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# AGING AFFECTS MORPHOLOGY BUT NOT STIMULATED SECRETION OF SALIVA IN RATS

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**ABSTRACT**

***Background:*** **The role of aging on the salivary gland function still remains controversial and inconclusive. This study was undertaken to determine the effects of aging on the morphology and secretion of salivary glands using male Wistar rats.**

***Method:*** **There were three age groups; group A (3 months old; n = 8), group B (6 months old; n = 8), and group C (9 months old; n = 8). Body weights, salivary gland weights, salivary flow rates, pH and salivary levels of sodium, potassium, calcium, chloride, bicarbonate, phosphate and total protein were measured and compared. Hematoxylin-eosin stained histological slides of the salivary glands were assessed for morphological changes.**

***Results:*** **Body weights increased with age while mean parotid gland weight was significantly higher in group B than in groups A and C. Mean salivary flow rate was significantly higher in group B and C than in group A, and mean salivary pH was significantly higher in group B and C than group A. Analysis of salivary electrolytes and total protein showed that mean levels of sodium, potassium and bicarbonate increased with age significantly while mean levels of calcium, chloride, phosphate and total protein did not show significant change among the groups.**

***Conclusion:*** **These findings showed that varying changes were observed in the morphology of salivary glands of aging rats without impaired function.**

**Keywords**: Aging, Salivary Glands, Salivary flow rates, Salivary electrolytes, Salivary total protein

## INTRODUCTION

Saliva is a watery fluid secreted by the salivary glands. Salivary fluid is an exocrine secretion consisting of approximately 99% water and a variety of electrolytes and proteins. The components interact and are responsible for the various functions attributed to saliva[1]. The physiological functions of saliva include initial food digestion, taste perception, maintenance of tooth integrity, oral clearance, lubrication, and protection of the oral cavity against infections. At present, saliva represents an increasingly useful auxiliary means of diagnosis and the salivary glands of rats and other rodents have been used extensively over the past years as models for the study of physiological and biochemical processes associated with secretion of saliva[2,3]. An interesting and useful characteristic of rat salivary glands is that they are essentially undeveloped at birth but undergo progressive development into mature organs during the first few weeks of life[4]. This also makes them useful models for the study of the developmental aspects of the secretory functions of the salivary glands.

Aging is a normal physiological phenomenon that affects almost all organs including the salivary glands[5]. Age related changes in the salivary glands have been documented in humans[6-8] and animals[9-11] with varying results. However, it has not been fully determined how aging influences the biochemical composition of saliva. Some functional studies on healthy individuals showed that aging does not diminish the ability of salivary glands to produce saliva[12,13]. On the other hand, some studies reported that there might be a progressive, but minor reduction, in flow of saliva from the glands due to aging[14,15]. Furthermore, the effects of aging on biochemical composition of saliva are not clear; therefore, the aim of this study was to evaluate age-related changes in the salivary glands histopathology and secretion in male Wister rats, and to add to available information for preclinical experimental research on age-related changes in the composition of saliva.

## METHODS

*Experimental Animals*

Twenty four five weeks old 24 male Wistar rats were purchased from the Animal Research House of the College of Medicine, University of Ibadan, Ibadan, Nigeria for the study. The animals were housed in a temperature, humidity, and light controlled environment on a standard diet with free access to water. They were randomly allocated to three groups (groups A, B and C) and used for the experiment at different ages. Group A (the young group, 3 months old, n = 8), group B (the adult group, 6 months old, n = 8) and group C (the old group, 9 months old, n = 8) had their evaluations at 3, 6 and 9 months old respectively. All experiments were carried out in accordance with The Code of Ethics of the EU Directive 2010/63/EU for animal experiments.

*Measurements of rat body weight, salivary gland weight, salivary flow rate and pH*

The rats were weighed using weighing balance (Citizen Scales, Mubai, India) and anesthetized with an intraperitoneal (i.p.) injection of ketamine (75 mg/kg). Each rat was positioned laterally after stimulation with pilocarpine (10 mg/kg, i.p.). Saliva was collected by free flow into sterile plain tubes for a period of 10 minutes for each animal. To reduce the effects of diurnal variation, saliva was collected between 8 and 10 am for all the groups. Salivary flow rate (ml/min) was calculated as total saliva volume (ml) divided by the collection time (min) while pH was determined using a digital pH meter.

