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CISPLATIN INDUCED INJURY ON RAT SUBMANDIBULAR SALIVARY GLAND (HISTOLOGICAL AND ULTRASTRUCTURAL EVALUATION)

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ABSTRACT

BACKGROUND: Cisplatin, a platinum-based chemical, is an exceedingly effective anti-cancer medication that is extensively utilized in treating several types of human neoplasms. Nevertheless, this treatment modality exhibits several drawbacks, including the occurrence of dose-dependent adverse effects such as cytotoxicity. The submandibular salivary gland is classified as one of the principal paired glands situated in an extraoral location. In humans, the submandibular glands are around half the size of the parotid gland. However, in rats, the submandibular gland is the largest among the three primary gland types.
OBJECTIVES: to evaluate the cytotoxic effects induced by cisplatin on the submandibular salivary glands of albino rats.
MATERIALS AND METHODS: A total of 30 adult albino male rats were divided into two groups, Gp I (control group) and Gp II (Cisplatin group). Rats in Gp II were administered a single intraperitoneal injection of 8 mg/kg of Cisplatin. At the end of the experiment (4 weeks), all rats were subjected to euthanasia. The submandibular salivary glands were removed and processed for histological and ultrastructural examination.
RESULTS: The submandibular salivary glands of the cisplatin group were observed using histological and ultrastructural analysis, revealing evidence of atrophy and degeneration in the acinar cells as apoptotic nuclei and cytoplasmic vacuolization. Striated and granular convoluted ducts showed pyknotic nuclei, partial loss of basal striations and loss of mitochondrial internal structure.
CONCLUSION: Cisplatin produced obvious degenerative changes on submandibular glands of albino rats.
KEYWORDS: Cisplatin, cytotoxicity, salivary gland.
RUNNING TITLE: cisplatin submandibular salivary gland

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INTRODUCTION

Cancer is the leading cause of death worldwide (1) and one of the world's leading health problems. Patients are typically treated with conventional approaches (2) such as radiation, chemical therapies and/or surgery. Chemotherapy, which makes use of medications known as alkylating agents, is a common and effective method of treating cancer (3). Cisplatin is the most potent of the standard chemotherapeutic medications for cancer treatment (4).
Cisplatin cis-[Pt(II)(NH(3))(2)Cl(2)] ([PtCl2(NH3)2] or CDDP) also known as cis-diamminedichloroplatinum(II), One of the best and original metal-based chemotherapy medicine used in cancers patients (5).
However, Cisplatin's cytotoxicity and lack of selectivity for cancer cells can have major consequences, since it can also harm normal tissues despite the drug's potent chemotherapeutic actions (6). The substance's chemical composition, dosage, and duration; all play a role in the extent and severity of the damage caused by the drug used (5).
The prevailing consensus among experts is that the principal factor contributing to the effects of cisplatin is its interaction with mitochondrial DNA (mtDNA) or genomic DNA (gDNA), leading to the formation of DNA lesions. This process delays the synthesis of mRNA, DNA, and proteins, ceasing DNA replication, and triggers the activation of many transduction pathways (7, 8). Subsequent alterations to the processing of DNA would initiate cytotoxic processes, leading to apoptotic cell death (9).
It is also cause oxidative stress that is a widely recognized mechanism behind the cytotoxic effects of cisplatin. Cisplatin has been observed to elicit oxidative stress through the generation of reactive oxygen species (ROS), as superoxide and hydroxyl

radicals. The extent of ROS formation is contingent upon the concentration of cisplatin and the duration of exposure (10).

Various chemotherapy drugs function by inducing apoptosis, a programmed cell death process, in order to destroy cells that harbor deleterious mutations and so prevent the growth of cancer (11).

Apoptosis can be defined as a biological procedure that involves the elimination of damaged or undesirable cells from an organism while minimizing harm to the surrounding cells.(12) As well, it refers to the cellular phenomenon wherein a cell undergoes growth arrest and division cessation, thereafter entering a meticulously regulated program that finally culminates in the cell's controlled and intended demise (13).This process is often accomplished within a very brief timeframe of a few hours (14).

The submandibular gland (SMG) is recognized as the second-biggest among the major salivary glands in humans, although in rats, it is considered the largest among the three primary gland types (15). The glandular structure consists of epithelial cells, also known as glandular parenchyma, as well as connective tissue components (16).

