

Premature Age Changes Associated with Cimetidine Treatment: A Histological Study

¹Yasear A.Y. ¹Sultan, A. ²Elramli, A.H.

¹Department of Oral Biology, Faculty of Dentistry, ²Department of Anatomy and Histology, Faculty of Medicine, Benghazi University, Benghazi, Libya

Abstract: Cimetidine is histamine H₂ receptor antagonist, its main clinical use is as an inhibitor of gastric acid secretion. For this study 12 (6 months old) female rabbits were used in this experiment. The rabbits were divided into four groups, three rabbits each.; viz:G1 received therapeutic dose of the drug (55.5 mg); G 2 received double therapeutic dose; G 3 received triple therapeutic dose; the fourth group served as a control. The injection was given twice daily for four weeks. The parotid glands were removed, fixed in neutral buffered formalin and processed for paraffin embedding. The sections were stained with haematoxyline and eosin, one step trichrome, and PAS stains Histometric evaluations of diameter of secretory end pieces were conducted. Data, expressed as means \pm SEM, were analyzed by ANOVA and multiple comparison tests. Probability less than 0.05 is considered significant. In cimetidine treated rabbits the pronounced observations in the parotid gland were the decrease in the diameter of the acini; increase in the connective tissue fibers; lymphocytic infiltration; increase in the amount of fat. The accumulation of fat cells and connective tissue fibers was considered as premature age changes in the gland. Fat deposition and fibrosis seen in cimetidine treated group may be related to parenchymal destruction caused by lymphocytic infiltrations. Our histological findings have demonstrated that the use of cimetidine has an inhibitory action on the activity and nature of saliva synthesized by the parotid glands in rabbits.

Key words: Cimetidine, premature age changes, parotid gland, rabbit

INTRODUCTION

Cimetidine is a histamine H₂- receptor antagonist, competitively inhibit histamine at all H₂- receptors but their main clinical use is as an inhibitor of gastric acid secretion, this drug is used for treatment of gastroesophageal reflux disease, Zollinger-Ellison syndrome and prevention and treatment of heartburn symptoms associated with acid indigestion and sour stomach (Katzung B. 2004) Using of this drug may causes headache, diarrhea, dizziness, confusion, excitement, depression, hallucination, gynaecomastia (Enlargement of breasts in males), and xerostomia with prolonged use of the drug (Brody T. M., *et al.*, 1998; Rang H.P., *et al.*, 2003)

Administration of most antihistamines causes a decreased salivary secretion (Liu, F.T.Y and Lin, H.S 1970). This interference with glandular function, which is due to histamine blocking and parasympatholytic effects, accounts for mouth dryness (Garrett, J. and Kyriacou, K 1988) and for increased incidence of dental caries in humans and in rats treated with antihistamines for prolonged periods (Pedersen A.M. *et al.*, 2002)

Structural changes develop in the secretory tissues of most salivary glands in man with advancing age (Scott, J. 2008)

Histomorphometric examination of "normal" salivary gland tissue revealed age-associated decreases in the number of acinar cells (Scott, J. *et al.*, 1987)

There have been several studies reporting age-related decremental changes in the morphologic appearance of human salivary glands (Andrew, W. 1952; Waterhouse, J.P. *et al.*, 1973; Scott, J. 1977; Nagler, R.M 2004)

Salivary flow has been shown to be age dependent, Nagler, (2004) measured salivary flow in 661 healthy individuals from 5 to 96 years of age. Although there was a wide scatter in the distribution, there was an increase in flow between the ages of 5 and 29.

The most obvious changes occur in the parotid gland, where the parenchymal tissue is gradually replaced by adipose tissue and fibrous connective tissue (Fatty degeneration) (Davis W. L. 1986; Bhaskar S. N. 1991).

Morphological and physiological age changes in parotid, submandibular and minor salivary glands include epithelial degeneration, atrophy, loss of acini and fibrosis occurs as age increases (De Oliveira, L A M, *et al.*, 2002).

