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Effect of Dexamethasone on the Histology and Histochemistry of Rabbit Submandibular Salivary Gland

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ORIGINAL ARTICLE

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Effect of Dexamethasone on the Histology and Histochemistry of Rabbit Submandibular Salivary Gland

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Abstract

***Background*: Dexamethasone is a synthetic long acting and potential glucocorticoids. Dexamethasone has a wide spectrum clinical application. It is used in diagnosis of Cushing's syndrome. It is also used for treatment of many inflammatory and autoimmune conditions, e.g. rheumatoid arithritis. It is also given to cancer patients undergoing chemotherapy or organ transplantation, and asthma treatment. In dentistry it is used before and after some form of dental surgery.**

***Objective:* Because salivary glands are tissues sensitive to hormones, this work was planned to study the effect of dexamethasone on the submandibular salivary gland.**

***Materials and methods*: Twenty six male rabbit were chosen to conduct the experiment. The rabbits were divided into control group (6 rabbits) which were injected (I.P) with normal saline; and treated group. The treated group was divided into two subgroups. The first one received a daily therapeutic dose (0.6 mg/kg) of dexamethasone injection for two weeks (subgroup 1 (10 rabbits). The second subgroup had received an overdose (1.2 mg/kg) from the same drug. The submandibular salivary glands of the rabbits were collected and fixed in neutral buffered formalin to be processed for the different histological routine techniques in order to be stained with H&E; Gomori's one step trichrome; PAS, and PAS/Alcian blue (pH 2.5) combination. For statistical analysis the mean and standard deviation (SD) have been adopted for the diameter of the secretory units of the glands.**

***Results:* The results have revealed swelling and an increase in diameter in the mucous acini of the secretory units. Other prominent changes were the atrophy and disruption of granular nature of the serous tubules. With PAS stains, the reaction was restricted to the serous tubules of the secretory units. The Alcian blue staining was restricted to mucous acini only.**

***Conclusion:* The observations of the present study have revealed significant salivary changes in rabbits received dexamethasone even for short period of time.**

The consequences will be undesirable effect on teeth and oral cavity health due to decrease in serous secretion coming from one of the major salivary glands.

Keywords: Rabbit, Dexamethasone, Submandibular salivary gland

1. Introduction

Dexamethasone is a synthetic long acting and potential glucocorticoids. The drug is among the most active member of its class being about 25–30 times as potent as hydrocortisone [1]. Dexamethasone has a wide spectrum clinical application. It is used in diagnosis of Cushing's syndrome [2]. Dexamethasone as a steroidal anti-inflammatory is used to treat many inflammatory and autoimmune conditions, e.g. rheumatoid arithritis. It is also given to cancer patients undergoing chemotherapy or organ transplantation [3, 4]. It is a common practice to administer dexamethasone to pregnant women whenever

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delivery before 34 weeks is anticipated [5–7]. The most wide spread use of glucocorticoids is in asthma treatment [8]. In dentistry the drug is used before and after some form of dental surgery such as pain resolution in periapical abscess [9], and management of complications in postoperative third molar surgery [10].

Salivary glands are tissues sensitive to hormones [11]. Biochemical changes in salivary protein were noticed to be associated with administration of dexamethasone [11–13]. Due to the wide spread use of dexamethasone in clinical practice, we decided to undertake a histological and histochemical study to verify the possible changes in the submandibular salivary gland. Rabbit was chosen as an experimental animal. Its submandibular salivary gland comprised of mucous and serous secretory units [14].

2. Materials and methods

2.1. Experimental animals

Twenty six mixed breed male rabbits weighing 1.5–2 kg, aged 4–6 months were selected for this study. Male rabbits have only been selected for this study to avoid any discrepancy in the results, which might come due to sexual dimorphism [15, 16].

2.2. Calculation of the drug dose

The drug dexamethasone concentration was calculated according to formula presented by Hicks [17]. for the rat. The dose was adjusted for the rabbit according to the formula of Pagat and Barnus [18]. It was found to be 0.6 mg/kg as therapeutic dose and 1.2 mg/kg as an overdose.

The rabbits were housed in cages under identical conditions. They were fed the usual rabbit food and allowed a free access to water. Following several days of acclimatization, the animals were assigned into three groups.