As the submandibular salivary glands were mixed salivary gland with predominantly serous in humans, whereas in rats it was exclusively consists of serous acinar cells. The glandular cells are organized in a structure consisting of acini and ducts (17).

In humans, the ductal system is comprised of striated ducts, intercalated ducts and excretory ducts. The granular convoluted ducts in rats are observed as a distinct segment of the ductal system within the SMG, positioned between the striated ducts and intercalated ducts (18).

The current investigation was undertaken to elucidate the impact of cisplatin induction on the cytotoxicity of the submandibular salivary glands in albino rats.

MATERIALS AND METHODS

Experimental animals

Thirty adult male albino rats weighing 200- 250 g, pathogen free, were utilized in this study. The population size of these animals was determined based on sample size calculations conducted in the Department of Biomedical Informatics and Medical Statistics at the Medical Research Institute of Alexandria University. The experimental protocol used in this study was approved by the Ethics Committee of Alexandria University- Egypt, Institutional Animal Care and Use Committee (ALEXU-IACUC) (Approval number: 0464-6/2022)

The animals utilized in this study were acquired from the animal house of the Medical Research Institute at Alexandria University. Rats were subjected to a controlled environment consisting of 12-hour light and dark cycles, with temperature maintained at 22±3ºC and relative humidity ranging from 55% to 60%. During the duration of the one-month study period, all rats were provided with unrestricted access to a standard meal for rodents and tap water.

MATERIALS

The cisplatin utilized in this investigation had been purchased from Liba Drug Company (Istanbul-Turkey).

Experimental design

Rats were divided into two distinct groups:

Group A: (Control gp): rats were kept under normal conditions. In order to mitigate the potential impact of injection stress or buffer-induced effects on rats, a solution of normal saline was administered through injection.

Group B: (Cisplatin gp): rats were administered a single dosage of 8 mg/kg body weight intraperitoneally once at the beginning of the experiment (19).

All animals across all groups were euthanized with overdose anesthesia after the experimental period which was lasted for one month. Submandibular glands were rapidly dissected out and kept for further analysis.

Histological procedures (20)

Right SMGs were removed and fixed immediately in 10% neutral buffered formalin solution for 48 hours. Subsequently, a gradual dehydration process was carried out using increasing concentrations of ethyl alcohol. The specimens were then cleaned with xylene, infiltrated with paraffin wax, and ultimately embedded in paraffin wax. Histological investigation was conducted by cutting serial sections with a rotary microtome, each measuring 5µ in thickness. These sections were subsequently stained using the hematoxylin and eosin stain.

Tissue preparation for Transmission Electron Microscopy (21)

The left SMGs were promptly sectioned into 1mm cubes and then immersed in a solution containing 4% formaldehyde and 1% glutaraldehyde for fixation. Subsequently, the specimens were subjected to a buffer rinse and subsequently immersed in a 1% osmium tetroxide solution for post-fixation. Following this fixation step, the samples underwent dehydration using a succession of ethyl alcohol solutions with increasing concentrations. Finally, the specimens were embedded in Epon. The ultrathin sections were cut, followed uranyl acetate and lead citrate staining. Then, these sections were examined at the Electron Microscope Unit at the Faculty of Science, Alexandria University.

RESULTS

Histological result

Control gp (Figure 1)

Upon examination under light microscope, the SMG of the control group shows normal histological structure and architecture.

Serous acini looked spherical in shape, lined with pyramidal shaped cells surrounding a narrow lumen. Acinar cells had large spherical basally located nuclei and apical cytoplasm with secretory granules.

Striated ducts were lined with tall columnar cells. Ductal cells showed large, rounded nuclei located at the center of the cell and eosinophilic cytoplasm. The typical basal striations were also prominent.

Granular convoluted duct (GCD) lined by columnar cells that contain massive, strongly stained secretory granules in its supranuclear cytoplasm.

Cisplatin group (Figure 2)

Histological examination of this group revealed pronounced degenerative changes of the gland structure.

Serous acini appeared atrophic with ill-defined boundaries. Acinar cells exhibited severe cytoplasmic vacuolations associated with apoptotic or pyknotic (crescent shaped) nuclei.