Kim (1984) and Scott *et al.*, (1987) stated that, as an age changes in the parotid salivary gland, the amount of fat is markedly increased in the connective tissue stroma.

A premature age changes in the parotid salivary have been noticed in patients suffered from Sjogren's syndrome (Izumi M., *et al.*, 1997). Infact most of the previous studies were dealing with clinical aspects of the effect of cimetidine on the salivary glands. Thus the present study was planned as a histological approach, in

order to have more complete picture on the effect of this drug on the parotid salivary. We have chosen the rabbit as an experimental animal for this study.

MATERIALS AND METHODS

For this study 12 female rabbits, aged six months, were used in this study. The rabbits were maintained on standard rabbit diet. They were divided into four groups (three animals each); viz: three experimental groups G1; G2 ; G3 , the last group(G4) was left as a control group.

Calculation Of The Drug Dose:

Human dose of Cimetidine is 20 mg/kg body weight.

For human of 70 kg body weight the required dose was $20 \times 70 = 1400$ mg twice daily. According to Pagat and Barnus formula (Pagat, G. E and Barnus, J. H 1964); the dose for rabbit weighing 1.5 kg = Human dose (70 kg) $\times 0.07 = 98$ mg, so the therapeutic dose used in this study was 55.5 mg twice daily.

The three treated groups were given an intramuscular injection of cimetidine for **four weeks** as follows:

- 1- G 1 group has received an injection of therapeutic dose of Cimetidine (55.5 mg) twice a day.
- 2- G 2 has received an injection of double therapeutic dose of Cimetidine (111 mg) twice a day.
- 3- G 3 group has received an injection of triple therapeutic dose of Cimetidine (166.5 mg) twice a day.
- 4- G 4 group served as a control, they have received equivalent injections of isotonic saline for the same period of time.

The rabbits were anaesthetized and perfused through the heart with normal saline followed by neutral buffered formalin. The parotid glands were removed, sliced into smaller pieces and further fixed in the same fixative for 48 hours. Salivary glands sections were stained with haematoxylin and eosin (H &E), one step trichrome (Culling C. F. A., *et al.*, 1985). Some sections were stained with periodic acid Schiff (PAS) method for identification of mucosubstances. The PAS method was preceded by diastase for possible presence of glycogenic mucosubstances (Mowry ,R .W. 1963).

Statistical Analysis:

Histometric evaluations of diameter of secretory end pieces were conducted on ten slides stained by Haematoxylin and eosin stain.

For the measurement, an eyepiece equipped with graticule having standard scale was used. The readings obtained were multiplied by a factor to get the result reads in micrometer.

Data, expressed as means \pm SEM, were analyzed by ANOVA and multiple comparison tests. Probability less than 0.05 is considered significant.

Results:

The parotid gland in rabbit of control group was composed of purely serous acini. The acinar cells contained apical acidophilic part with acidophilic granules. Their nuclei tend to be displaced toward the basophilic distal third of the cells (figure 1). Strong positive reaction was obtained after staining with PAS (with and without pretreatment with diastase) (figures 5 and 6).

In cimetidine treated rabbits the pronounced observations in the parotid gland were the decrease in the diameter of the acini (see table 1); there was a noticeable increase in the connective tissue fibers around the acini and different levels of the duct system (figure 2); there was pronounced lymphocytic infiltration on the parenchymal cells (figure 3); increase in the amount of fat in the connective tissue around the acini (figure 4).

The results of PAS stain, with and without pretreatment with diastase, were the same (figures 5 and 6). In G1 group, the reaction was mild. In G2 group the reaction was moderate. With increasing of the dose of the drug (G3 group) there was abolishing of the reaction in the secretory acini and duct system (figures 3 and 4).

The statistical analysis has revealed a change in the diameter of the acini of the parotid gland (see tables 1, 2, and 3 and figures 7 and 8). There was a difference between the three treated groups and the control with P value ≤ 0.05 .

