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Histological and Biochemical Evaluation of the Effect of Desloratadine Drug in Parotid Gland Tissues

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ABSTRACT

Background: The functional deterioration of salivary glands is a consequence of a wide range of factors and significantly interfered with life quality. Desloratadine (an antihistamine) is among the drugs listed to cause dry mouth; however, its effect on major salivary gland tissues has not been well studied.
Objectives: To evaluate the effects of desloratadine treatment on parotid gland tissues, histological features, and their impact on serum oxidative and antioxidant markers.
Materials and methods: Thirty rats were used in this study. They were divided into three groups (each containing ten rats). Group A: control rats. Group B and C have received desloratadine at dose 0.142 and 0.245 mg/kg of body weight respectively for three weeks. After three weeks, serum levels of sialic acid, malondialdehyde, catalase, lactate dehydrogenase, superoxide dismutase, creatin kinase, and glutathione were estimated for three groups. Then, animals were sacrificed and five μm formalin-fixed paraffin-embedded tissue sections were prepared routinely from parotid glands for histological evaluation under light microscope.
Results: The histological evaluation of salivary gland tissues in both treated-groups was revealed a remarkable cytoplasmic vacuolization, atrophy, and degranulation in acinic cells. The serous acinar cells were showed autolysis and nuclear changes (pyknosis, karyorrhexis, and karyolysis). There was an increase in the interstitial spaces between each parenchymal element associated with few mononuclear cell infiltrations. The intra-lobular ducts were reduced in size and were indistinct throughout lobes. The severe changes were associated with higher desloratadine dose. Regarding biochemical analysis, the treated-groups had significantly increased serum levels of malondialdehyde, sialic acid, lactate dehydrogenase and creatin kinase, and significantly reduced serum levels of superoxide dismutase, catalase, and glutathione.
Conclusion: Desloratadine administration produces noticeable histological changes in a dose-dependent manner associated with increased oxidative stress markers and decreased antioxidative activity.

Keywords: Desloradatine; Salivary Glands; Parotid gland; Xerostomia; Malondialdehyde; glutathione; Catalase; Superoxide dismutase.

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INTRODUCTION

One of the main functions of the major salivary glands is the secretion of more than 90% of unstimulated saliva, which is primarily responsible for electrolyte content and salivary volume [1]. Saliva

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is a vital fluid, that has confined and total protective mechanisms, as it has cleansing, lubricating, buffering, antibacterial effects on the oral cavity, pharyngeal region, and upper part of the gastrointestinal duct [2]. These protective properties are related to salivary flow. Therefore, any alteration of salivary glands' integrity and activity could change the flow and composition of saliva [3]. Xerostomia is a dry mouth syndrome resulting from the reduction in salivary flow per second, it might be due to systemic diseases such as Sjögren's Syndrome (a pathological condition affecting salivary glands), head and neck radiotherapy, and uses of certain medications [4].

Desloratadine (des- carboethoxy-loratadine, CAS 100643 − 71 − 8), a basic metabolite of loratadine, is the second-generation antihistamine drug that is commonly used for the treatment of allergic rhinitis and has nasal decongestant effect in patients with intermittent allergic rhinitis. This drug is highly selective for histamine H1 receptors [5]. It is metabolized by isoenzymes of the cytochrome P450 system; including mostly CYP3A4 and CYP2D6 [4, 5]. It is almost totally (97−99%) bound to plasma proteins. Its structure is closely related to tricyclic antidepressants, such as imipramine, and is distantly related to the atypical antipsychotic quetiapine. Loratadine's peak effect occurs after 1−2 hours, and its biological half-life is on average eight hours (range 3−20 hours) with desloratadine's half-life being 27 hours (range 9−92 hours), accounting for its long-lasting effect. About 40% of Loratadine is excreted as conjugated metabolites into the urine, and a similar amount is excreted into the feces [6]. This new-generation antihistamine (desloratadine) which has very low antimuscarinic effects, leads to xerostomia via their H1-receptor antagonistic effects [7].

To date, little is known about the effects of premedication with desloratadine on the salivary gland tissues. The few previous studies have mostly clinical in their approach. Only one published paper has studied the effect of desloratadine on the histopathological and biochemical features of the submandibular gland [8]. Therefore, this is the first study was conducted to evaluate the histopathological alterations of parotid gland tissues concerning the serum oxidative stress which might occur during the premedication with this drug.