Striated ducts lined with atrophic epithelium and pyknotic nuclei. The characteristic basal striations of the ductal cells were partially lost.

Granular convoluted ducts showed severe degenerative changes with loss of basic characteristics of the ductal unit.

Ultrastructural results

Control group (Figure 3)

Serous acini is pyramidal shaped cell that revealed normal basely located euchromatic nuclei. Cytoplasm basely surrounding the nucleus contained well organized rouph endoplasmic reticulum. Apical cytoplasm near the lumen occupied by spherical electron spherical zymogen granules (secretory granules). Numerous mitochondria were observed with normal internal cristea.

The intercalated duct is rounded in shape lined with cuboidal epithelial cells surrounding a narrow lumen. The ductal cells have centrally located euchromatic nuclei with few cell organells. Few secretory granules detected at the luminal surface of the cells. Myoepithelial cells were noticed at the base of the duct.

The striated ducts were lined by tall columnar epithelial cells surrounding a wide lumen. Basal membrane infoldings with numerous organized arranged mitochondria were found. Blood vessels were found around the duct.

Granular convoluted ducts were lined by tall columnar cells surrounding wide lumen. The basal part of the ductal cell with several membrane infolding accommodating large number of well-organized mitochondria. The apical part of the ductal cells occupied by secretory granules with different sizes and electron density.

Cisplatin group (Figure 4)

Sever degenerative changes in the acinar cells, their nuclei appeared heterochromatic and apoptotic. The outline of nuclear membranes was appeared irregularly. Severe dilatation of rough endoplasmic reticulum was obviously noticed. Few zymogen granules were filled the cytoplasm of the cells in different sizes and densities. Ductal cells showed pyknotic and dense chromatic nuclei with degenerated mitochondria.

Striated ductal cells exhibited vacuolization and altered in their organization, their nuclei appeared pyknotic and irregular outline. Loss of regularity of basal membrane infoldings.

Granular convoluted ducts represent extensive cytoplasmic vaculations of the ductal cells.

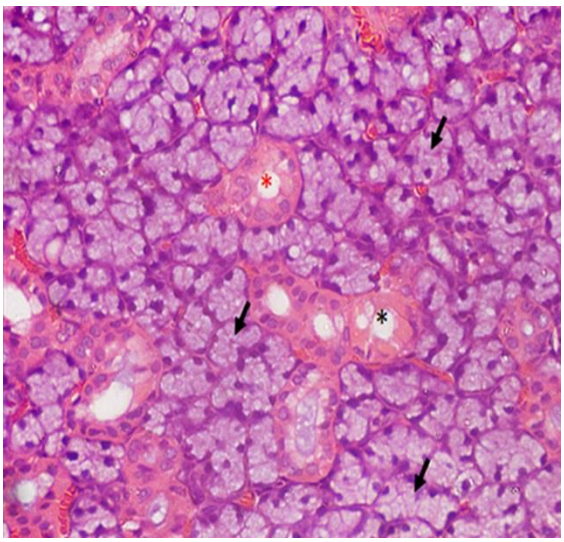

Figure 1: Light micrograph : (control group) showing normal appearance of densely packed serous acini (arrows), striated duct (Red asterisk), and granular convoluted tubules (Black asterisk) [H&E stain x 400].

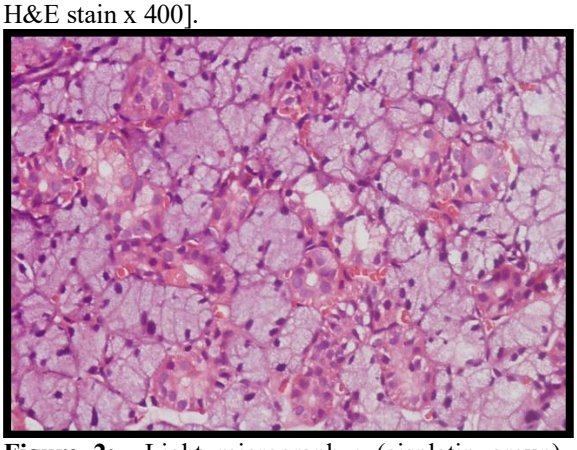

Figure 2: Light micrograph : (cisplatin group) showing massive degenerative changes in the acinar and glandular cells .Excessive intraacinar and intraductal cells vaculations(asterisk)with pyknotic (apoptic) nuclei (arrows).[H&E stain x 400].