1. The test group (G1): It includes ten rabbits. They were injected with therapeutic dose of dexamethasone (0.6/kg). The animals of this group were further divided into two subgroups:
   a. G1A (five animals): which received daily single intraperitoneal (i.p) injection of the drug for one week.
   b. G1B (five animals): which received daily single i.p injection of the drug for two weeks.
2. The test group (G2): It includes ten rabbits. They were injected with an overdose of dexamethasone (1.2 mg/kg). As in G1 group, the animals of this group were further subdivided into two subgroups:
   a. G2A (five animals): They were given i.p daily injection for one week.
   b. G2B (five animals): which received the intraperitoneal injection (i.p) injection of the drug for two weeks.
3. The control group (G3): It includes six rabbits. They were given i.p injection of normal saline, in order to simulate the effect of injection. As in the other two groups, the animals of the control group were further divided into two subgroups.
   a. G3A (three animals): received the saline injection for one week.
   b. G3B (three animals): received the saline injection for two weeks.

2.3. Collection of samples and processing of tissue

The rabbits were anesthetized next day to the end of time of treatment allocated for each group. Perfusion fixation method for the whole body via the left ventricle was followed. The fixative used is neutral buffered formalin. The submandibular gland was dissected out and sliced into smaller sized pieces, and placed in the fresh fixative for 72 hours.

After fixation the samples were dehydrated in graded ethanol, cleared in xylene and embedded in paraffin. Sections 5–7 μm thick were cut to be stained for histology and histochemistry. Serial sectioning was used for statistical analysis.

2.4. Stains used in this study

1. Haematoxylin and eosin (H&E) stain for general histology.
2. Periodic acid Schiff's stain (PAS) for neutral mucuosubstances in general. Some sections were pretreated with diastase to discriminate between glycogenic and nonglycogenic mucosubstances [19].
3. AB (pH 2.5)/PAS combination to discriminate between acid mucosubstances (blue color) and neutral mucosubstances (red color) [18].
4. Gomor's one step trichrome stain [21].

2.5. Statistical analysis

For the statistical analysis, ten sections from each rabbit, stained with H and E, were selected for measuring the diameter of the mucous acini and serous tubules, using the graded ocular micrometer. The mean and standard deviation (SD) have been adopted for the diameter of the serous tubules and the mucous acini. Differences among means were analyzed for

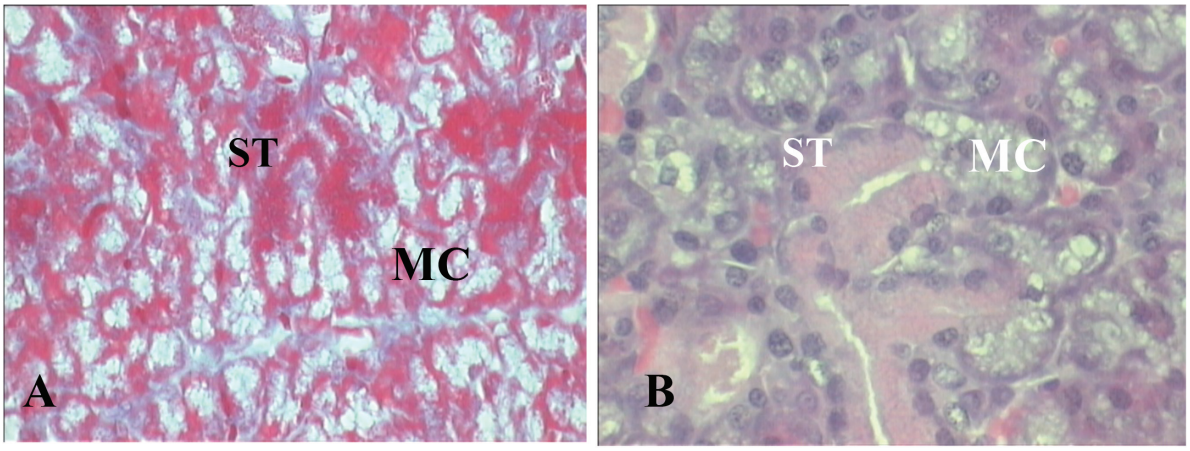

Fig. 1. Section from the control group demonstrates the predominance of the serous tubules (ST) which are outnumbering the mucous acini. 1A section stained with one step trichrome stain;1B H&E stained section. X400. Control group.

statistical significance by one–way analysis of variance (ANOVA), followed by calculation of critical differences ($P < 0.05$) for multiple comparisons (using LSD method).