MATERIALS AND METHODS

This experimental study was conducted in the animal house of the Veterinary Medicine Faculty in Tikrit University, in January 2020. The set of rules applied in this study was agreed by the Animal Ethics Committee of Tikrit University/Veterinary Medicine College (4644/18/7 in 14/3/2019). Thirty male Sprague-Dawley rats were used in this study. Rats were five and a half months old and (205-220 g) weight. Rats were kept under conventional animal housing conditions, at normal temperature, and in a satisfactory ventilated room, under 12h light/ 12h dull cycle, with free access to nourishment and water until the end of the study.

The animals were randomly divided into three groups, each one composing from ten rats as shown in Figure 1

1. Group A: 1ml of normal saline (0.9%) was orally administered (control group).
2. Group B: Desloratadine was orally administered at a dose of 0.142 mg/kg in saline every day for three progressive weeks.
3. Group C: Desloratadine was orally administered at a dose of 0.245 mg/kg in saline every day for three progressive weeks.

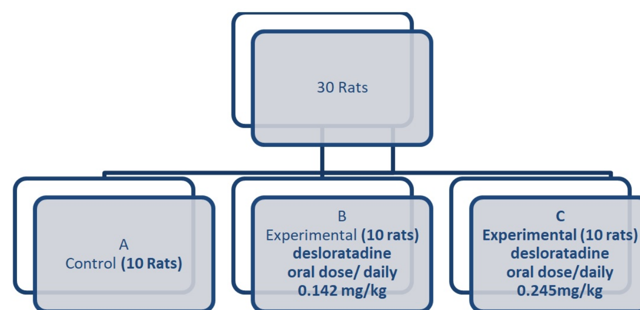

Figure 1. Study design.

To evaluate the levels of oxidative and antioxidative serum factors, blood samples were collected from the heart of rats after three weeks, then centrifuged for five minutes at 3000 rpm, and clear serum was separated. Biochemical analysis of sialic acid (SA) was estimated by Ehrlichs reagent in a spectrophotometer (APLE PD-303 UV Japan) using commercially available kits. Malondialdehyde (MDA), catalase (CAT), lactate dehydrogenase (LDH), superoxide dismutase (SOD), creatine kinase (CK), and glutathione (GSH) levels were measured and their levels were assayed using RANSEL kit (RANDOX laboratories). The animals were anesthetized by using intraperitoneal sodium pentobarbital (40 mg/kg) before rats' scarification.

The excised parotid glands were immediately fixed in 10% formalin and processed for paraffin embedding according to standard procedure. Serial sections of five μm thickness were cut and stained with hematoxylin and eosin (H&E). Two examiners unaware of experimental details independently determined the histomorphological changes in the parotid glands using a light microscope (Leica Microsystems). Statistical analyses were performed using SPSS version 10.0. The results were expressed as mean ± SD, and tested by Student's t-test. The statistical differences were considered significant at $p < 0.05$.

RESULTS

The histological observation of the parotid gland tissues in the control group (A), was showed normal lobular structure with densely packed serous acini and a well-developed duct system represented by intralobular (intercalated and striated ducts), interlobular and excretory ducts, with small amounts of loose stromal connective tissue (Figure 2). On the other hand, altered histological changes were observed in the salivary gland tissues in both groups received desloratadine (B and C). More severe changes were associated with higher desloratadine dose.

The most remarkable change detected in experimental groups was cytoplasmic vacuolization in acinar cells. These vacuoles were more frequently seen in group C. These vacuolizations were occurred in most gland lobes and even involved some epithelial lining of intralobular ducts (Figures 3 and 4). Acinar cell atrophy was seen in many areas of the gland. It is worth pointing out, that nuclear alterations (pyknosis, karyorrhexis, and karyolysis) were also noticed in the serous cells (Figure 5). Moreover, acinar autolysis of the serous acini was observed in different areas of parotid glands in group C. It was showed serous cells with partial or complete damage of limits and a disordered cytoplasm, with partial

