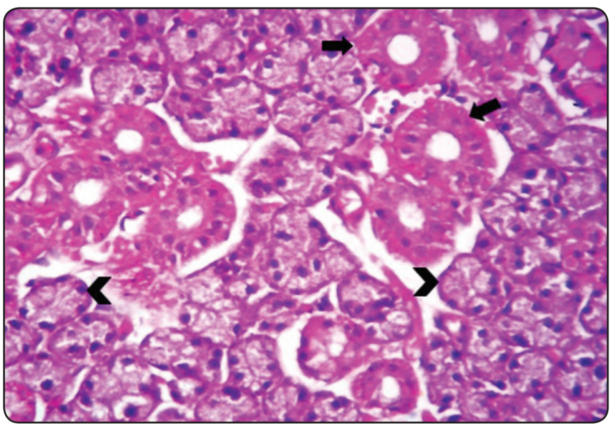

Fig. (2) Light micrograph (LM), group A, demonstrating: Normal architecture of the serous acini where the acinar cells are pyramidal in shape surrounding a narrow lumen with spherical nuclei and normal cytoplasm (arrow heads). Note the normal secretory striated ducts lined by tall columnar cells with well defined basal striations (arrows) (H&E stain; x 400).

Group B (Zinc oxide nanoparticles):

The cells of the serous acini showed, atrophic changes as the affected acini became small and lost its nornal architecture and their lining cells had vacuolated cytoplasm **(Fig. 3).** Striated duct showed degenerative changes as they lost their basal striation and had vacuolization of the cytoplasm and dilated lumina .The cells also showed, apoptotic nuclei **(Fig.4)**.

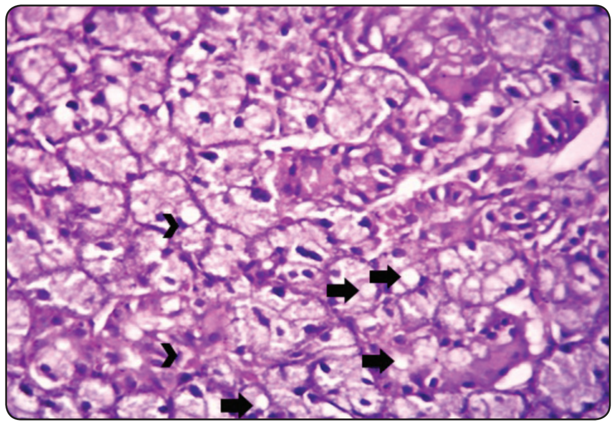

Fig. (3) Light micrograph (LM), group B, showing: Loss of the normal structural features of the acini, loss of the cell boundaries. Most of the nuclei appear pyknotic or apoptotic (arrow heads). The cytoplasm also show extensive vacuolization (arrows). (H&E stain; x 400).

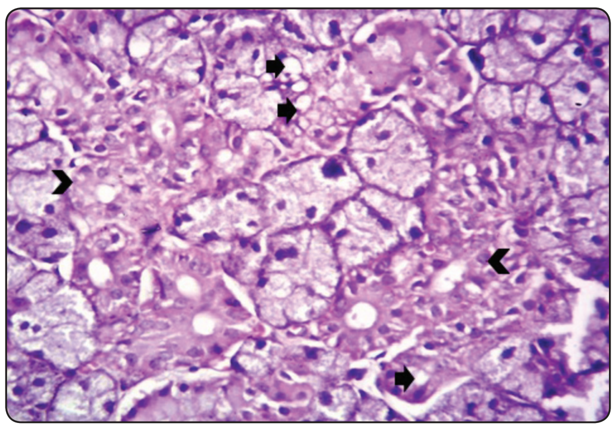

Fig. (4) Light micrograph (LM), group B, showing: Shrunken acini, atrophic vacuolated cytoplasm, (arrows). Note the slight dilatation of the secretory striated ducts with loss of their striations (arrow heads) (H&E stain; x 400).

Group C (Zinc oxide nanoparticles together with Quercetin):

The serous acini showed well preserved architecture comparable to the normal one. The acinar cells revealed , normal nuclei. The cytoplasm exhibited, few vacuolization **(Fig.5)**. The secretory striated duct showed normal lining cells with normal nuclei, well preserved basal striations and normal lumen **(Fig.6)**.

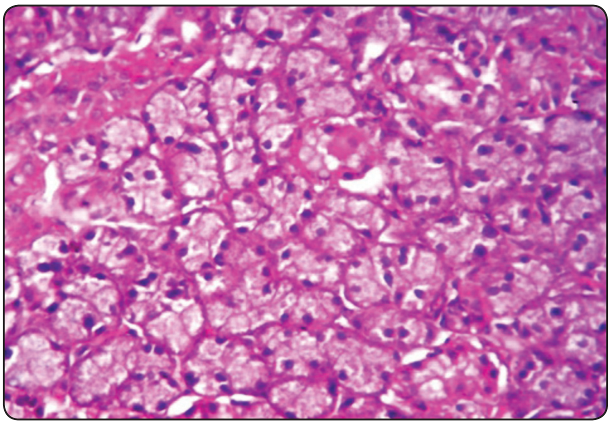

Fig. (5) Light micrograph (LM), group C, showing: Preservation of the acinar architecture and their cell boundaries. The acinar cells appeared normal in shape with spherical nuclei. (H&E stain; x 400).

