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EFFECTS OF CHEMO-THERAPEUTIC DRUGS USED IN HEAD AND NECK CANCERS ON SUBMANDIBULAR GLANDS OF RATS

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ABSTRACT

Introduction: Chemo-therapeutic drugs can cause morphological damages to the salivary glands.

Objectives: This study aimed to analyze and elucidate 5-fluorouracil (5-FU), cisplatin, methotrexate (MTX), and adriamycin (ADR)-induced morphological changes on submandibular glands (SMGs) of the rats.

Material and methods: Forty male Wistar albino rats were divided into five groups, with 8 rats per group. In the control group, 1 ml/kg 0.9% sterile saline solution; in the second group, 100 mg/kg dose of 5-FU; in the third group, 5 mg/kg dose of cisplatin; in the fourth group, 15 mg/kg dose of MTX; in the fifth group, 15 mg/kg dose of ADR were injected once a day for three consecutive days intra-peritoneally. In all groups, SMGs were excised, histopathological analyses were done using a light microscope, and total protein content was analyzed according to Lowry method. Data were examined using analysis of variance (ANOVA), Tukey HSD, Kruskal-Wallis, and Dunn's tests. Results were evaluated at 95% confidence interval and significance level of $p < 0.05$.

Results: There was a statistically significant decrease in gland weight and total protein content of the 5-FU, ADR, and cisplatin groups compared to that of the control ($p < 0.001$). However, the decrease in gland weight and total protein content of MTX group was not statistically significant ($p > 0.05$). Acinus areas of 5-FU, ADR, cisplatin, and MTX groups were lower than that of the control significantly ($p < 0.001$).

Conclusions: According to morphological parameters of our study, the most toxic effects were found to be caused by 5-FU, followed by ADR and cisplatin, while MTX presented the least toxic effects on the rats' SMGs.

Key words: 5-FU, cisplatin, methotrexate, adriamycin, submandibular gland.

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INTRODUCTION

Malignancies of the head and neck region constitute about 5-10% of all neoplasms [1, 2]. Chemotherapy is administered for the treatment of many tumors, or before radiotherapy and surgery. Chemo-therapeutic drugs used in the treatment of head and neck cancers can damage healthy tissues due to their side effects [3-5].

Because of its' high level of mitotic activity, the oral mucosa is an important area where the presence of chemotherapy toxicity has often been encountered. Chemo-therapeutic drugs can cause indirect or direct changes

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in the oral mucosa. Oral mucosal toxicity and increased incidence of mucositis are the main topics of studies on oral side effects of chemo-therapeutic drugs [6-14].

However, the mechanism underlying other side effects, such as those involving salivary glands, is largely unknown. Saliva and salivary glands are also adversely affected by chemotherapy toxicity. Chemo-therapeutic drugs can cause morphological damage to the salivary glands [4, 15-17]. In patients treated with chemo-therapeutic drugs, living conditions deteriorates because of the side effects, such as the symptom of dry mouth commonly associated with the hypofunction of salivary glands, taste disorder (hypogeusia), and swallowing problems, saliva being susceptible to candida infections due to the decrease in its' anti-microbial potential [18-24]. However, the mechanisms that cause these effects have not been explained yet, and further research are needed [25].

Submandibular glands (SMGs) of rats have a mixed structure consisting of both serous and mucous secretory units, and are the largest salivary glands of rats [26]. Therefore, SMGs of the rats are a convenient model for investigating morphological integrity [27]. 5-fluorouracil (5-FU), cisplatin, methotrexate (MTX), and adriamycin (ADR) are the most commonly used chemo-therapeutic drugs in head and neck cancers [28]. The objective of this research was to analyze and elucidate the effects of 5-FU, cisplatin, MTX, and ADR on SMGs of the rats. In this study, we evaluated these effects according to morphological parameters.

MATERIAL AND METHODS

ANIMALS

This study was performed following the National Institutes of Health guide for the care and use of laboratory animals (NIH Publications No. 8023, revised in 1978). All procedures of the study were reviewed and approved by the Animal Research Local Ethics Committee in the Aziz Sancar Institute of Experimental Medicine, Istanbul University, Turkey (approval No. 2020/31).

Forty male Wistar albino rats weighing 220 ± 10 g, 8 weeks of age were used in the experiment. The animals were kept separately in well-ventilated metal cages (floor areas of 250 cm^2 per animal) with controlled temperature (22 ± 2°C) and humidity (55 ± 10%), following a 12 hour light/dark cycle. All animals were fed with standard laboratory rat chow, and allowed food and water ad libitum. Animals were monitored daily.

EXPERIMENTAL DESIGN

Following several days of acclimatization, animals were randomly divided into five groups of 8 rats per group. In the control group, 1 ml/kg 0.9% sterile saline solution; in the second group, 100 mg/kg dose of 5-FU (5-Fluorouracil®, Sandoz, Turkey); in the third group, 5 mg/kg dose of cisplatin (Cisplatin-Kocak®, Atafarm, Turkey); in the fourth group, 15 mg/kg dose of MTX (Methotrexate-Kocak®, Atafarm, Turkey); in the fifth group, 15 mg/kg dose of ADR (Adrimisin®, Saba, Turkey) were injected once a day for three consecutive days intra-peritoneally. 5-FU, cisplatin, MTX, and ADR solutions were diluted in normal saline to a total volume of 2 ml before injection [29-32].

At the end of the third day, 10 mg/kg xylazine hydrochloride (Rompun®, Bayer, Turkey) and 50 mg/kg ketamine hydrochloride (Ketalar®, Eczacıbaşı, Turkey) anesthesia were applied before intra-peritoneal surgery. Both left and right SMGs were excised, dissected free of connective tissues, and weighed immediately. Tissue samples were taken from each gland for microscopic examination, and the rest of the gland was processed for total protein assessment. All animals were sacrificed with 10 mg/kg xylazine hydrochloride and 150 mg/kg ketamine hydrochloride anesthesia at the end of the surgery.

HISTOLOGICAL PREPARATION

Specimens from the SMGs were fixed in a 10% neutral formaldehyde solution, then dehydrated by a graded ethanol series. After the fixation procedure, the tissues were trimmed and processed using standard paraffin-embedding methods. From the paraffin blocks of each rat, sections were cut at 5-µm sections using a microtome (Leica, Germany); then, representative sections were stained with hematoxylin and eosin (H&E), and periodic acid-Schiff (PAS) for conventional histological assessment using a light microscope (Olympus BX-21, Japan).

HE-stained sections on 650× magnification and PAS-stained sections on 650× and 1,400× magnification were evaluated to investigate the morphological changes of SMGs of all groups. During microscopic examination, vacuolization and degeneration in the cells of the excretory ducts in all groups were observed.

Cover-slipped sections were photographed with a camera attached to a light microscope (Nikon D-70, Japan). Images were then transferred to a computer system for analysis, and the acinus areas of SMGs were analyzed using Image-Pro Plus software program (Image-Pro Plus 7, Media Cybernetics, Inc., Rockville, MD, USA). Next, the collected data were tabulated in Microsoft Excel sheet to be further statistically analyzed.