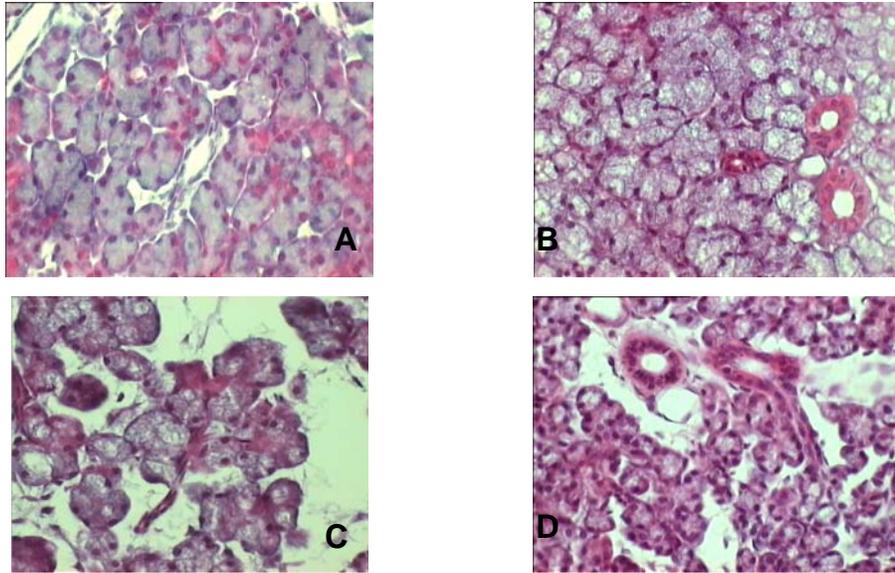


Fig. 1: H&E stained sections

A- The photomicrograph shows normal structure and appearance of secretory acini and duct system. **G 4 group.** Original magnification $\times 200$.

B- The photomicrograph shows beginning of vacuolation in acinar cells. **G 1 group.** Original magnification $\times 200$.

C- The photomicrograph shows same appearance of acini as in G1 group **G 2 group.** Original magnification $\times 200$.

D- The photomicrograph shows marked decrease in size of acini in **G 3 group.** Original magnification $\times 200$.

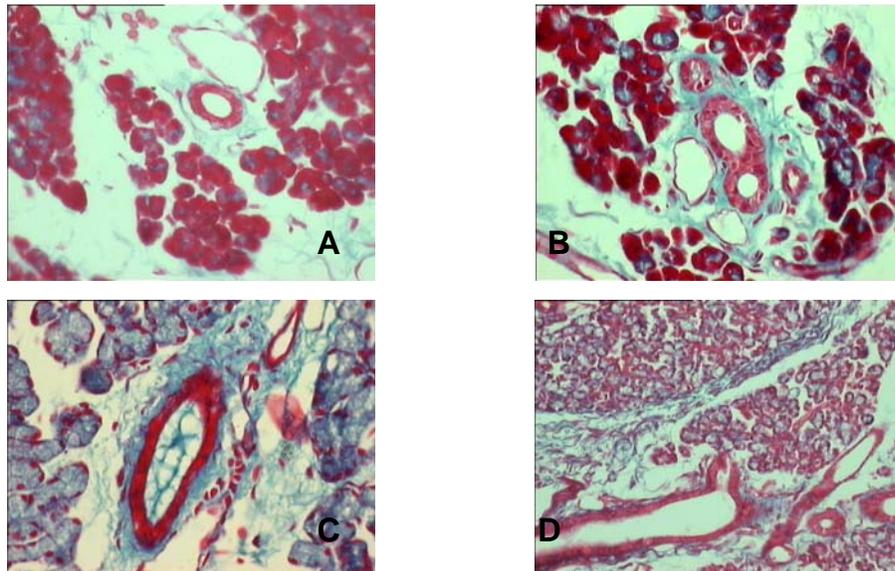


Fig. 2: One step trichrome stained sections

A- The photomicrograph shows secretory acini with normal C. T. septa.

G 4 group. Original magnification $\times 200$.

B- The photomicrograph shows increase in the amount of C.T. fibers especially around the duct system. **G 1 group.**

Original magnification $\times 200$.