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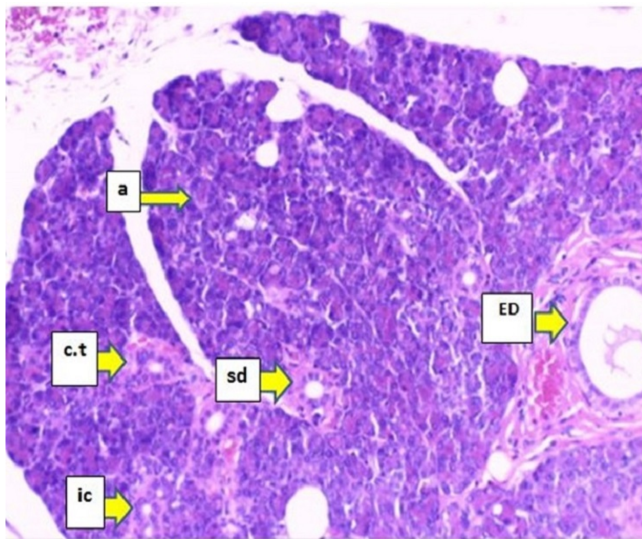

Figure 2. Parotid gland tissue of control group (A), the glandular parenchyma was found to be compact and consisted of serous acini (a), with small amounts of loose stromal connective tissues(c.t.). Besides, a network of intralobular ducts[intercalated (ic) and striated (sd)], and excretory(ED) duct system. H&E ×15.

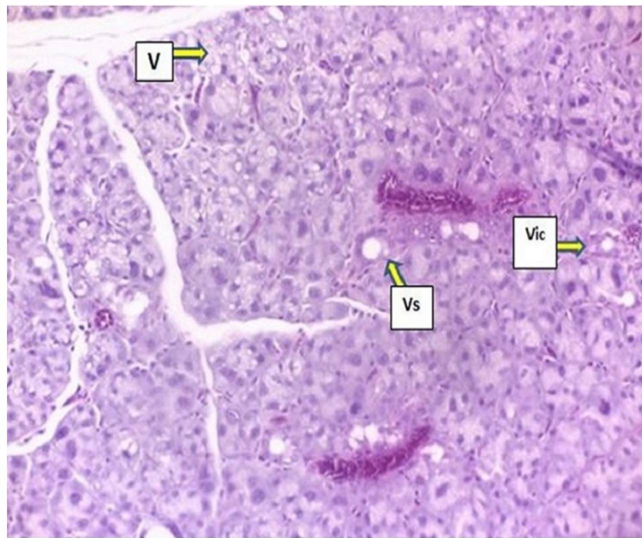

Figure 3. Parotid gland tissue of group B. Notice the cytoplasmic vacuolization of the cell acini (V), the lining of striated (Vs) and intercalated ducts (Vic) are affected by vacuoles. H&E ×20.

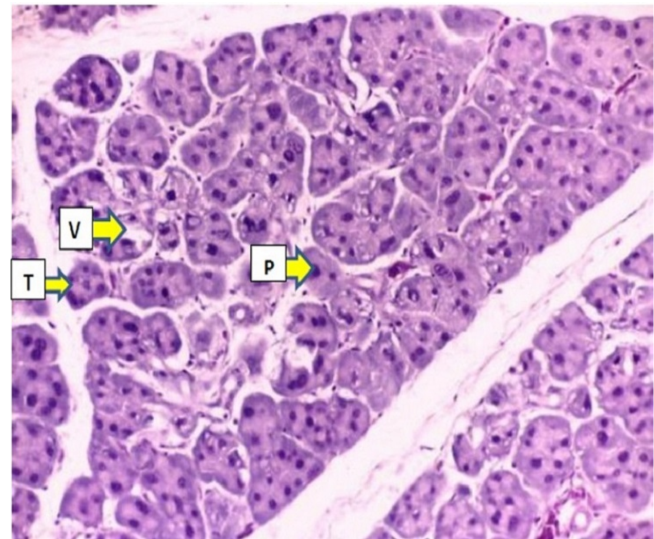

Figure 4. Parotid gland tissue of group C. Aggressive cytoplasmic vacuolization of cell acini (V) is seen, with atrophied acini (T) and pyknosis of nuclei (P) and reduced number of intralobular ducts. H&E ×20.