After saliva collection, mid line incisions were made on the necks of the rats to expose the submandibular glands. Skins were reflected and the right and left submandibular glands located at both sides of the trachea were carefully removed and placed in 10% formosaline. Fatty lymph nodes and sublingual glands were carefully separated and removed from the submandibular glands. Laterally, another incision was made in the pre auricular area and the parotid glands were exposed and removed. Because of the light weights of the glands, they were carefully weighed using a semi-micro analytical balance (Citizen Scales, Mubai, India).

*Morphological analysis of tissues*

The glands were immediately placed in 10% formalsaline, embedded in paraffin, sectioned at 4 mm, and stained with haematoxylin and eosin (H–E) using standard tissue processing protocols.

*Biochemical analysis of saliva*

The saliva samples collected were stored at -20$^0$C until laboratory analysis. The samples were defrosted at room temperature and then centrifuged at 6000 rpm for 10 minutes before being used in order to remove extrinsic contamination elements. For the determination of salivary ions, the sample was diluted at 1/100 and sodium, potassium and calcium concentrations in mmol/L were determined using flame emission spectrophotometry. Concentrations of chloride and bicarbonate in mmol/L were determined by Schales method using mercuric nitrate. Total protein concentration in g/dl was determined by colorimetry with the use of Helios spectrophotometer (Thermo Scientific, Waltham, USA) by reading samples at 720nm. Bovine serum albumin was used for calibration.

*Statistical analysis*

The main outcome variables were mean salivary gland weights, flow rates, pH, and mean salivary levels of sodium, potassium, calcium, chloride, bicarbonate, phosphate and total protein. Data were expressed as mean ± SD. One way ANOVA model and Turkey's post hoc tests were employed in comparing the values among the groups. Results with p-value less than 0.05 were considered significant.

## RESULTS

*Body weights and salivary gland weights*

Group C (9 months old) had the highest mean body weight (303.5 ± 10.06 g) followed by group B (228.75 ± 14.39 g) and group A had the least mean weight (152.3 ± 5 g). There was significant increase in body weights with age among the groups (p = 0.00, F =

**Table 1:** Weights of salivary glands (g) among the groups

| | Group A | Group B | Group C | P value |
|---|---|---|---|---|
| Right Submandibular | 0.35 ± 0.08 | 0.43 ± 0.04 | 0.47 ± 0.10 | 0.6 |
| Left submandibular | 0.36 ± 0.08 | 0.48 ± 0.04 | 0.39 ± 0.01 | 0.5 |
| Right Parotid | 0.08 ± 0.01$^a$ | 0.12 ± 0.02$^b$ | 0.06 ± 0.01$^a$ | 0.01* |
| Left Parotid | 0.09 ± 0.02$^a$ | 0.12 ± 0.01$^b$ | 0.08 ± 0.01$^a$ | 0.04* |

** mean values with the same superscript are not significantly different at 0.05 level.*

**Table 2:** Salivary flow rate, pH and total protein concentrations among the groups

| | Group A | Group B | Group C | P value |
|---|---|---|---|---|
| Flow rate (mls/min) | $0.12 \pm 0.01^{b}$ | $0.19 \pm 0.01^{a}$ | $0.21 \pm 0.02^{a}$ | 0.01* |
| pH | $8.35 \pm 0.14^{b}$ | $9.15 \pm 0.02^{a}$ | $8.93 \pm 0.03^{a}$ | 0.001* |
| Total protein (mg/dl) | $0.35 \pm 0.06$ | $0.64 \pm 0.18$ | $0.39 \pm 0.05$ | 0.17 |

** mean values with the same superscript are not significantly different at 0.05 level.*

**Table 3:** Levels of salivary electrolytes and total protein among the groups

| | Group A | Group B | Group C | P value |
|---|---|---|---|---|
| Sodium (mmol/L) | $39.63 \pm 2.65^{a}$ | $56.63 \pm 4.84^{b}$ | $75 \pm 11.02^{c}$ | 0.01* |
| Potassium (mmol/L) | $24 \pm 2.23^{a}$ | $42.63 \pm 3.04^{b}$ | $46.94 \pm 7.4^{bc}$ | 0.01* |
| Calcium (mg/dl) | $4 \pm 0.29$ | $5.03 \pm 0.67$ | $5.6 \pm 0.48$ | 0.1 |
| Chloride (mmol/L) | $31.38 \pm 2.16$ | $28.13 \pm 4.08$ | $44.25 \pm 9.4$ | 0.16 |
| Phosphate (mmol/L) | $0.66 \pm 0.14$ | $0.54 \pm 0.11$ | $0.83 \pm 0.08$ | 0.23 |
| Bicarbonate (mmol/L) | $34.5 \pm 2.93^{a}$ | $60.63 \pm 3.79^{b}$ | $64.34 \pm 10.84^{bc}$ | 0.01* |

** mean values with the same superscript are not significantly different at 0.05 level.*

51.54). The weights of the right and left parotid glands were significantly higher in group B than groups A and C ($p = 0.01$, $F = 6.27$; $p = 0.04$, $F = 4.01$ respectively), while the weights of the submandibular glands did not show any significant difference as shown in Table 1.