TOTAL PROTEIN ASSESSMENT

SMGs were placed in Falcon tubes for total protein assessment. Tissue samples were homogenized by diluting 1 : 10 with 0.15 M KCl solution, and were centrifuged. After discarding the supernatant fluid, the total protein concentration of precipitate was analyzed (Luminex xMAP® Technology, Luminex Co., Austin, TX,

USA) according to Lowry method [33, 34], using Folin phenol as the reagent, and bovine serum albumin (Folin-Ciocalteu's phenol reagent; Merck Co., Darmstadt, Germany) as the standard.

STATISTICAL ANALYSIS

Normality of data distribution was assessed by Kolmogorov-Smirnov and Shapiro-Wilk normality tests. When data followed normal distribution, statistical comparisons among groups were performed using one-way analysis of variance (ANOVA), followed by Tukey HSD test to determine the group that caused the difference. Otherwise, Kruskal-Wallis test was used for the comparisons of parameters in non-parametric distribution between the groups, and Dunn's test was applied to determine the group that caused the difference. Results were evaluated at 95% confidence interval and significance level of $p < 0.05$. Statistical analysis of all the data was performed using Microsoft Excel and IBM SPSS (IBM SPSS Statistics v.22.0; IBM Corporation, and Somers, NY, USA) software programs.

RESULTS

QUANTITATIVE MEASUREMENT OF CHEMOTHERAPY-INDUCED CHANGES IN GLAND WEIGHTS, TOTAL PROTEIN CONCENTRATIONS, AND ACINI

Acinus areas of 5-FU, cisplatin, MTX, and ADR-treated groups were lower than that of the control group, and this decrease in experimental groups was statistically significant ($p < 0.001$) (Table 1).

The gland weights (Table 2) and the total protein concentrations (Table 3) of SMGs of the rats were found to have a decrease in ADR, 5-FU, and cisplatin-treated rats when compared to the control group, and the decreases observed in these groups were statistically significant ($p < 0.001$). However, a decrease in the gland weights and in the total protein concentrations of MTX-treated rats were not statistically significant when compared to the control group ($p > 0.05$) (Tables 2 and 3).

Statistical comparisons of the SMG weights, acinus areas, and total protein concentrations of all experimental groups are presented in Table 4.

TABLE 1. Acinus areas of all groups

Groups	*n*	Acinus areas (μm²)	χ^2	*p*-value
ADR-treated group	8	4645609 ± 231343.50	150.36	0.000***
Cisplatin-treated group	8	4763003 ± 237112.15		
5-FU-treated group	8	3367649 ± 167619.99		
MTX-treated group	8	5478163 ± 272901.00		
Control group	8	8026697 ± 399629.48		

*Kruskal Wallis test was used. ***p-value < 0.001. Values are mean ± SD*
ADR – adriamycin, 5-FU – 5-fluorouracil, MTX – methotrexate

TABLE 2. Submandibular gland (SMG) weights of all groups

Groups	*n*	SMG weights (g)	*F*	*p*-value
ADR-treated group	8	0.48 ± 0.035	90.908	0.000***
Cisplatin-treated group	8	0.53 ± 0.038		
5-FU-treated group	8	0.44 ± 0.049		
MTX-treated group	8	0.64 ± 0.042		
Control group	8	0.68 ± 0.040		

*One-way ANOVA test was used. Values are mean ± SD. ***p-value < 0.001*
ADR – adriamycin, 5-FU – 5-fluorouracil, MTX – methotrexate

TABLE 3. Total protein concentrations of all groups

Groups	*n*	Total protein (μg)	*F*	*p*-value
ADR-treated group	8	48.80 ± 2.39	767	0.000***
Cisplatin-treated group	8	57.40 ± 3.68		
5-FU-treated group	8	27.50 ± 2.46		
MTX-treated group	8	76.30 ± 2.88		
Control group	8	78.70 ± 3.14		

*One-way ANOVA test was used. Values are mean ± SD. ***p-value < 0.001*
ADR – adriamycin, 5-FU – 5-fluorouracil, MTX – methotrexate

LIGHT MICROSCOPY OF THE RATS' SUBMANDIBULAR GLANDS

CONTROL GROUP

The mucous glands were stained paler than the serous acinar cells in the control group stained with H&E (Figure 1A). The lumens of the mucous acini were wider than those of the serous glands, their nuclei were at the basal part of the cell, and their cytoplasms were observed to be faintly stained (Figure 1A). In the parenchyma, the excretory ducts formed by cubic cells with nuclei located in the center of the cytoplasm were observed in the form of normal lumen structures (Figures 1A and B). The nuclei of the serous glands with narrow lumens and slightly basophilic cytoplasm were larger and more rounded (Figure 1A).

5-FU-TREATED GROUP

Vacuolization (Figures 2A-C) was observed in the cells of the excretory ducts, and degeneration (Figures 2C and D) was observed in both serous and mucous acini in the 5-FU-treated group. The nuclei of most cells in the acini were significantly larger than that of the control group (Figure 2D), and the nucleus chromatin was

TABLE 4. Statistical comparisons of the submandibular gland (SMG) weights, acinus areas, and total protein concentrations of all experimental groups

Groups	Acinus areas	Total protein	SMG weights
ADR-treated group			
Cisplatin-treated group	0.406	0.000***	0.014*
5-FU-treated group	0.000***	0.000***	0.077
MTX-treated group	0.027*	0.000***	0.000***
Control group	0.000***	0.000***	0.000***
Cisplatin-treated group			
5-FU-treated group	0.996	0.000***	0.000***
MTX-treated group	0.153	0.000***	0.000***
Control group	0.000***	0.000***	0.000***
5-FU-treated group			
MTX-treated group	0.000***	0.000***	0.000***
Control group	0.000***	0.000***	0.000***
MTX-treated group			
Control group	0.000***	0.181	0.077

*Significance: ***p < 0.001, **p < 0.01, *p < 0.05*
ADR – adriamycin, 5-FU – 5-fluorouracil, MTX – methotrexate