3. Results

3.1. Control group

Staining sections of submandibular gland of control group with H&E revealed the presence of compound secreting units; they were composed of mucous acini which converge to open into serous tubules. (Fig. 1). The serous tubules were predominant in the submandibular gland, outnumbering the mucous acini. With one step trichrome stain the serous tubules have taken red coloration, while mucous acinar cells were expressing pale faint appearance. The mucous acinar cells were pyramidal in shape, having pale unstained cytoplasm with foamy appearance. The serous cells of the tubules were strongly acidophilic with H&E stain. They were particularly rich in granules localized predominantly in the supra-nuclear region of the cell (Figs. 2 and 3).

The reaction with PAS/AB combination was showing bright red magenta colored granules in supranuclear position of the serous tubular cells. The mucous acini have revealed alcinophilic blue coloration (Figs. 3 and 4).

3.2. Treated groups

3.2.1. G1 group

The H&E stained sections have revealed swelling of the cells in the mucous acini, indicated by presence of vacuolated cytoplasm with hydropic degeneration

Table 1. The mean and standard deviation of the mucous acini diameter (μm) at different doses (N = number of readings for each subgroup).

Descriptive statistics				
Dependent variable: _Mucous acini				
Dose	Period	Mean	Std. deviation	N
Control	1 Week	29.8875	7.2585	100
	2 Weeks	28.9909	7.0408	100
	Total	29.4392	7.1466	200
Therapeutic	1 Week	46.7625	8.8140	100
	2 Weeks	49.4625	9.0091	100
	Total	48.1125	8.9921	200
Over dose	1 Week	46.2000	6.6546	100
	2 Weeks	50.6625	7.7330	100
	Total	48.4312	7.5355	200
Total	1 Week	40.9500	10.9214	300
	2 Weeks	43.0386	12.7408	300
	Total	41.9943	11.9021	600

(Fig. 5 and Fig. 6). There was an increase in the diameter of the mucous acinar (Table 1 and Fig. 7). On the other hand there was a pronounced reduction in the number and diameter of the serous tubules (Table 2 and Fig. 8). With PAS/AB combination, the PAS reaction was restricted to the serous tubules, while alcinophilic reaction was expressed in the mucous acini only (Fig. 9).

3.2.2. G2 group

The results presented for this group have revealed no differences from the results obtained for the G1 group. Thus the description for the two groups would be the same (Fig. 10).

3.3. Statistical analysis

The change in the diameter of the secretory units of the submandibular gland of the rabbit are shown in Tables 1 and 2 and in Figs. 7 and 8.