*Salivary flow rate, pH and total protein*

The mean salivary flow rates of the three groups were $0.12 \pm 0.01$ mls/min; $0.19 \pm 0.01$ mls/min and $0.21 \pm 0.02$ mls/min respectively. The mean salivary flow rates in groups B and C were significantly higher than group A ($P = 0.01$). The mean salivary pHs of the three groups were $8.53 \pm 0.14$; $9.15 \pm 0.02$ and $8.93 \pm 0.03$ respectively. The mean pH was significantly higher in groups B and C than group A ($P = 0.001$). The mean values of salivary total protein concentrations did not show any significant change among the groups (Table 2).

*Salivary levels of electrolytes*

The mean levels of sodium, potassium and bicarbonate increased significantly with age while the mean levels of calcium, chloride and phosphate did not show significant change among the groups (Table 3).

*Salivary gland morphology*

Histological analysis of the H–E stained salivary glands in groups A and B showed the normal lobular structure

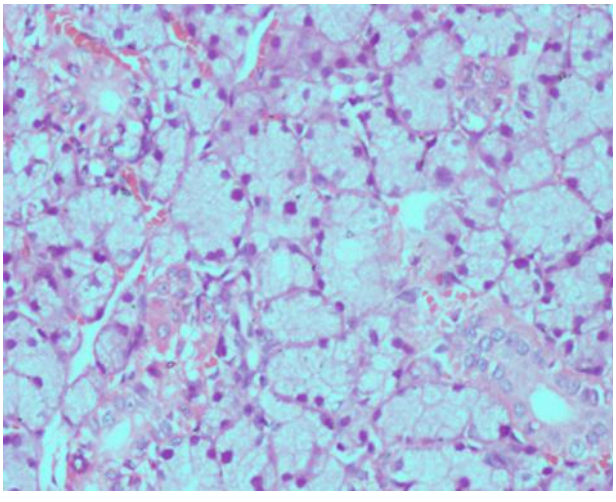

**Figure 1:** Photomicrograph showing normal submandibular gland tissue in group A (H & E X400)

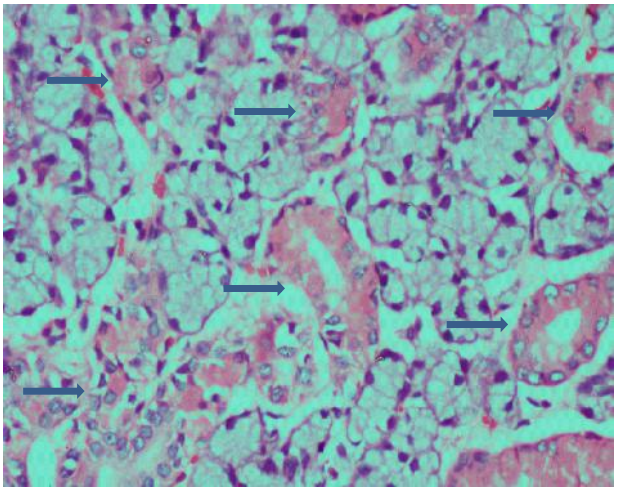

**Figure 2:** Photomicrograph showing submandibular gland tissue in group B with numerous and more prominent striated ducts (H & E X400)