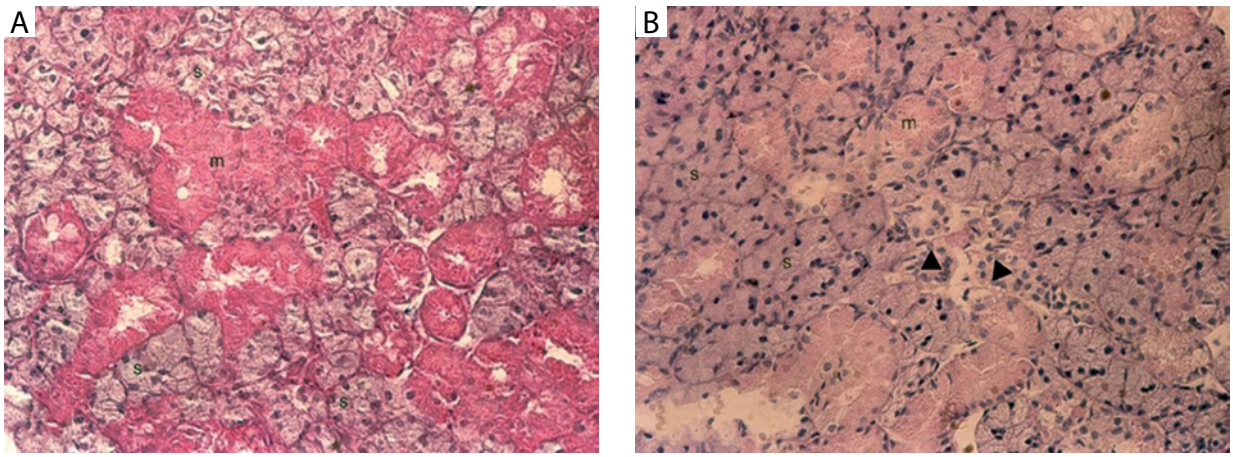

FIGURE 1. Light micro-photography of the rats' submandibular glands (SMGs) of the control group. Typical histological features of the rats' SMGs. **A**) The mucous glands were stained paler than the serous acinar cells in the control group (H&E; 650×). **B**) In the parenchyma, the excretory ducts formed by cubic cells with nuclei located in the center of the cytoplasm were observed in the form of normal lumen structures (PAS; 650×). Abbreviations: m – mucous glands, s – serous glands; arrowheads indicate excretory ducts

fragmented in some cells (Figure 2C). In PAS-stained sections, the nuclei of the acini were in irregular oval shape (Figure 2B).

CISPLATIN-TREATED GROUP

Vacuolization in acinar cells and inflammation in some areas were observed in the cisplatin-treated group (Figures 3A-D). The nuclei of the acinus cells were localized at the basal part of the cells. The morphology of the nucleus was similar to the control group (Figures 3A and B). Chromatin in the nucleus was dispersed (chromatolysis) in some acini (Figures 3B and D).

MTX-TREATED GROUP

Extensive and intensive vacuolization were observed in the mucous cells predominantly in the MTX-treated group (Figure 4A). Also, acinus degenerations (Figures 4A-C) and apoptotic bodies (Figures 4D and E) were observed in some areas. In H&E-stained sections, focal inflammation areas were more prominent in the inter-

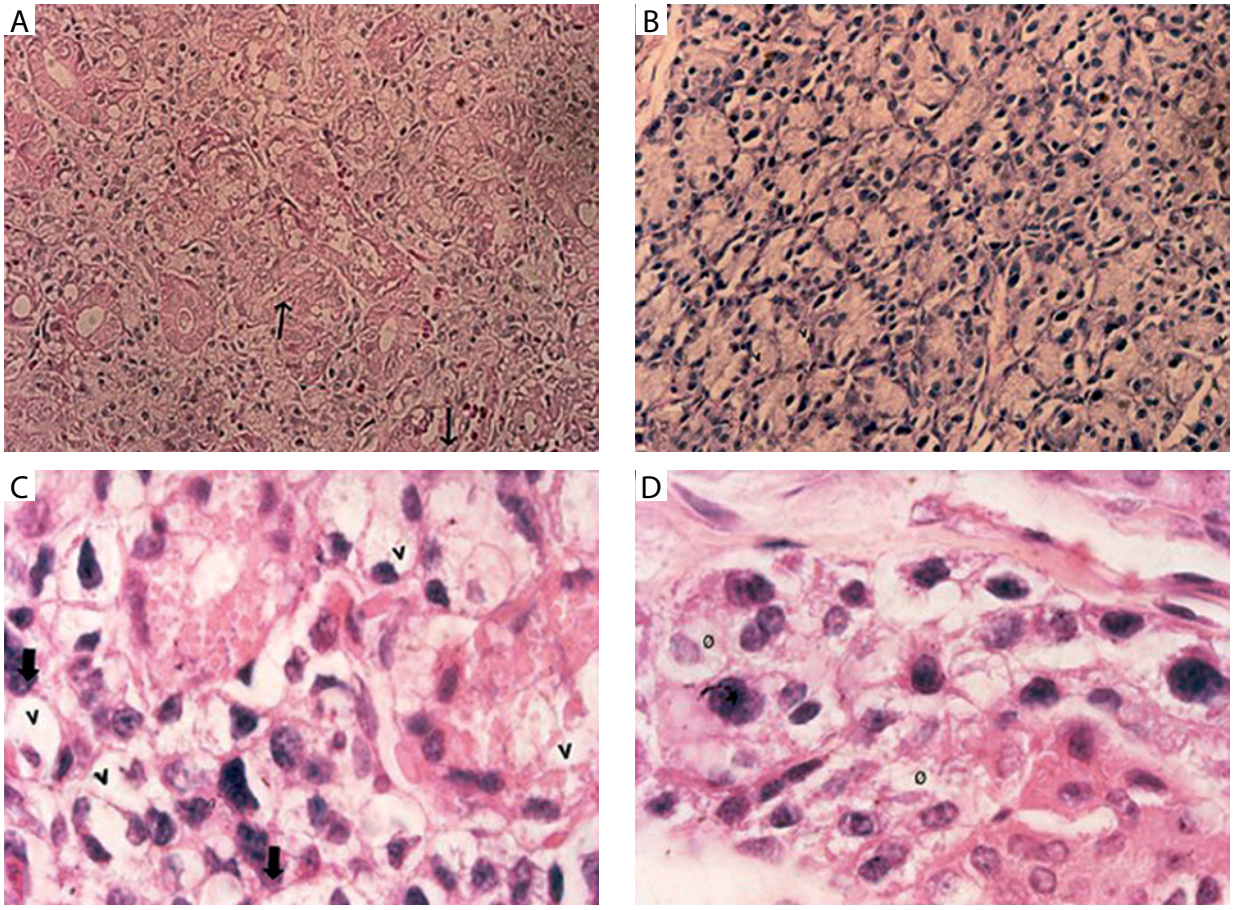

FIGURE 2. Light micro-photography of the rats' submandibular glands (SMGs) of the 5-FU-treated group. **A-C**) Vacuolization was observed in the cells of the excretory ducts. **A**) The arrow indicates vacuolization (H&E; 650×). **B**) The nuclei of the acini were in irregular oval shape (PAS; 650×). **C**) Vacuolization and degeneration were observed in all acini. Nucleus chromatin was fragmented in some cells (PAS; 1,400×). **D**) The nuclei of most cells in the acini were larger than that of the control group significantly. Acinus degeneration was observed in both serous and mucous acini (PAS; 1,400×). Abbreviation: v – vacuolization, Ø – acinus degeneration. The arrow indicates nucleus chromatin

stitial area (Figure 4B), and dilatation was observed in the excretory ducts in PAS-stained sections (Figure 4C).