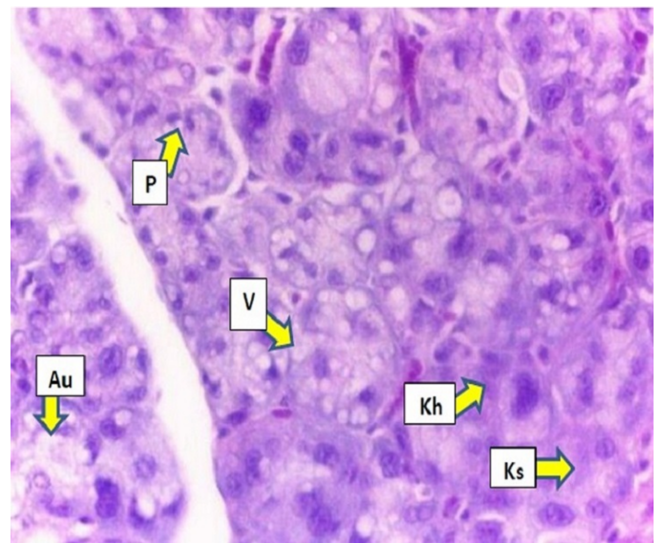

Figure 5. Parotid gland of group C, vacuolization(V) is obvious, notice the nuclear alteration of acini, pyknosis (P), karyorrhexis (Kh) and karyolysis (Ks) of nuclei are detected with autolysis (Au) affecting many serous acini. H&E ×100.

degradation of their contents, and in some areas, the complete absence of the nuclei and organelles (Figure 6). Group C was also showed discrete circumscribed areas of acinar shrinkage and degranulation, accompanied by an increase in the interstitial space between each parenchymal element (Figure 7). The intralobular ducts were small and indistinct as a general feature throughout the lobes of groups B and C (Figure 7). The mononuclear cell infiltration; although discrete, was noted in interstitial tissues of the acini in both treated groups (Figures 7 and 8).

Regarding the biochemical analysis, the serum levels of MDA, SA, LDH, and CK in rats treated with desloratadine in both doses (0.142 and 0.245mg/kg) were significantly increased when compared to the control rats. On contrary, desloratadine treatment was significantly decreased the level of SOD, CAT, and GSH in the treated groups when compared to the control group as shown in Table 1.

DISCUSSION

The current study was revealed significant histomorphological alterations within the parotid gland

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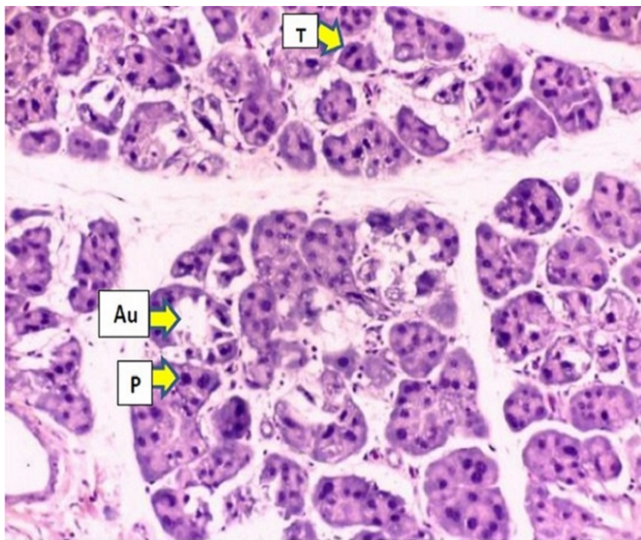

Figure 6. Parotid gland of group C, notice the atrophied (T) acini with autolysis (Au) affecting many serous acini associated with pyknosis (P) of nuclei. H&E ×20.