C- The photomicrograph shows more fibrosis occurs around the duct system. **G2 group.** Original magnification $\times 200$.

D- The photomicrograph shows more fibrosis around the duct system and between the lobules of the gland. **G3 group.** Original magnification $\times 200$.

Note the destruction of some of the acini. The acini have been heavily infiltrated with inflammatory cells.

Rabbit injected with double therapeutic dose of Cimetidine (G2 group)
H&E stain. X 200

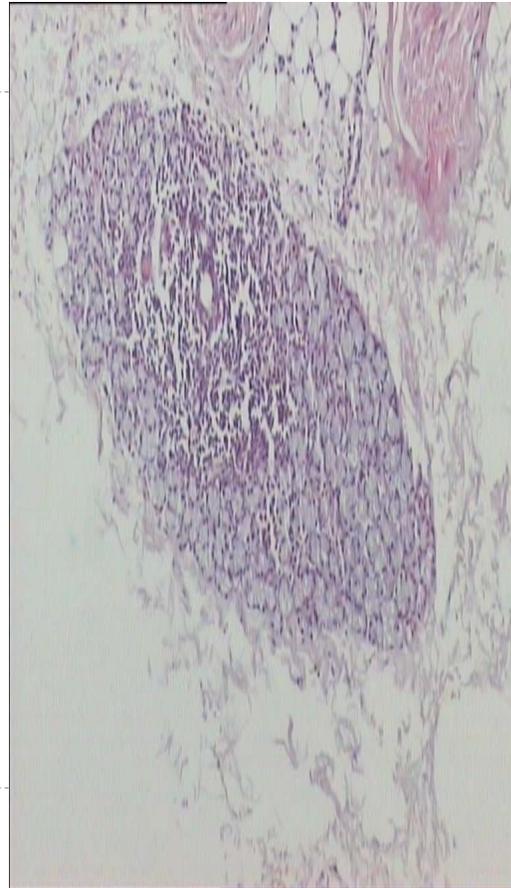


Fig. 3:

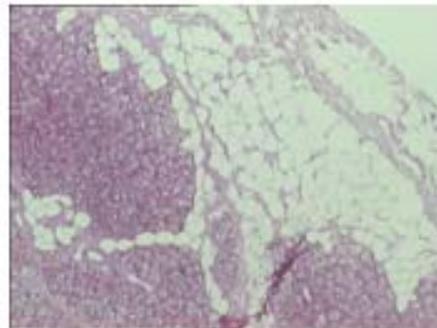
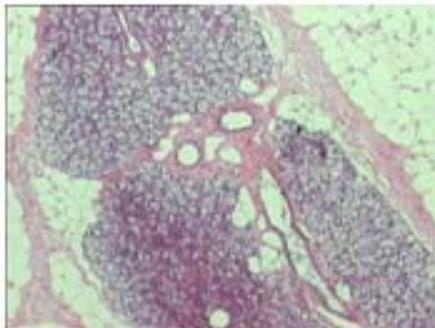


Fig. 4: The Photomicrographs is showing increase in amount of fat accumulation in parotid gland obtained from rabbit receiving triple of the therapeutic dose of cimetidine. H &E stain. X 100.