ADR-TREATED GROUP

A large number of pleomorphic nuclei of the mucous glands and inflammation foci were observed in ADR-treated group (Figures 5A-D). Inflammatory cell groups were observed in the interstitium (Figures 5A and E).

DISCUSSION

Romaniuk *et al.* [35] observed dose-related changes in rat salivary glands due to methotrexate administration. They reported that acinar and ductal cell vacuolization and swelling, apoptosis in acinar cells with pyknosis in the nuclei, decrease in secretion granules, chronic inflammation, interstitial fibrosis, and shrinkage of acinar cells occurred as early pathological findings within the first seven days after a single-dose of intra-peritoneal injection of methotrexate to the rats. The researchers also reported that the severity of pathological changes increased as chemo-therapeutic doses increased. They stated that the SMG was more resistant than the parotid gland to the cytotoxic effects of methotrexate.

In our study, the effect of methotrexate on SMGs was lower than the other drugs. Vacuolization, apoptosis, inflammation, and shrinkage in acinar cells of the rats' salivary glands, reported by Romaniuk *et al.* [35] are consistent with our results concerning methotrexate-induced morphological changes. Methotrexate exerts its' cytotoxic effect by blocking the cell cycle. In our study, the presence of apoptotic bodies in the SMGs' acini of the methotrexate-treated group may be explained by the inhibition of DNA synthesis. It is known that apoptosis occurs as a response to negative processes that can affect DNA. At this point, it may be thought that apoptotic cell death should be within 12-24 hours after methotrexate administration, and should not be seen after a long

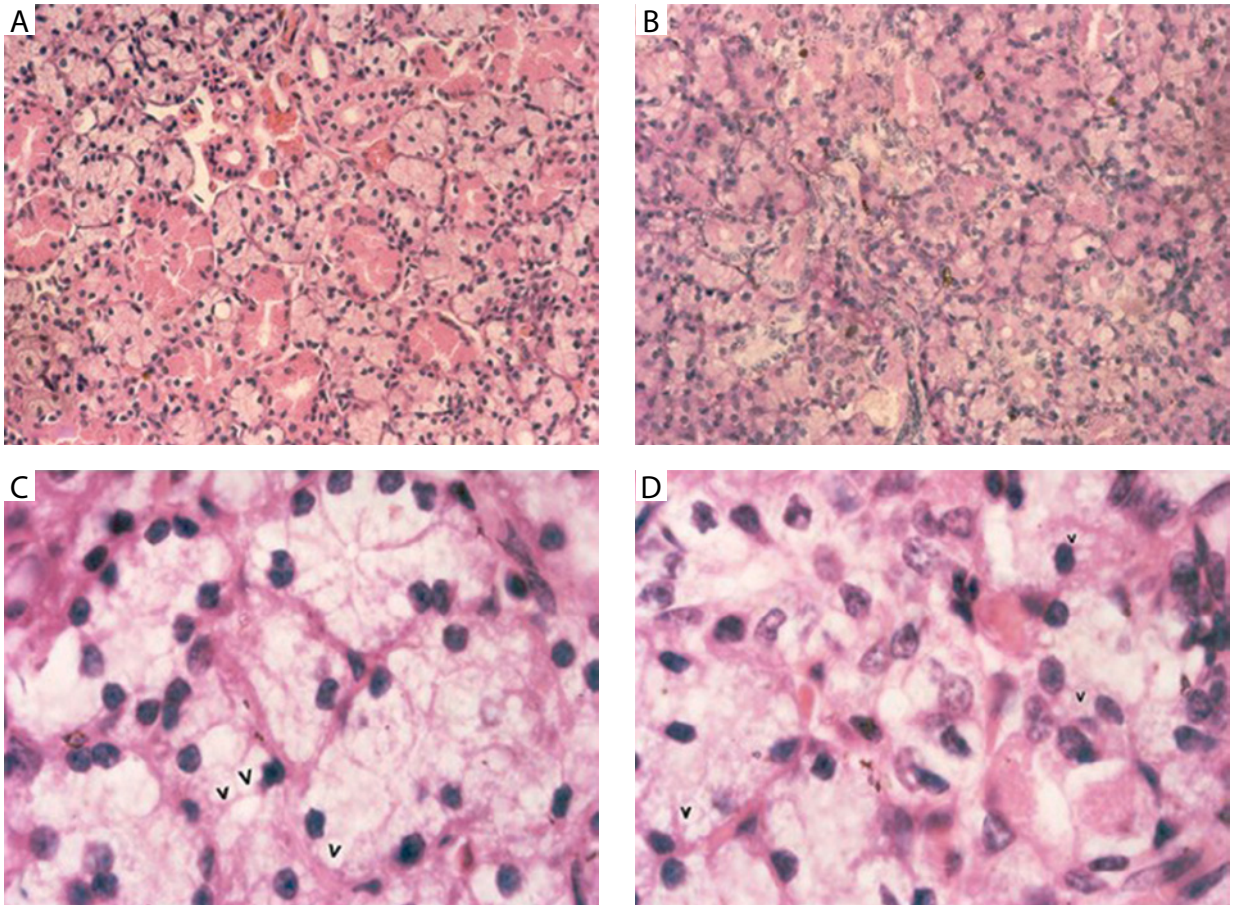

FIGURE 3. Light micro-photography of the rats' submandibular glands (SMGs) of the cisplatin-treated group, stains, and magnifications: **A**) H&E, 650×; **B**) PAS, 650×; **C**) PAS, 1,400×; **D**) PAS, 1,400×. **A, B**) The morphology of the nucleus was similar to the control group. **B-D**) Intense vacuolization was observed in all acinar cells prominently. **A, D**) The nuclei of the acinar cells were localized at the basal part of the cells and inflammation sites were observed but not intensely. **B, D**) Chromatin of the nucleus was dispersed (chromatolysis) in some acini. Abbreviation: v – vacuolization

time, such as 3 days. Therefore, the inflammation areas in the tissue imply that a different process is working. Observation of these two different findings together may be considered as a result of the drug administration protocol in our study. Apoptosis of a certain group of cells is expected as a result of the methotrexate administration on the first day and apoptosis of a certain group of cells is expected after the following doses. The apoptotic cell death observed in our study could occur due to the last dose of methotrexate. On the other hand, inflammation may be due to necrosis that occurred secondary to apoptosis. Also, the fact that inflammation was more dominant than the apoptotic cells in the methotrexate-treated group supports this idea.

There are studies reported that total protein increases, decreases, or does not change during chemotherapy [31, 32, 36-41].

In the present study, the decrease in total protein and SMGs weight in the methotrexate-treated group was not statistically significant compared to the control group ($p > 0.05$). Mc Bride *et al.* [39] and Wolff *et al.* [32] reported similar results in their study, examining the effects of methotrexate on the total protein concentration of the rats' SMGs. The common point of all these studies is that structural degeneration has not yet been reflected in the function.