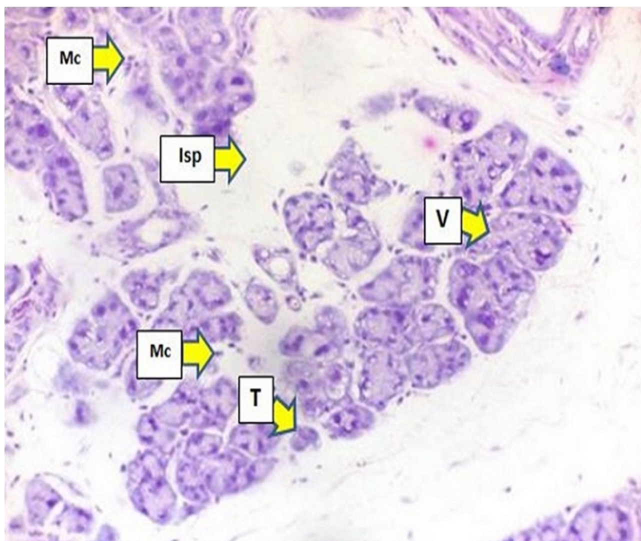

Figure 7. Parotid gland of group C, notice the damage affecting all structure of the lobe associated with an increase of interstitial spaces (Isp), nuclear alteration, vacuolization (V), acini atrophy (T), mononuclear cell infiltration (Mc), as well as a decreased number of intralobular ducts. H&E ×20.

parenchyma in the groups treated with desloratadine in a dose-dependent manner. These alterations were reflected the tissue degeneration and atrophy. There were marked cytoplasmic vacuolization, nuclear alteration, autolysis, and acinar cells atrophy, besides mononuclear cells infiltration, increase the interstitial spaces, and decrease the number of intralobular ducts. These findings, to a great extent, are mimic those observed with oxidative stress [9, 10], irradiation [11, 12], and age-related changes, which have reported in human sublingual glands [13], labial glands [14, 15] and salivary glands in mice [16].

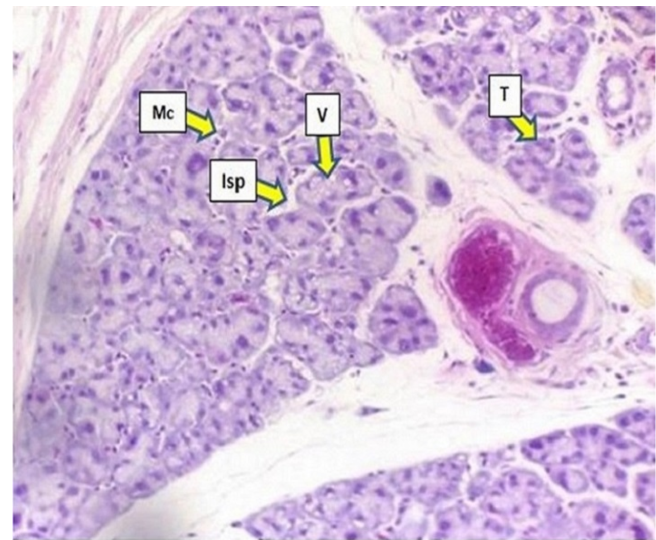

Figure 8. Parotid gland tissue of group B. Affected by cytoplasmic vacuolization of acini cells (V), many atrophied acini (T), reduced number of intralobular ducts, increase interstitial spaces (Isp) and mononuclear cells infiltration (Mc). H&E ×20.

Table 1. Effect of Desloratadine administration on biochemical parameters of rats.

Biochemical parameters	Control	Desloratadine (0.142mg/kg)	Desloratadine (0.245mg/kg)
MDA (mol/L)	3.4±0.33	5.35±0.35	6.7±0.34*
SOD (U/mL)	22.72±0.13	15.27±1.65	9.26±0.04†
GSH (mg/mg protein)	78.07±6.7	57.1±6.15	49±5.31*
CAT (K/ml)	6.969±0.763	3.197±0.151	1.9±0.313†
CK (U/L)	74.64±5.87	78.41±3.72	86.56±11.6*
LDH (U/L)	486.0±3.3	578.0±2.3‡	742.0±3‡
SA (mg/dl)	30±7 ‡	56.21±7.34	61±7.44

* P< 0.05
† P< 0.001
‡ P< 0.01

Each morphological alteration is a consequent event for molecular changes that affect many reactions. The defect in the nucleus is usually related to alterations affecting the total cell biological activity. On the other hand, changes in the cytoplasm occur when there is a defect in the cell's functional activity [17]. Oxidative stress is the most important causative factor for the induction of cellular apoptosis [18]. A decrease in parenchyma volume is accompanied by an increase in connective tissue, which exerts obstructive compression on the ducts and vessels, leading to functional and nutritional disturbance in acini and finally resulting in atrophy [14]. It could explain the decrease in the number of intralobular ducts found in treated groups of the current study. This finding confirms the observation of Scott J, that the striated ducts with advancing age have shown a reduction of about one-third of their initial value [19].