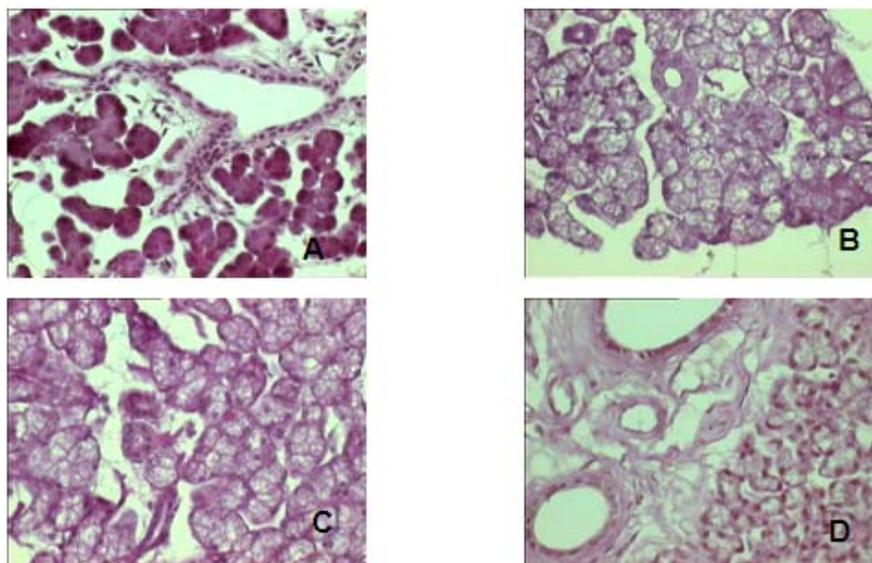


Fig. 5: PAS stain without diastase pretreatment stained sections

A- The photomicrograph shows normal strong **positive** reaction of secretory acini and duct system in the control (**G 4**) **group**. Original magnification $\times 200$.

B- The photomicrograph shows normal structure of secretory acini and duct system showing **mild** reactivity of mucosubstances with PAS. **G 1** **group**. Original magnification $\times 200$

C- The photomicrograph shows normal structure of secretory acini and duct system showing **moderate** reactivity of mucosubstances with PAS. **G 2** **group**. Original magnification $\times 200$

D- The photomicrograph is showing **negative** reaction for mucosubstances with. **G 3** **group**. Original magnification $\times 200$.

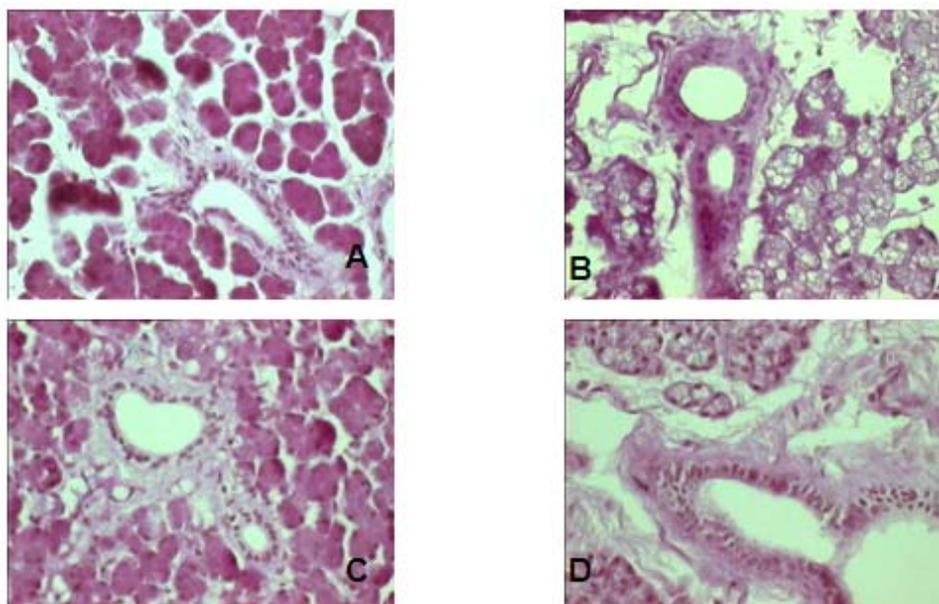


Fig. 6: PAS stain with diastase pretreatment stained sections

A- The photomicrograph of **normal positive reaction** in secretory acini and duct system. **G 4** **group**. Original magnification $\times 200$

B- The photomicrograph shows normal structure of secretory acini and duct system showing **mild** reactivity of mucosubstances with PAS. **G 1** **group**. Original magnification $\times 200$.

C- The photomicrograph shows normal structure of secretory acini and duct system showing **moderate** reactivity of mucosubstances with PAS. **G 2** **group**. Original magnification $\times 200$.