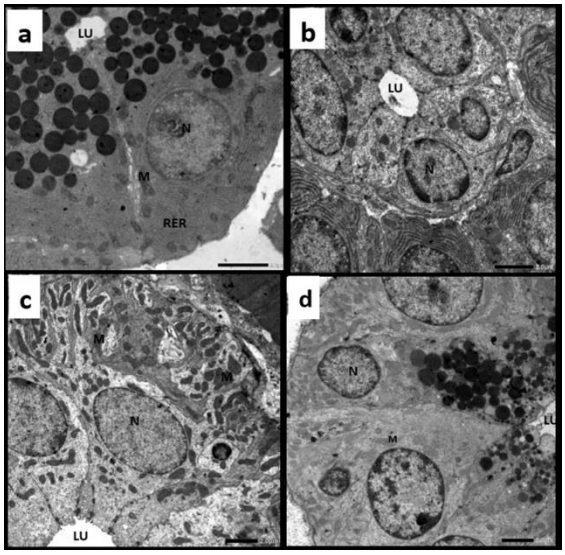

Figure 3: EM, (Control group): (a)showing pyramidal shaped acinar cell of normal euchromatic nucleus(N) surrounded by basal well-organized RER, numerous mitochondria (M). The zymogen granules numerous directed toward the lumen of the cell. (b) intercalated duct lined with cuboidal cells surrounding a narrow lumen (Lu). Ductal cells exhibiting a centrally placed euchromatic nucleus (N) with few cell organelles. (c) striated ducts lined with tall columnar cells surrounding a wider lumen (Lu). Ductal cells revealed centrally placed euchromatic nucleus (N) with prominent basal infoldings with longitudaly arranged mitochondria (M). (d)Granular convoluted ducts with tall columnar cells surrounding a lumen (Lu). Ductal cells revealed basely placed euchromatic nucleus (N). The apical cytoplasm filled with secretory granules with different sizes and electron densities [original magnification (a)x3000 (b) x2500 (c) x800 (d)x2000].

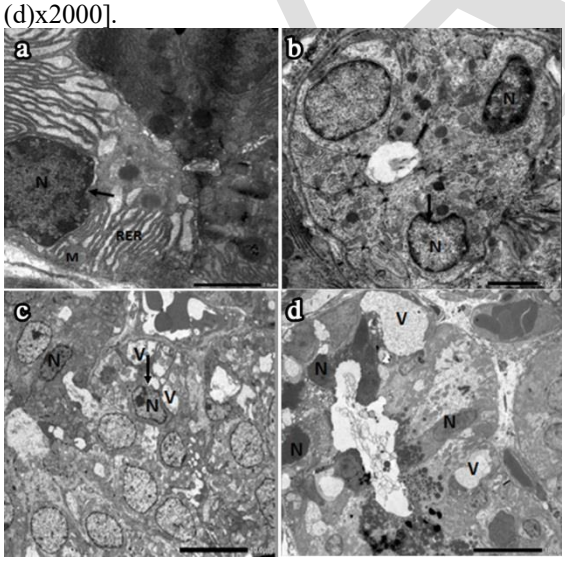

Figure 4: EM (Cisplatin group): Showing (a) part of acinar cell containing heterochromatic nucleus (N) with irregular outline(arrows), abnormal extensively dilate RER and degenerated mitochondria(M) cristae. (b)intercalated ductal cells exhibiting variation in the shape of nuclei (N), margination of chromatin (arrow head). (c)The striated ductal cells show pyknotic nuclei(N) with irregular outline (arrows). vacuolization of the cytoplasm could be detected in ductal cells (V). (d) degenerated granular convoluted ductal cells show dense heterochromatin atypical nuclei(N) and loss of structural details in the cytoplasm. Extensive cytoplasmic vacuolization of the ductal cell(V).[original magnification (a)x4000 (b) 1500 (c) &(d)x800].

DISCUSSION

The current investigation aims to elucidate the degenerative consequences of chemotherapy on the submandibular salivary gland of rats. SMG was considered an ideal model for investigating these effects due to its ability to secrete approximately 60% of saliva. Typically, chemotherapy that targets salivary glands has a number of serious adverse effects (22).