Sandborg and Siegel [31] examined the effects of 5-FU on protein synthesis and secretion of the rats' parotid glands. They reported that the total protein and salivary gland weights decreased significantly. Ozel *et al.* [42] reported that, in a histopathological study with light microscopy, the first sign of the effect of 5-FU on acinar cells was changed in the cytoplasmic secretory granules of the rabbits' SMGs. Degenerative changes in organelles that produce protein, have also been observed in the Brunner glands, in sublingual glands, and pancreas as a result of 5-FU chemotherapy [31, 43-45]. Martin *et al.* [44] stated that 5-FU affects DNA, RNA, and protein synthesis, and inhibits the secretion mechanism of the cells in the pancreas. Enlargement in aci-

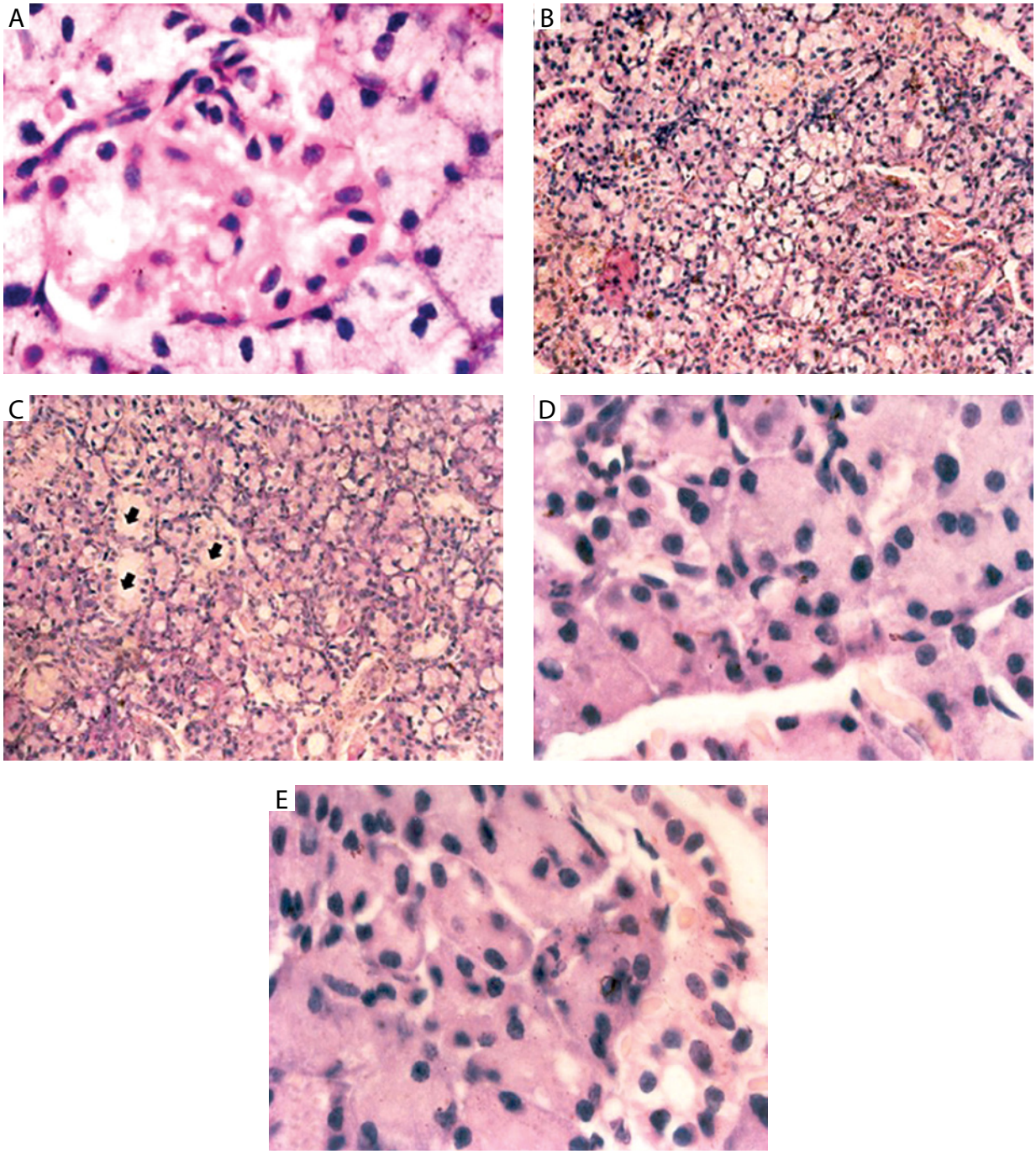

FIGURE 4. Light micro-photography of the rats' submandibular glands (SMGs) of the methotrexate (MTX)-treated group. **A**) Vacuolization was observed in the mucous cells predominantly (PAS; 1,400×). **B**) Focal inflammation areas were more prominent in the interstitial area (H&E; 650×). **C**) Dilatation was observed in the excretory ducts (PAS; 650×). Also, acinus degenerations (**A-C**) and apoptotic bodies (**D**), PAS; 1,400× were observed in some areas. **E**) Apoptosis was observed in the cell nucleus (PAS; 1,400×). Abbreviation: The arrow indicates dilatation in the excretory ducts

nar cells, reported by Martin *et al.* [44] may be due to inflammatory edema. The absence of inflammation in the 5-FU-treated group in our study can be explained by the difference between the protocols due to the duration of administration of the drugs and the period of the study. Vacuolization and acinar degeneration observed in the 5-FU group may occurred due to toxic effect of the drug. In our study, although we could not compare the cell nucleus and cytoplasm ratio due to intracellular vacuolization; this contrast can be explained by the effects of 5-FU on the intra-nuclear chromatin material. Therefore, further studies are needed to determine at what level the effects of the drug on the nucleus are reflected in morphology.