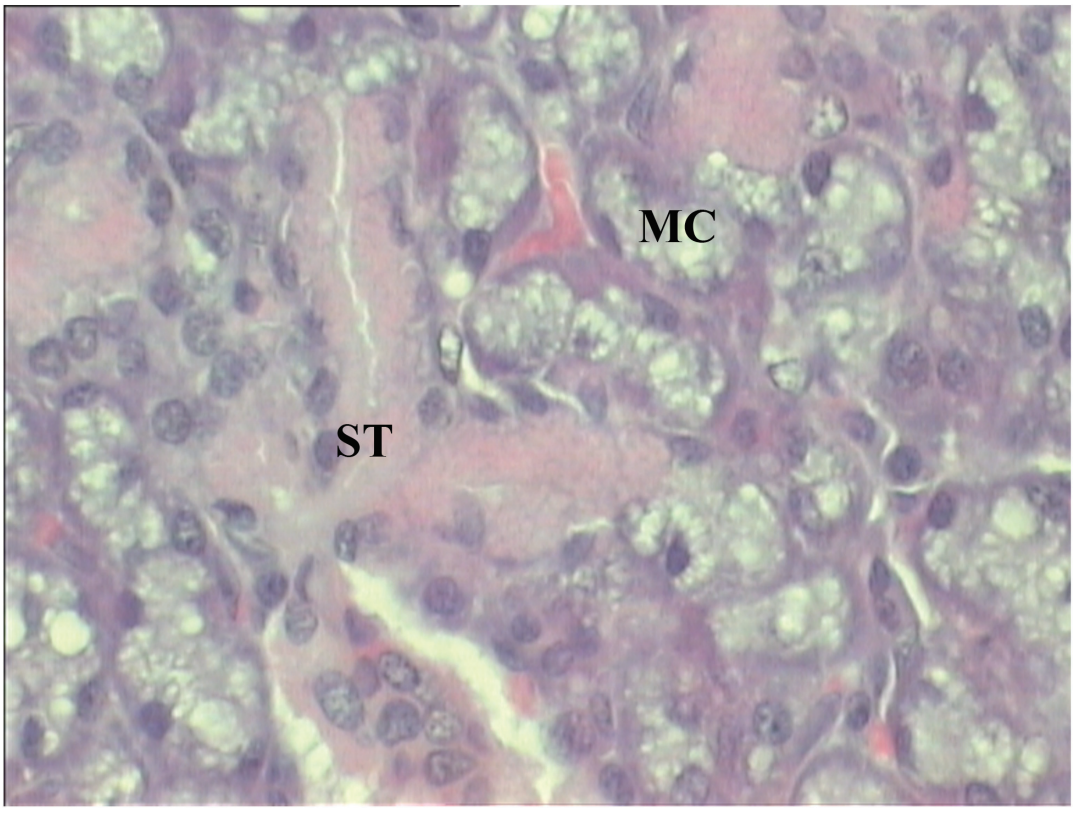

Fig. 2. The micrograph is showing mucous acinar cells (MC) of control group, with foamy appearance cytoplasm. Note also serous (ST) with acidophilic granules localized in apical part of the cells. H&E stain, X400.

Table 2. Showing the mean and standard deviation of the diameter of the serous tubules (μm) for each subgroup. N = number of readings for each subgroup.

Descriptive statistics				
Dependent variable: Serous tubules				
Dose	Period	Mean	Std. deviation	N
Control	1 Week	45.9375	6.7499	100
	2 Weeks	44.5594	6.5474	100
	Total	45.2484	6.6686	200
Therapeutic	1 Week	36.8625	6.8269	100
	2 Weeks	32.0250	8.2645	100
	Total	34.4438	7.9401	200
Over dose	1 Week	35.7375	7.9675	100
	2 Weeks	35.4375	5.7499	100
	Total	35.5875	6.9319	200
Total	1 Week	39.5125	8.5122	300
	2 Weeks	37.3406	8.7089	300
	Total	38.4266	8.6723	600

From the statistical analysis presented in Table 1 it has been shown that the F-test between doses was 88.559 (P value < 0.001), so there was a difference between the three groups used in this study. The F-test between periods of treatment was 10.751 (P value = 0.001). So there was a difference between the time. The F-test which represent the interaction between doses and period was 6.129 (P value = 0.002) so there was a difference between the different kinds of treatment employed in this study.

After using the multiple comparison tests and using LSD method. It has been found that there was a difference between control group and the treated groups (P value < 0.001). On the other hand there was no difference between therapeutic and overdose groups (P value = 0.683).

The results presented in Table 2 were also showing the mean and SD of interaction between the time and dose of different groups.

The use of multiple comparison and LSD method have revealed that there was a difference between the control group (G3A&B) with the treated groups used in this experiment (G1A&B together with G2A&B) with the P value < 0.001. There was no difference (P value = 0.610) between the therapeutic dose group for one week and overdose for one week.

Differences with P value < 0.001 between the treated groups for one week and control of two weeks were noticed. But the differences were negligible between the therapeutic groups for one week and those for two weeks (the P value = 0.015).