D- The photomicrograph is showing **negative** reaction for mucosubstances with. **G 3** **group**. Original magnification $\times 200$.

C- The photomicrograph is showing **moderate** reactivity of mucosubstances with PAS in **G 2 group**. Original magnification $\times 200$.

D- The photomicrograph is showing **negative** reaction for mucosubstances with PAS. **G 3 group**. Original magnification $\times 200$.

Table 1: Describe the means and standard deviations for Group 2 that treated with different doses of cimetidine and their control group.

Group	Number of reading	Mean	Standard deviation SD	Minimum	Maximum
G4	150	40.325	7.722	26.25	52.50
G1	150	39.675	6.790	22.50	63.75
G2	150	33.675	5.689	26.25	52.50
G3	150	39.075	6.545	22.50	45.00

Table 2: Describe the ANOVA test applied on the diameters of the acini between the subgroups of group 2 that treated with cimetidine.

	Sum of Square	df	Mean Square	F	Sig.
Between Groups	4441.031	3	1480.344	33.160	0.000
Within Groups	26606.625	596	44.642		
Total	31047.656	599			

Table 3: describe the degree of significance between the diameters of G4 group (control) and G 1, G2, and G3 groups (treated) using multiple comparison test.

(I) Control	(J)Treated	Mean Difference (I-J)	Std.Error	P value
G4	G1	-1.2500	0.7715	0.106
	G2	-1.1000	0.7715	0.154
	G3	5.4000*	0.7715	0.000

*The mean difference is significant if P value ≤ 0.05

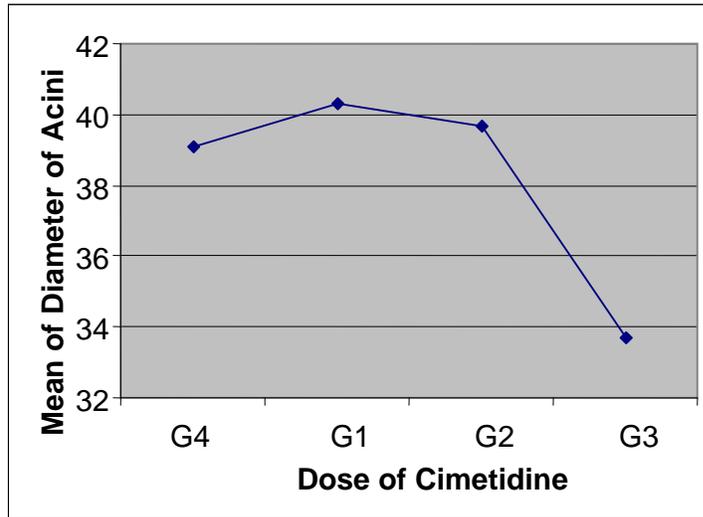


Fig. 7: showing the relation between the diameter of the acini and the dose of the cimetidine.

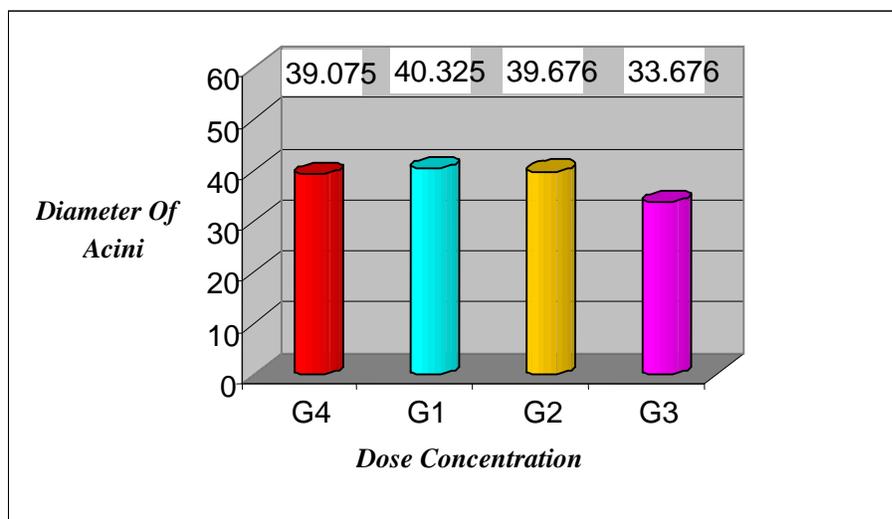


Fig. 8: showing the relation between the diameter of the acini and the dose of the cimetidine.