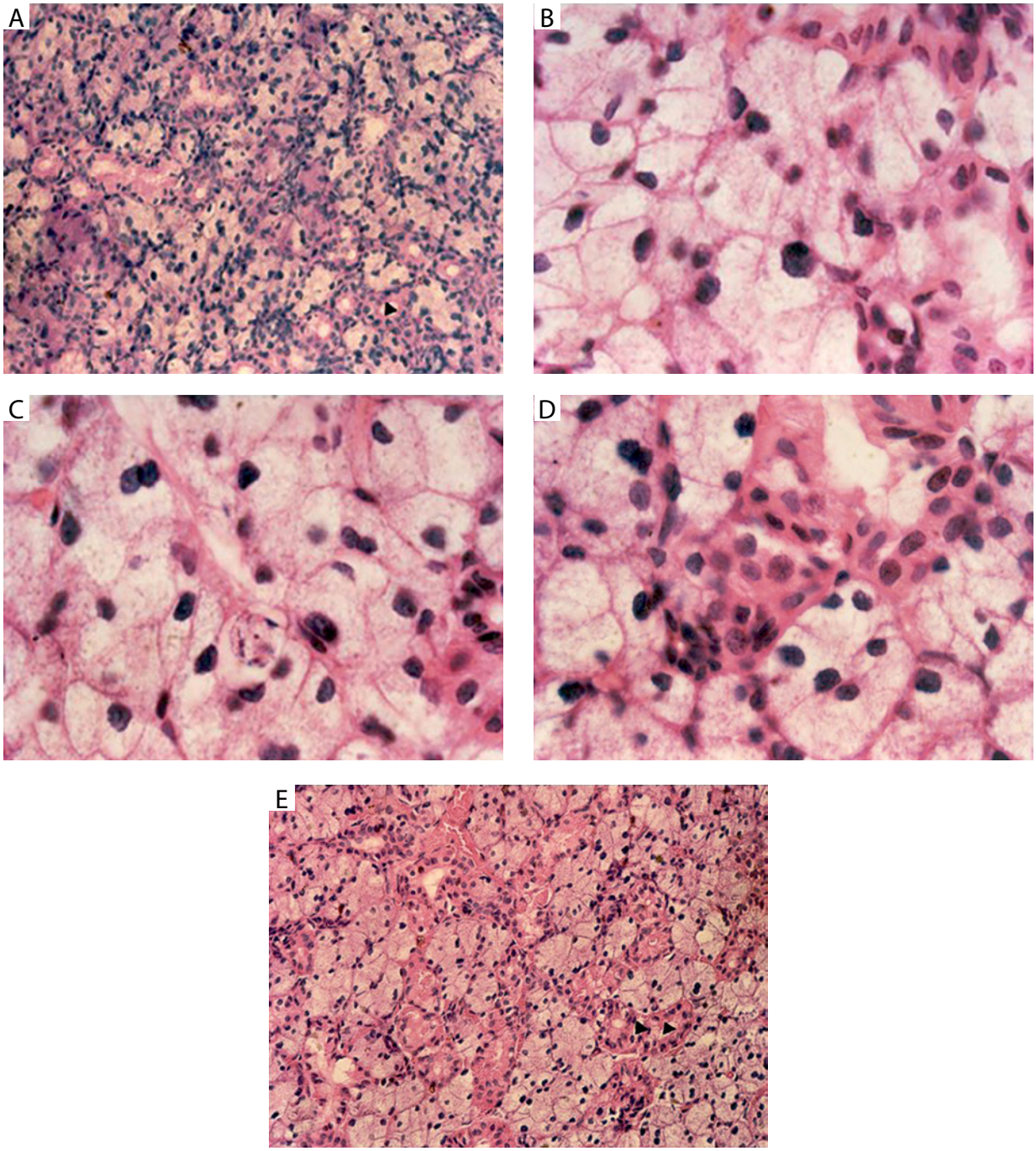

FIGURE 5. Light micro-photography of the rats' submandibular glands (SMGs) of the adriamycin (ADR)-treated group. **A**) The arrowhead indicates excretory channels (PAS; 650×). **B, C**) The nuclei of the cells were observed pleomorphic in all acini (PAS; 1,400×). **D**) Inflammation foci was commonly observed (PAS; 1,400×). **E**) The arrowheads indicate excretory channels (H&E; 650×). **A, E**) Inflammatory cell groups were observed in the interstitium

Massieh *et al.* [2] in a histological and immunohistochemical study reported that 5-FU administration showed features of severe degenerative changes in parotid glands of the rats, which increased over time. 5-FU administration showed ill-defined acinar outline, multiple different sized vacuolization, and disfigured nuclear pattern. These changes were more obvious 10 days after 5-FU administration. The findings were in accordance with previous studies, which demonstrated that chemotherapeutic agents, such as 5-FU and cisplatin, could induce salivary gland acinar degeneration in the form of acinar and ductal cell vacuolization, nuclear pyknosis, and subsequent apoptosis of the cells [8, 30, 42, 46].

Jirakulsomschok *et al.* [29] reported that there was a significant increase in the size of the acinar cells of the SMGs, a significant decrease in the parotid glands, and

a 43% decrease in the total protein of the SMGs compared to the control group of ADR-treated group. In addition, they reported that salivary gland weights decreased by 20-30% compared to the control group, and this decrease was due to a loss of glands' mass. Our study results were consistent with the study of Jirakulsomschok *et al*. [29] in terms of the salivary glands' weight and total protein concentration. In our study, acinus areas and SMG weights were lower than the control group. This result may be explained by the decrease in total protein as well as compatible with the decrease in the functional secretion amount of the glands. The increase in nucleus size and vacuolization in cells in our study were also consistent with cell swelling in the literature. However, Jirakulsomschok *et al*. [29] reported conflicting results in two different salivary glands. They reported a significant increase in the size of acinar cells of the SMGs, and a decrease in the size of the acinar cells of the parotid glands of the ADR-treated rats. Also, Jirakulsomschok *et al*. [29] stated that, despite the decrease in the total protein, the enlargement of acinar cells may be related to the increase in the number, size, and cluster pattern of the secretion granules as well as the process that decelerates the excretion of the gland. The decrease in acinar areas in our study may be explained by cell degeneration. The decrease in the number of cells should be considered as a process that would result in a decrease in the area of the acinus, regardless of the size of the remaining cells.

Doxorubicin, such as ADR, is a cytotoxic anthracycline group, chemo-therapeutic drug. It shows its' effect by disrupting DNA and RNA synthesis, such as ADR. Lockhart and Sonis [47] reported acinar degeneration, ductal dilation, cyst formation, and inflammation in the minor salivary glands due to the effect of doxorubicin. Acinus degeneration and inflammation observed in our study were consistent with the study of Lockhart and Sonis [47] on the effects of doxorubicin in minor salivary glands.

Cisplatin is a highly effective chemo-therapeutic drug that is commonly used in the treatment of head and neck cancers. Despite its' potent antitumor effects, cisplatin is known to cause serious side effects reported in a prospective non-randomized study, in which the influence of concomitant radio-chemotherapy with cisplatin tends to cause a higher probability of complication compared to radiotherapy alone [17, 48, 49]. Yamamoto *et al*. [50] reported that cisplatin has a significant potential to damage the salivary glands' tissue *in-vitro*. Cisplatin can initially reduce saliva production by blocking aquaporin expression, or conventionally, by stabilizing DNA strands. Then, it averts regeneration of glandular tissue when progenitor cells are damaged.

Kitashima [30] reported in an experimental study that cisplatin-induced morphological changes in the SMGs of rats were in both acinar cells and ductal system. In that study, on the fifth day after cisplatin infusion, a series of protein synthesis processes were damaged, resulting in a decrease in secretory granules. On the seventh day after infusion, the shape of acinar cells remained irregular, but the number of secretory granules increased.