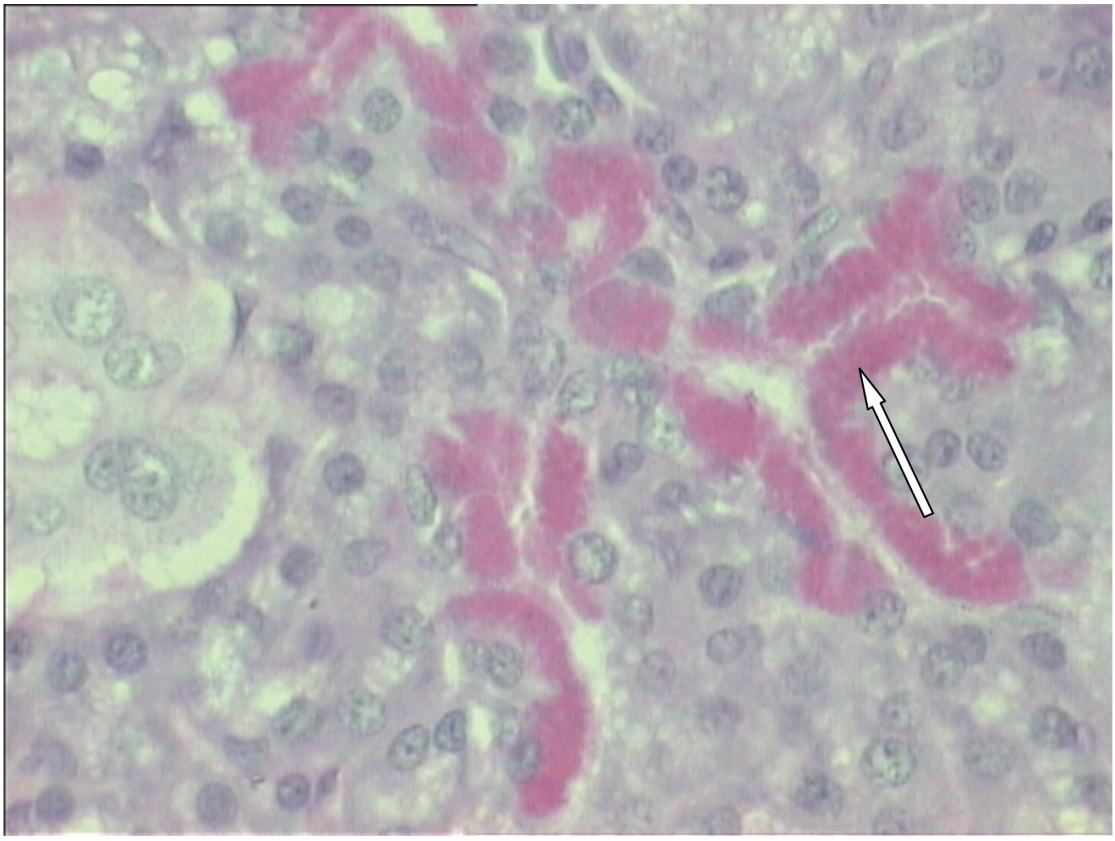

Fig. 3. Serous tubular cells of control group with red magenta colored granules in supranuclear position (arrow). PAS/hematoxylin countered stained section. X400.

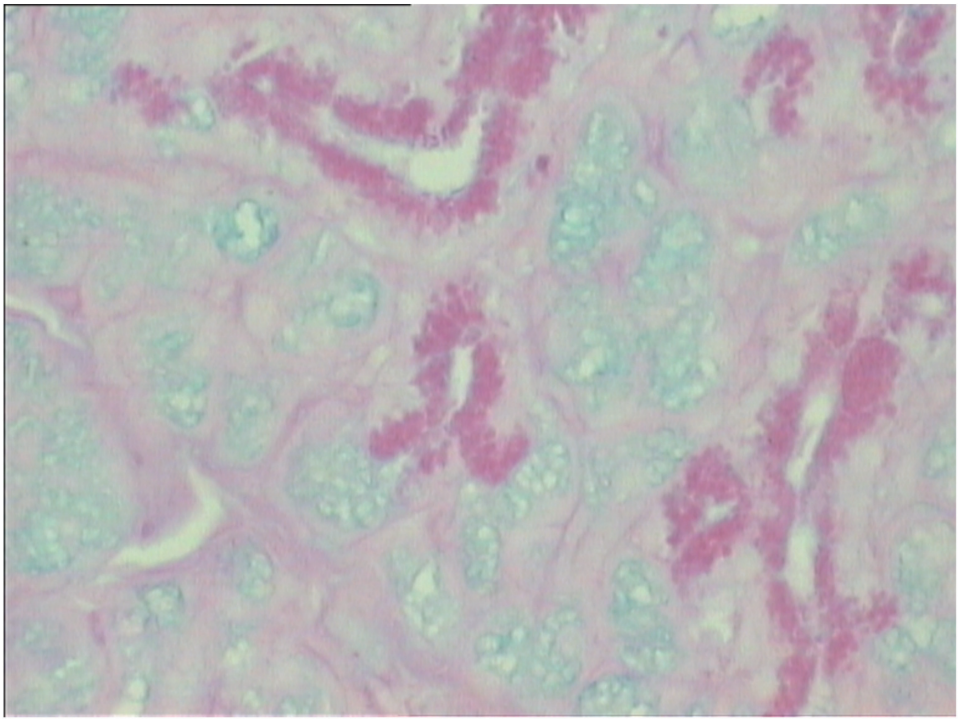

Fig. 4. PAS/AB stained section of control group demonstrates red magenta granules localized in supranuclear position of serous tubular cells. The mucous acini are AB positive. X:400.