Discussion:

The results of cimetidine effect on the parotid gland in the rabbits of the present work have demonstrated a reduction in the size of acini and deposition of fat cells, fibrosis and inflammatory cells infiltration especially lymphocytic infiltration. In fact, the accumulation of fat cells and connective tissue fibers in and around the acini have been suggested to be associated with salivary glands undergoing changes in structure and function with age (Kim S.K. 1984; Block J.H., Beale J. M. 2004). However, premature age changes have been seen in the parotid glands of patient suffering from Sjogren's syndrome (Izumi M., *et al.*, 1997; Fox P.C., *et al.*, 1986). In addition to that, stressful circumstances (*e.g.*, disease, surgery, pharmacotherapeutics) strain the reserve capacity of the salivary glands, thus hindering their ability to compensate for increased metabolic demand, resulting in compromised function (Evers BM, *et al.*, 1994).

At present, no explanation could be given about the exact mechanism of fat deposition in salivary glands of treated group. The most straight forward scenario may be related to the reduced level of cellular secretory activity as has been suggested to be the case during atrophy of the gland caused by a liquid diet (Schneyer, L. H. & Schneyer, C. A. 1960; Daly M. J., Humphray J. M. and Stables R 1982). Another explanation for the fat deposition and fibrosis seen in cimetidine treated group may be related to parenchymal destruction caused by lymphocytic infiltrations is followed by fibrosis. The proliferating fibroblasts are in turn induced to differentiate to adipocytes by cytokines and other differentiation mediators released by surrounding lymphocytes or other cell types. Supporting this notion are several reports implying that immunologic factors play a role in fat synthesis (Vernet C, Boretto J, Mattei MG, *et al.* 1993; Pham-Dinh D, Mattei MG, Nussbaum JL 1993).

The presence of acinar cells with reduced size supports the suggestion that the cellular activity of secretion has reduced in the gland due to the use of antihistamine (Liu, F.T.Y and Lin, H.S. 1970). Degenerating secretory granules of similar appearance have been observed in parotid acinar cells following starvation of rat for about a day (Hand A.R. 1979), with chronic treatment with reserpine (Soussa,E.F. and Saad EL-Din, T.A. 1996) and following treatment with receptor selective agonist (Peter,B *et al.*, 2007)

On the other hand, the gradual decrease in diameter of acini and in the intensity of staining reaction with PAS ,of the present work, may be due the action of cimetidine. In parotid glands of G 3 (with triple dose injection) group there was a complete abolishing in the reaction with PAS in the acinar cells. Infact , the serous cells of canine salivary glands (Reifel, C.W. and Travill,A.A. 1970); human parotid gland (Harrison,J. D. *et al.*, 1987); and the serous demilunar cells of goat submandibular (El_Shafey,S.M. *et al.*, 1980) and rabbit's sublingual glands all produce neural mucosubstances which were PAS positive. The inhibitory action of antihistamine was appreciated due to the fact that the histamine has been considered as one of the neurotransmitters and plays a positive role in process of secretion in gastric gland (Angus ,J .A. *et al.*, 1967) and salivary glands (Emmilin, N.1960; Abbas ,M.G. and Salah, H. 1985). Thus our histological findings have demonstrated that the use of cimetidine has an inhibitory action on the activity and nature of saliva synthesized by the parotid glands in rabbits, that was in contrast to early pharmacological reports (Daly M. J., *et al.*, 1982) which mentioned that antihistamine has no effect.

In view of the results of the present study the authors' advice to all persons taken antihistamine, should pay good attention to oral hygiene.

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