Consequently,the present work provides confirmation that the SMGs of rats exclusively possess serous acini, as previously documented by Amano et al.,2012 in his investigation on the anatomical and histological characteristics of major salivary glands in rodents and humans (17). In contrast to the findings of Yasser et al.,2020 (23), who characterized the nature of rats' SMGs as mixed acini that exhibited neither pure serous nor pure mucous properties.

Cisplatin is a chemotherapeutic agent known for its potent anticancer properties. However, it has been observed to induce structural injury to the SMG, leading to morphological abnormalities in both the ductal system and acinar cells (24).

As a result ,in this present study, histological examination of cisplatin group revealed degenerative changes affecting the serous acini. Also, Acinar and ductal cells showed apoptotic nucleus and extensive cytoplasmic vacuolations.

These observations are consistent with the findings of Hey et al.,2009 who proposed that cisplatin can impact salivary secretion by either inhibiting the production of aquaporins or by influencing DNA strands (25). Additionally, the binding of platinum to DNA resulted in the formation of intra-stranded and inter-stranded crosslinks, hence impacting the structure and function of the DNA molecule. The occurrence of DNA damage subsequently ceases the progression of the cell cycle and triggers apoptosis in rapidly dividing cells. This theory has been acknowledged by Ali et al.2021 (26) and Romani et al.,2022(27).

Moreover, the observed alterations in nuclear morphology and chromatin condensation, as documented in the present study, could potentially be associated with the apoptotic modifications occurring within the epithelial cells. The aforementioned findings, as postulated by Dasari et al.,2014 provide justification for the assertion that

cisplatin elicits oxidative stress within the cell. This particular mechanism represents a significant factor contributing to the manifestation of cisplatin-induced toxicity. Consequently, oxidative stress has the potential to disrupt normal biological processes and initiate cellular death in addition to causing DNA damage (28)

Likewise , Obeng et al.,2021 provided an explanation for the nuclear behavior observed in apoptotic cell death. This process involves the gradual margination and compaction of chromatin, which subsequently forms aggregates beneath the nuclear membrane (29).The electron-dense regions are then arranged in tight shapes resembling caps (30), causing nuclear spliiting (31).

Apoptosis has been regarded as a mechanism that serves to offset the impact of cell proliferation through mitotic division, in the context of tissue kinetics. Conversely, an overabundance of apoptosis can lead to the degeneration and dysfunction of organs (31).

In both in vivo and in vitro studies, it was illustrated that Cisplatin induced the excessive overproduction of free radicals (32). Free radicals, often referred to as reactive oxygen species, exhibit a high reactivity towards various biological components, leading to the initiation of a series of oxidation and reduction events. Under specific circumstances, the process of oxidative stress might potentially lead to changes in the structure and function of mitochondria in normal cells, resulting in organ malfunction (28).

Furthermore, Oropesa et al.,2013 explained the morphological changes in the degenerated cells. Cell cytoskeletons play an active role in cells undergo morphological changes. Apoptotic cells exhibit significant remodeling of the cytoskeleton, and the correct dismantling of the dying cell is facilitated by the caspase-mediated digestion of cytoskeleton proteins (33).

As well as, cytoplasmic vacuolations in the serous acinar cells are responsible for collecting the toxic injurious elements and preventing them from interfering with the normal biological activities of the affected cells as explained by Cheville et al.,2009(34).

Autophagy, characterized by the presence of cytoplasmic vacuoles, is also induced in response to cellular stress as a defensive mechanism. Once cellular stress reaches an irreversible state, the cell will undergo programmed cell death, commonly known as apoptosis, due to excessive amounts of autophagy (35).

Finally , our results confirmed that cisplatin induce degeneration of the submandibular salivary glands, which lead to alternations in submandibular glands acini and ductal cells.These alternations that happen may explain the clinical manifestations facing cancer patients. As this glands responsible for saliva production ,cisplatin lead to disterbance in salivary secretions that deteriorate the condition of oral tissues (36).

CONCLUSION

According to the findings of this study, the treatment of cisplatin in albino rats has been associated with significant degenerative alterations in the acini and ducts of the submandibular salivary gland.

CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

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