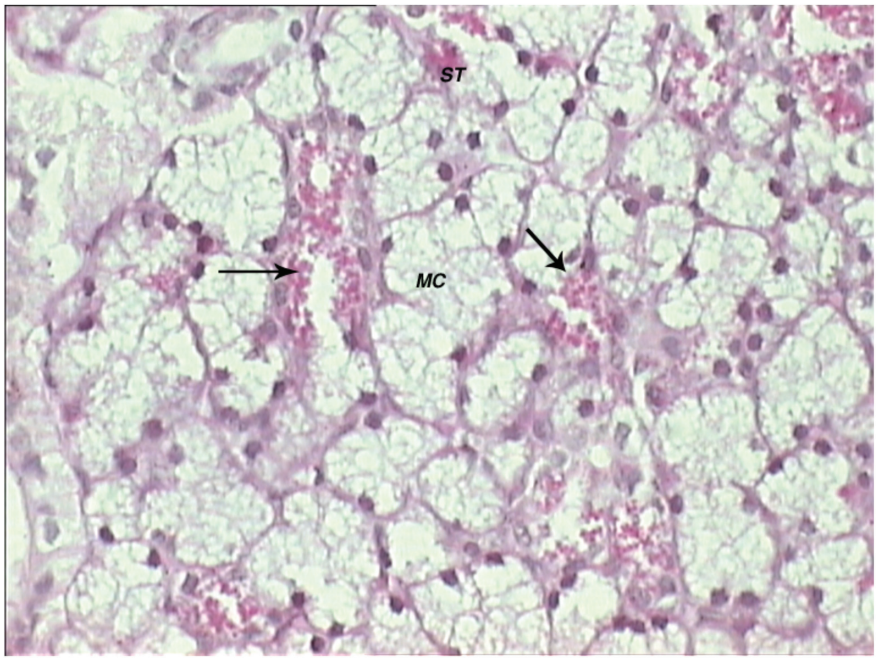

Fig. 5. Foamy vacuolated appearance of the mucous acinar cells(MC) from submandibular gland of G1A subgroup treated rabbit. One step trichrome stain. X400.

There was difference noticed (P value < 0.001) between control group for one week and treated groups for two weeks.

4. Discussion

The effect of drugs on salivary the glands have been a matter of interest for the physiologist for more than a century.

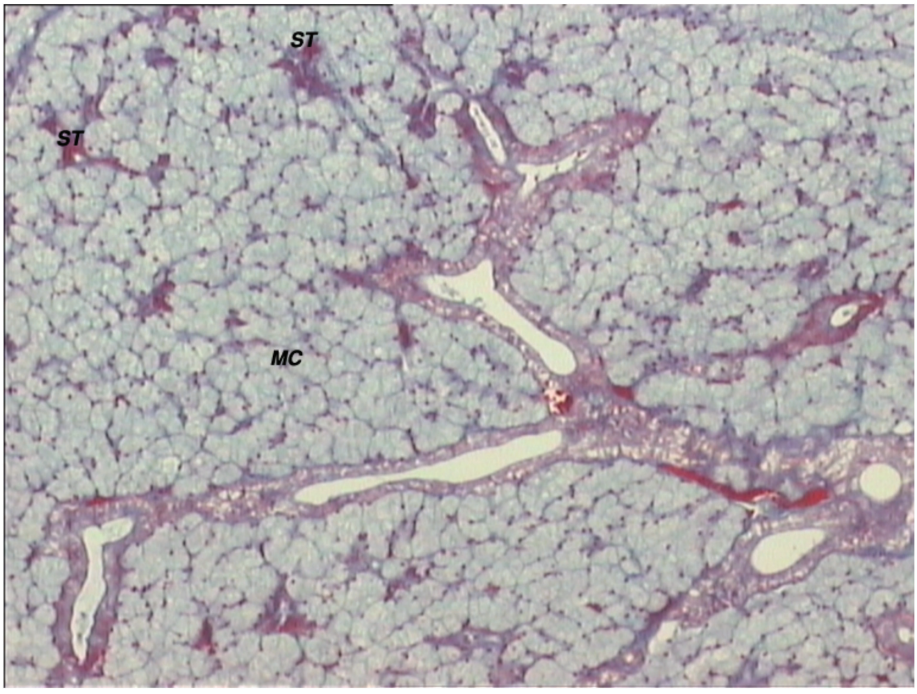

Fig. 6. Micrograph of section taken from submandibular gland of treated group (G1A) showing the predominance of mucous acini (MC; ST=serous tubules). One step trichrome stain. X100.