Elgamily *et al*. [46] reported that the injection of cisplatin affected the histological picture of the rats' parotid glands. Signs of acinar degeneration appeared represented by disfigured lobular structure and loss of acinar outline, severe vacuolization, and disfigured nuclear pattern; these changes increase with time as the glands were more affected after 40 days. These results were in accordance with Kitashima [30] and Ozel *et al*. [42], who demonstrated that chemo-therapeutic agents could induce salivary glands' acinar degeneration in the form of acinar and ductal cell vacuolization, nuclear pyknosis, and subsequent apoptosis in the cells.

In a histological and ultrastructural study, Labah *et al*. [4] reported that cisplatin injection had adversely affected the histological structure of the rats' SMGs. In their study, signs of acini degeneration represented by disfigured lobular structure and loss of normal architecture of the secretory portions were observed. These results corresponded with the findings of Ozel *et al*. [42].

Dessoukey *et al*. [19] showed that cisplatin revealed apparent shrinkage of both serous acini and glandular lobules with the presence of interstitial edema. Serous acini, intercalated ducts, striated ducts, granular convoluted tubules, and excretory ducts showed multiple vacuoles, pyknotic, and hyperchromatic nuclei as well as many degenerated cells. These results were in line with Terzi *et al*. [17], and could be attributed to accelerated acinus cell apoptosis and degeneration induced by cisplatin administration [30]. The presence of intra-cytoplasmic vacuoles might be caused by the released free radicals, which cause damage to cellular components [48].

In our study, the acinus areas and the salivary gland weights of the cisplatin-treated group were lower than in the control group significantly ($p < 0.001$). Moreover, the total protein decreased by 27% compared to the control group due to the effect of cisplatin. The chromatolysis in the nucleus and decrease in total protein observed in the cisplatin-treated group can be explained by the binding of cisplatin to nuclear and cytoplasmic proteins and its' effects on DNA. It can be concluded that the decrease in the acinus areas was due to degeneration. However, this degeneration was not as prominent as in other groups.

Methotrexate had the least adverse effects on the rats' SMGs compared to the other groups in this study. A decrease in total protein and glands' weight as a result of vacuolization and acinus degeneration was an expected result in the methotrexate-treated group of this study. The results of Romaniuk *et al*. [35] and Mc Bride *et al*. [39] studies are also in accordance with our findings. In this case, it is possible to say that the morphological changes are not reflected to the cell function in the methotrexate-

treated group. While adverse effects of the 5-FU, cisplatin, and ADR on acinus cells appeared also on cell function, it may be thought that there was no such interaction in the methotrexate-treated group of our study. This result may explain why the total protein concentration that reflects the function was lower in the methotrexate-treated group than in the control group. On the other hand, a decrease in gland weight may be due to a severe impairment of the gland function and inflammation due to interstitial edema related to methotrexate.

The number of the biochemical parameters was the limitation of this study. Biochemical parameters used in this morphological study could be enhanced and supported by functional parameters, such as secretion rate, secretion flow rate, and secretion composition. Furthermore, in this study, the findings of salivary glands' damage can be supported by molecular parameters for the evaluation of mechanism of the damage and quantitative evaluation of the degree of the damage. Therefore, additional research and long-term observations are needed to evaluate the putative effects of chemo-therapeutics on salivary glands' function and oral mucosa more accurately.

CONCLUSIONS

According to the results of the present study, the most toxic effects on SMGs of the rats are found to be caused by 5-FU, followed by ADR and cisplatin. Also, MTX has the least toxic effects on the SMGs of the rats.

The data on this subject is important in terms of presenting clues that may guide in clinical practice. The effects of chemo-therapeutic drugs on exocrine glands may contribute to the revision of the indications of these drugs. The results of this study might be important for increasing quality of life of patients during chemotherapy.

CONFLICT OF INTEREST

The authors declare no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

References

1. Gupta N, Gupta R, Acharya AK, et al. Changing trends in oral cancer-a global scenario. Nepal J Epidemiol 2016; 6: 613-619.
2. Massieh C, El-Zainy MA, Amin RM, Fathy I. Systemic versus local injection of bone marrow mesenchymal stem cells on 5-fluorouracil treated parotid glands of albino rats. Braz Dent Sci 2021; 24 (4 Suppl 1): 1-13.
3. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin 2018; 68: 394-424.
4. Labah DA, Wahba OM. Flaxseed oil: an emerging nutraceutical decimates cisplatin-induced submandibular salivary glands damage in rats. Tanta Dent J 2017; 14: 105-111.
5. Rosenberg B. Fundamental studies with cisplatin. Cancer 1985; 55: 2303-2315.
6. Al-Ansari S, Zecha JA, Barasch A, de Lange J, Rozema FR, Raber-Durlacher JE. Oral mucositis induced by anticancer therapies. Curr Oral Health Rep 2015; 2: 202-211.
7. Deboni AL, Giordani AJ, Lopes NN, et al. Long-term oral effects in patients treated with radiochemotherapy for head and neck cancer. Support Care Cancer 2012; 20: 2903-2911.
8. Hitomi S, Ujihara I, Sago-Ito M, et al. Hyposalivation due to chemotherapy exacerbates oral ulcerative mucositis and delays its healing. Arch Oral Biol 2019; 105: 20-26.
9. Jensen SB, Pedersen AM, Vissink A, et al. A systematic review of salivary gland hypofunction and xerostomia induced by cancer therapies: prevalence, severity and impact on quality of life. Support Care Cancer 2010; 18: 1039-1060.
10. Skeff MA, Brito GA, de Oliveira MG, et al. S-nitrosoglutathione accelerates recovery from 5-fluorouracil-induced oral mucositis. PLoS One 2014; 9: e113378.
11. Villa A, Sonis ST. Mucositis: pathobiology and management. Curr Opin Oncol 2015; 27: 159-164.
12. Vissink A, Mitchell JB, Baum BJ, et al. Clinical management of salivary gland hypofunction and xerostomia in head-and-neck cancer patients: successes and barriers. Int J Radiat Oncol Biol Phys 2010; 78: 983-991.
13. Wang ZH, Yan C, Zhang ZY, et al. Radiation-induced volume changes in parotid and submandibular glands in patients with head and neck cancer receiving postoperative radiotherapy: a longitudinal study. Laryngoscope 2009; 119: 1966-1974.
14. Wardill HR, Sonis ST, Blijlevens NMA, et al. Prediction of mucositis risk secondary to cancer therapy: a systematic review of current evidence and call to action. Support Care Cancer 2020; 28: 5059-5073.
15. Al-Refai AS, Khaleel AK, Ali S. The effect of green tea extract on submandibular salivary gland of methotrexate treated albino rats: immunohistochemical study. J Cytol Histol 2014; 5: 212-220.
16. Mahmoud EF, Mahmoud MF, Haleem MAA. Royal jelly ameliorates oxidative stress and tissue injury in submandibular salivary gland of methotrexate treated rabbits: immunohistochemical study. J Am Sci 2012; 8: 501-508.
17. Terzi S, Özgür A, Mercantepe T, Çeliker M, Tümkaya L, Dursun E. The effects of astaxanthin on salivary gland damage caused by cisplatin in the rat. Int J Res Med Sci 2017; 5: 1410.
18. Chaveli-López B, Bagán-Sebastián JV. Treatment of oral mucositis due to chemotherapy. J Clin Exp Dent 2016; 8: e201-e209.
19. Dessoukey S, Halawa A, Fathy I, Kashkoush D. The effect of adipose derived stem cells versus platelet rich plasma in ameliorating cisplatin-induced Injury on the submandibular salivary gland. A comparative histological study. Egypt J Histol 2021; 44: 406-417.
20. Hong CHL, Gueiros LA, Fulton JS, et al. Systematic review of basic oral care for the management of oral mucositis in cancer patients and clinical practice guidelines. Support Care Cancer 2019; 27: 3949-3967.
21. Jensen SB, Mouridsen HT, Bergmann OJ, Reibel J, Brünner N, Nauntofte B. Oral mucosal lesions, microbial changes, and taste disturbances induced by adjuvant chemotherapy in breast cancer patients. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2008; 106: 217-226.
22. Jensen SB, Mouridsen HT, Reibel J, Brünner N, Nauntofte B. Adjuvant chemotherapy in breast cancer patients induces temporary salivary gland hypofunction. Oral Oncol 2008; 44: 162-173.
23. Mazzeo MA, Linares JA, Campos ML, et al. Oral signs of intravenous chemotherapy with 5-Fluorouracil and Leucovorin calcium in colon cancer treatment. Med Oral Patol Oral Cir Bucal 2009; 14: E108-E113.
24. Mittal BB, Pauloski BR, Rademaker AW, et al. Effect of induction chemotherapy on swallow physiology and saliva production in patients with head and neck cancer: a pilot study. Head Neck 2015; 37: 567-572.