The present study findings confirmed the results of Tatar et al. [8], who have observed vacuolization of the acinar and

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ductal cells when they were evaluating the effect of antihistamine on submandibular gland tissues. Thus, cytoplasmic vacuolization might be an independent process planned to preserve cells against oxidative stress [9]. The results of the current study are consistent with the findings of Prestifilippos colleagues [20], in their experimental periodontitis study, they have concerned the associated morphological alteration in the submandibular gland. They have noticed many changes, as loss of some secretory granules and edema of periductal areas with a slightly more vacuolization of acinar cells cytoplasm. After treating with histamine, it prevented both morphological and functional changes in treated glands, that considerably have reduced the enhanced apoptosis, and partly reversed the reduced proliferation created by experimental periodontitis. The same findings were found in irradiated submandibular glands, which treated with histamine and prevented ionizing radiation-induced decreased proliferation and apoptosis [21].

Desloratadine has a role in the progressive deterioration associated with the process of acinar atrophy. It causes mitochondrial oxidative stress and the generation of intramitochondrial reactive oxygen species (ROS), which induces an imbalance of oxidants and antioxidants status [22]. The biochemical results have revealed a significant increase in serum MDA level (which is a biomarker for lipid peroxidation and oxidative stress) in desloratadine overdose groups. The finding was indicated enhanced lipopolysaccharides leading to tissue injury and failure of the antioxidant defense mechanisms to prevent the formation of excess free radicals [23].

Catalase and SOD are the two scavenging enzymes that enhance superoxide anion's breakdown by converting them into H_2O_2 and are catalytically converted by catalase into ground-state oxygen and hydroxyl radicals [24, 25]. The SOD and catalase levels were significantly decreased in the desloratadine treated rats, which might be due to the decrease in the capacity to eliminate increased H_2O_2, which is converted to highly reactive hydroxyl radical through Fenton reaction at the existence of enriched Fe^{2+}/Cu^{1+} [23]. These results were in agreement with a study done by Tatar et al. [8].

Glutathione is a predominant endogenous antioxidant and is used as a cofactor to remove hydrogen peroxide and lipoperoxides by the glutathione peroxidase during which GSH is converted into oxidized form of glutathione (GSSG). There was a marked reduction in non-enzymatic antioxidant (GSH) level in the desloratadine treated groups compared to the control. Depletion of GSH in tissue indicates its utilization and thus increased tissue susceptibility to oxidative stress [26, 27]. Normal healthy cells contain plenty of LDH and CK enzymes. Both of them are cytosolic enzymes used as a measure or marker of tissue damage [28]. The current study was showed that LDH and CK are increased after administration of desloratadine, which could be due to induction ROS levels and inhibition of antioxidant enzyme activities [29]. Sialic acid (SA) is the generic term given to a family of acetylated neuraminic acid derivatives. It occurs mainly at terminal positions of glycoprotein and glycolipid oligosaccharide side-chains. Treatment rats with desloratadine are induced a significant increase in SA. Such alteration may be due to the body's defense mechanisms and the releasing of sialic acid from the damaged cell into the bloodstream [30].

The results were suggested that premedication with this drug may contribute to the functional deteriorations of salivary glands that significantly interfere with the quality of life. However, the adverse effects of desloratadine could be acute or reversible. It is still preliminary findings; further research in this direction is indicated to analyze the significance of these findings by using additional investigating tool as histo or immunohistochemical assessment, and obtain more in-depth insights into desloratadine effects in parotid and other salivary glands.

Also, this experimental study has some limitations. It depends on subjective morphological evaluation, which is less accurate than the objective/automated interpretation of the image analysis system. The lack of measurement of saliva volume in this study might be considered another significant limitation.

CONCLUSION

The undesirable histologic changes observed in parotid gland tissues were associated with alteration in serum levels of oxidative stress and antioxidant activity, following the oral administration of desloratadine, in a dose-depending manner.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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