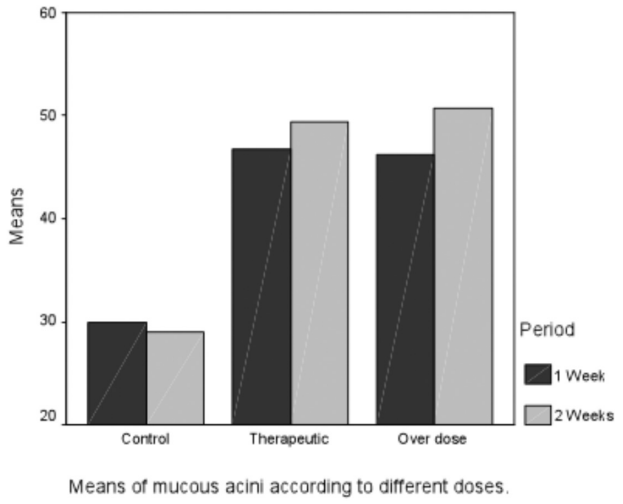

Fig. 7. Means of diameter of l mucous acini according to different doses of dexamethasone treatment. Note the increase in the diameter of the acini (the measurements for means are in micrometer).

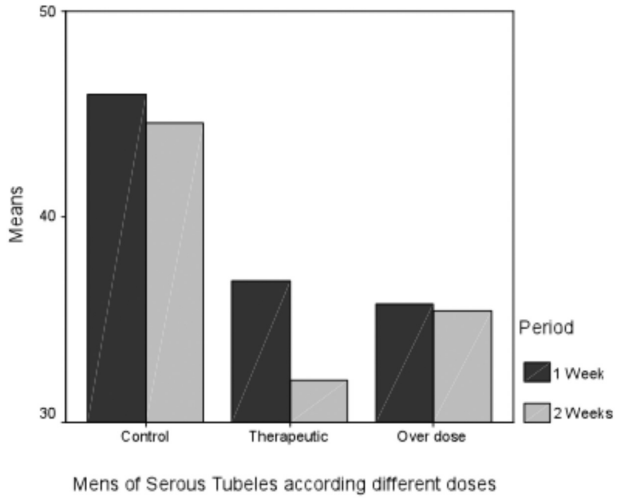

Fig. 8. Means of the serous tubules diameter at different doses of dexamethasone treatment. Note the great reduction in the diameter due to the treatment with dexamethasone (the measurements of means are micrometer).

It was apparent from this study that dexamethasone, even with short period of application, altered the morphological appearance of the submandibular salivary secretory unit cells. The most affected parts were the mucous acini and serous tubules. The statistical analyses have supplemented these finding with further support. The hydropic degeneration (Vacuolization) of the acinar cells was found in all treated rabbits. The hydropic degeneration was previously defined as reversible injury due to accumulation of water inside cells caused by many factors as chemicals and drugs [22].

An explanation for the mechanism of the former changes was proposed by [23]. They suggested that permeability of salivary gland cells to ions caused by significantly increase of intracellular sodium with intracellular decrease of potassium. The accumulation of sodium leads to water increase to maintain osmotic conditions and the cell swell.

The most recent studies on the side effect of dexamethasone on the mucous exocrine gland demonstrated decrease in the mucin production after administration of the drug [24, 25], that in fact explain the wide spread use of glucocorticoids in asthma treatment [8].

In the present study there was an increase of the size and diameter of the mucous acini. The increase in size does not means more mucin production, on the contrary mucin production by exocrine glands in the body has been noticed to be decreased after dexamethasone production in varieties of organs:gastric mucosa [26]; goblet cells of respiratory system [25].

The second most prominent change noticed in this work was the alterations in the serous tubules. As seen in the control group the serous tubules were dominating the scene of the gland, with their well organized and regularly arranged acidophilic granules. As dramatic changes after the injection of the drug, the serous tubules have reduced in size; their granules were irregularly arranged and disarrayed.

Thus it was judged from the histological findings supplemented by statistical analysis of the present work, that the serous tubules were subjected to the process of degeneration as attributed to the side effect of dexamethasone [22].

The alterations seen in serous tubules secretory granules morphology are in support of previous studies on the effect of dexamethasone on serous cells of parotid gland [14, 27].