25. Bomfin LE, Braga CM, Oliveira TA, et al. 5-Fluorouracil induces inflammation and oxidative stress in the major salivary glands affecting salivary flow and saliva composition. Biochem Pharmacol 2017; 145: 34-45.
26. Pinkstaff CA. The cytology of salivary glands. Int Rev Cytol 1980; 63: 141-261.
27. Tamarin A, Sreebny LM. The rat submaksillary salivary gland. A correlative study by light and electron microscopy. J Morphol 1965; 117: 295-352.
28. Kim R, Hahn S, Shin J, et al. The effect of induction chemotherapy using docetaxel, cisplatin, and fluorouracil on survival in locally advanced head and neck squamous cell carcinoma: a meta-analysis. Cancer Res Treat 2016; 48: 907-916.
29. Jirakulsomchok D, Yu JH, Sheetz JH, Schneyer CA. Effects of adriamycin on calcium concentration and morphology of mouse salivary glands. J Oral Pathol 1983; 12: 491-501.
30. Kitashima S. Morphological alterations of submandibular glands caused by cisplatin in the rat. Kurume Med J 2005; 52: 29-38.
31. Sandborg RR, Siegel IA. Effects of 5-fluorouracil on protein synthesis and secretion of the rat parotid gland. Arch Oral Biol 1990; 35: 991-1001.
32. Wolff A, Moreira JE, Marmary Y, Fox PC. Lack of acute effects of methotrexate on rat parotid salivary gland function. Arch Oral Biol 1989; 34: 109-115.
33. Brunotto M, Malberti A, Zárate AM, et al. Early phenotypic and genotypic alterations in submandibular gland oncogenesis in rats. Acta Odontol Latinoam 2006; 19: 13-21.
34. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the folin phenol reagent. J Biol Chem 1951; 193: 265-275.
35. Romaniuk K, Gavin JB, Adkins KF. The effect of methotrexate on salivary glands in the rat. J Dent Res 1983; 62: 678. Abst. No. 261 (IADR).
36. Dehpour AR, Ghafourifar P, Madani F, Mousavizadeh K, Abdollahi M. Effects of vinca alkaloids on rat parotid and submandibular glands function in vivo. Gen Pharmacol 1995; 26: 321-325.
37. Jensen SB, Pedersen AM, Reibel J, Nauntofte B. Xerostomia and hypofunction of the salivary glands in cancer therapy. Support Care Cancer 2003; 11: 207-225.
38. Månsson-Rahemtulla B, Techanitiswad T, Rahemtulla F, McMillan TO, Bradley EL, Wahlin YB. Analyses of salivary components in leukemia patients receiving chemotherapy. Oral Surg Oral Med Oral Pathol 1992; 73: 35-46.
39. McBride RK, Harper C, Siegel IA. Methotrexate-induced changes in rat parotid and submandibular gland function. J Dent Res 1987; 66: 1445-1448.
40. Meurman JH, Laine P, Keinänen S, Pyrhönen S, Teerenhovi L, Lindqvist C. Five-year follow-up of saliva in patients treated for lymphomas. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 1997; 83: 447-452.
41. Sagulin GB, Roomans GM. Effects of thyroxine and dexamethasone on rat submandibular glands. J Dent Res 1989; 68: 1247-1251.
42. Ozel O, Ayçiçek A, Kenar F, et al. Histopathologic changes in the rabbit submandibular gland after 5-fluorouracil chemotherapy. Turk J Med Sci 2010; 40: 213-220.
43. Anand C, Han SS. Effects of 5-fluorouracil on exocrine glands. III. Fine structure of Brunner's glands of rats. J Anat 1975; 119: 1-17.
44. Martin BF, Levin RJ, Kugler JH. A light and electron microscope study of the exocrine pancreas following administration of 5-fluorouracil. J Anat 1969; 104: 93-108.
45. Kim MK, Han SS. Effects of 5-fluorouracil on exocrine glands. I. gland weights in mice receiving synthetic polynucleotides. Proc Soc Exp Biol Med 1972; 139: 1246-1251.
46. Elgamily MF, Denewar M. Potential benefit of Annona muricata extract in combating cisplatin induced injury of parotid gland in albino rats. Egypt Dent J 2020; 66: 997-1007.
47. Lockhart PB, Sonis ST. Alterations in the oral mucosa caused by chemotherapeutic agents. A histologic study. J Dermatol Surg Oncol 1981; 7: 1019-1025.
48. Amin L, Fathy H. Role of ghrelin on cisplatin induced morphological changes on submandibular salivary glands. Egypt Dent J 2017; 63: 3225-3233.
49. Hey J, Setz J, Gerlach R, Vordermark D, Gernhardt CR, Kuhnt T. Effect of cisplatin on parotid gland function in concomitant radiochemotherapy. Int J Radiat Oncol Biol Phys 2009; 75: 1475-1480.
50. Yamamoto T, Staples J, Wataha J. Protective effects of EGCG on salivary gland cells treated with gamma-radiation or cis-platinum-(II)diammine dichloride. Anticancer Res 2004; 24: 3065-3073.