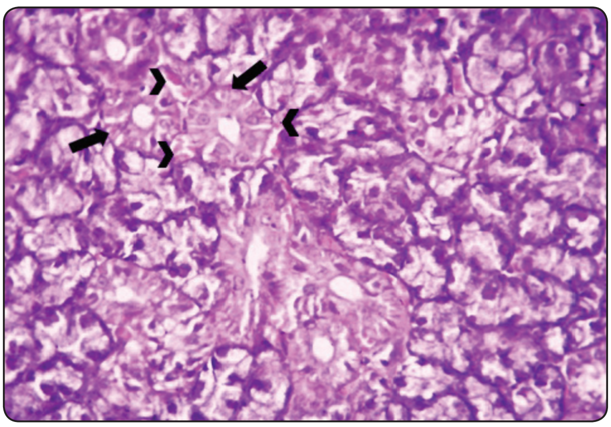

Fig. (6) Light micrograph (LM), group C, revealing: Apparent preservation of the epithelial lining of the secretory striated ducts and its basal striations (arrows). Note the normal blood capillary in association with secretory striated duct (arrow heads) (H&E stain; x 400).

DISCUSSION

Nano-toxicology is a study field that focuses on understanding the mechanism by which nanomaterials affect cellular function and lead to cytotoxicity. Recently, several researches have been conducted on NPs. The frequent use of ZnO Nps in food chains is and the continuous exposure to these NPs may affect human health[25]. Thus, it is important to raise the awareness about the possible side effects of these NPs. Therefore, the cytotoxic effect of these NPs became a significant threat that should be investigated, before increasing their applications [26].

ZnO NPs toxicity in mice occurred when exposed via digestive tract[27]. That is why the oral route was chosen in this study as human beings have a high are more subjected to ZnO NPs' ingestion in the tooth pastes and food products. Thus, they are at high risk for ingestion of high doses of ZnO NPs[28, 29].

We decided to choose the dose (300 mg/kg body weight/day) of the zinc oxide nanoparticles as it was according to Sharma et al[29] and Vandebriel et al[30]

The duration of twenty eight days was chosen according to Chung et al. [31] They revealed the accumulation of Zinc oxide nanoparticles in the blood circulation and in variable organs, after twenty eight days.

Light microscopic results of the current study showed that in group B, Zinc oxide nanoparticles have the capability to provoke a toxic effect on the cells of the acini as well as the ducts of the gland.

These were seen by the presence of different degrees of degeneration affecting most of the cells. The cells of the acini demonestrated, loss of their normal architecture with apoptotic nuclei. The cytoplasm revealed extensive degeneration and vacuolization. The secretory striated ducts showed degeneration in their epithelial lining with loss of the basal striations, vacuolation of the cytoplasm and expansion of the lumen. These results were also seen in other studies that used different doses of Zinc oxide nanoparticles[32].

Zinc oxide nanoparticles may affect the cells of the acini through many mechanisms, which suggest different possibilities for interpretation of the histological findings in the current work.

One of these important mechanisms of Zinc oxide nanoparticles' intoxication is the particle's dissolution in the biological environments[33].

Different studies have postulated that the high solubility of Zinc oxide nanoparticles may play accrual role in its toxic effect. Xia et al.[33] attributed the cytotoxic effect of these NPs to the release of free Zn^{2+} ions and its uptake by the cells. Other studies revealed that dissolved Zn^2+ ions triggers an oxidative DNA damage leading to apoptosis of the cells.

That concept was also approved by the study of Kao et al. [34]

This findings comes in accordance to the histological changes found in the current wok which showed apoptosis of the nuclei in aciniar cells. Another mechanism responsible for ZnONPs' toxicity is the oxidative stress, which leads to the failure of the cells to deal with the residues resulting from the metabolic and structural disturbances[29]. Thus, ROS generation and oxidative DNA damage plays a curial role in the genotoxicity and cytotoxicity of zinc oxide nanoparticles. Oxidative stress leads to accumulation of ROS. The oxidative stress induced by zinc oxide nanoparticles was evaluated and illustrated that the level of ·OH in zinc oxide nanoparticles' suspensions was higher than in bulks· OH is found to be the most toxic ROS species that is capable to provoke oxidative damage to the cell membranes which leads to cell death [35]. This issue might explain the cytoplasmic degeneration seen in cells of this study.

Cytoplasmic vacuolization of the rat serous cells was observed in group B. These findings were in accordance with Thakur et al[36] and Iavicoli et al[37]. This vacuolization may be due to disruption of membrane caused by zinc oxide nanoparticles leading to high influx of water and Na+ together with leaking of lysosomal enzymes resulting in cytoplasmic degeneration [38].

Meanwhile, the acini and ducts of rats in group C revealed a relatively similar histological appearance comparable to that of the controls in group A. These results declared the safe use of Qc as an anti-oxidant prophylactic agent, as observed at the histological level. This group showed relatively preserved cytoplasmic integrity, but with few vacuoles.

As mentioned before, that oxidative stress represents a common mechanism for cell damage induced by Zinc oxide nanoparticles [29, 33]. That is why the role of antioxidants as protective agents against ROS induced changes, has been considered in the present study.

Qc, is a flavone found in apples and berries that has been found to have an antioxidant activity[39-41]. Quercetin, is an antioxidants capable of scavenge ring free radicals present in the body which disrupts the cell membrane and leads to apoptosis[42]. Numerous recent researches have investigated the antioxidant and cyto-protective potentials of Quercetin[43-45] .

CONCLUSION

The results of this study aims to raise the awareness on the toxicity of zinc oxide nanoparticles when administrated through oral rout, and approved the possible prophylactic effect of Qc in counteracting this cytotoxilogical effect. Thus, Qc can be used safely to overcome ZnO NPs adverse effects.

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