In conclusion this study have demonstrated morphologically and statistically that rabbit treated for short period of time with pharmalogical doses of dexamethasone resulted in changes in submandibular gland saliva with subsequent alteration in submandibular saliva protein concentration, nature of mucin composition and concentration. Thus the present study in contrast with previous studies which pointed out that dexamethasone had no significant effect on submandibular gland structure of the rat [28] and mice [29]. The reason behind this discrepancy in the results might be attributed to the species variation reaction to the drug dexamethasone. The outcome from this investigation revealed significant changes in the submandibular salivary gland changes which affect its function and in turn alter the composition of saliva and salivary flow that could result in higher susceptibility to dental and oral diseases. These results suggest that patients, who are treated

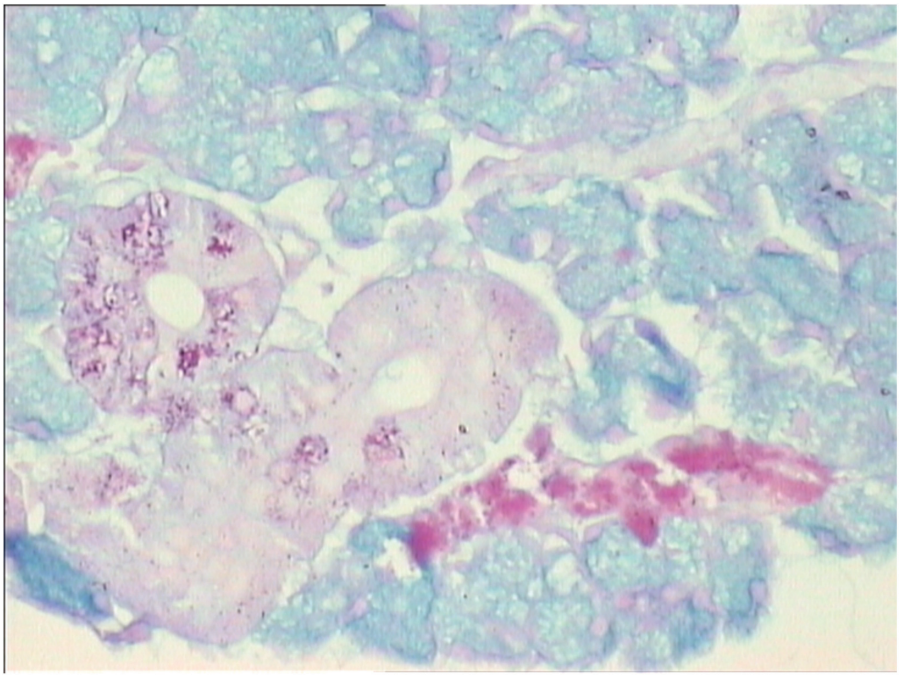

Fig. 9. Treated group (G1) PAS/AB stained section demonstrating the predominant mucous acini with alcinophilic reaction; red magenta coloration is only restricted to the serous tubules. X200.

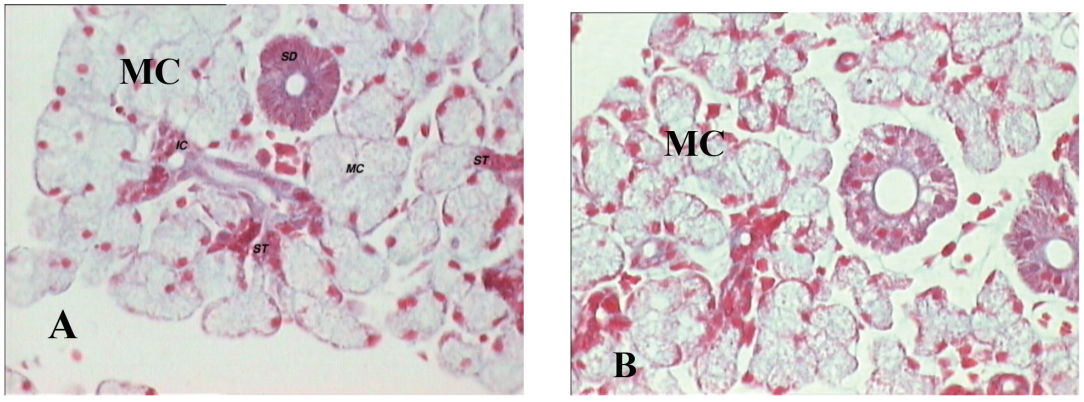

Fig. 10. Micrographs of treated groups (A from G1 group and B from G2 group) showing the predominance of mucous tubules (MC). One step trichrome stain. X200.

with dexamethasone, should be kept under continuous and dental health care, especially those of chronic diseases where the drug should be taken for a long period. Thus further investigation entailing the administration of dexamethasone for longer period to the experimental animals is required to elucidate further changes which may occur.

It is worth to mention also that the use of different histological and histochemical stains was aimed to draw the attention to the fact with pictorial evidence that dexamethasone treatment has affected both mucous and serous secretory unit of submandibular mixed predominantly serous salivary gland. The consequences will be undesirable effect on teeth and oral cavity health due to decrease in serous secretion coming from one of the major salivary glands.

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