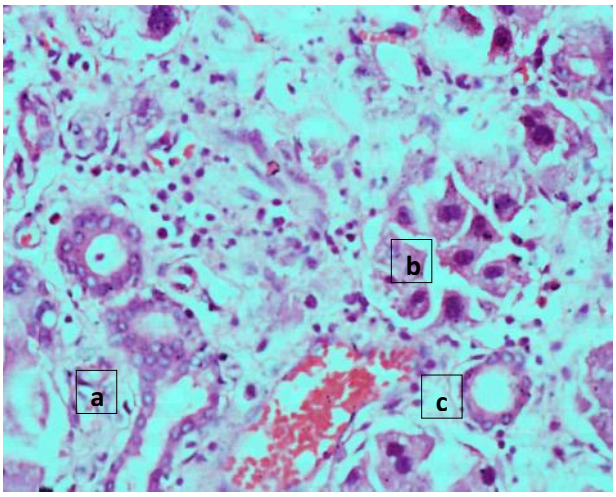


**Figure 3:** Photomicrograph showing submandibular gland tissue in group C with acinar atrophy (a), pleomorpism (b) and periductal fibrosis (c) (H & E X400)

with densely packed acini and a well-developed excretory duct system (Figure 1). There were more prominent striated ducts in the submandibular glands of rats in group B than those in group A (Figure 2). On the other hand, the glands in group C exhibited acinar cell atrophy with pleomorphism and fibrosis of the secretory ducts (Figure 3).

## DISCUSSION

The main findings of this study were increased flow rate, pH, and levels of sodium, potassium and bicarbonate in salivary secretion as well as increased weight of parotid, acinar atrophy, increased periductal fibrosis and reduced mucin content of the salivary glands of aging rats.

Regarding salivary flow rates the oldest group of animals had salivary flow rates greater than the other two groups. Similar to our findings, some studies[16,17] reported that the salivary volumes and flow rates of male and female rats in response to pilocarpine increased progressively with increasing age. The relationship between age and salivary flow rates is controversial, for instance some studies[18,19] have reported a progressive decrease in salivary flow; some studies[20,21] documented progressive increase while others[22,23] reported that salivary secretion and composition are largely age independent in human. In the present study, flow rates were significantly higher among the oldest compared with the younger age groups, which suggests that the aging process is positively related to salivary flow rate in rats. In humans, a functional study among healthy individuals reported that aging itself does not necessarily lead to diminished glandular capacity to produce saliva[15] but rather, reduced salivary output in the elderly results from their attendant systemic diseases and drug use. In contrary, Navazesh *et al.*[24] found that the total unstimulated salivary flow was insignificantly lower in healthy individuals between the ages of 65 and 83 years, in comparison to individuals between ages 18 and 35 years. Similarly, Percival *et al.*[25] reported that the total unstimulated salivary flow is inversely related to age being significantly reduced in healthy elderly persons, aged 80 years and above. However, no age related reduction was found in stimulated salivary flow from the parotid gland in the same individuals. This suggests that aging does not impair salivary gland ability to respond to stimulus; however the reduction in the unstimulated salivary flow could be attributed to the various diseases associated with aging and their drug therapies. In addition, Lima *et al.*[26] demonstrated that elderly persons presented with very low daily saliva production which appeared to be more related to systemic diseases and continuous use of medications than aging.

Salivary pH was higher in the older groups which may be attributable to the increased flow rate in these groups. In addition, bicarbonate level was increased with age which could have also contributed to the higher pH levels in the older group.

Similar to the findings of this study, histological analyses of salivary glands have demonstrated that with advancing age in mice, the parenchyma of the salivary glands was replaced by adipose and fibrovascular tissue with reduction in the volume of the acini[10]. In agreement with previous studies, we found that the salivary glands in group C showed acinar cell atrophy, and higher periductal fibrosis levels than those in groups A and B. Although our results support the view that the aging process adversely affects acinar cells expressed as loss of parotid gland weight, acinar cell atrophy, and higher levels of periductal fibrosis, the flow rates were increased significantly with increased age. This may suggest that the morphological changes observed in the oldest group did not affect the secretory function of the glands.

Few reports have been conducted on the biochemical composition of saliva in older persons and most studies have reported that of parotid secretion. Heft and Baum[7] reported changes in sodium but not potassium concentrations of parotid saliva among different age groups. Similar to our findings, animal[17] and human[18] studies have reported no change in the salivary protein levels among different age groups. Sevon *et al.*[27] reported that salivary calcium and phosphate concentrations increased with age in adult women with peak values at around 50-54 years of age whereas, age had no effect on salivary flow-rate,

sodium, potassium or protein concentrations. These variations can be attributed to different study groups (human and animals) and variability in methodology. In this study, biochemical and morphological assessment of age-related changes in salivary secretion and salivary gland function was conducted using male Wister rats. Rats are commonly used animals in research, and are popular for translational research studies because of their similarity to humans with respect to anatomy, physiology and genetics as well as their convenience. Rats have accelerated lifespan (one rat day equals about 30 human days), which minimizes the costs, space, and time required to perform research, especially research studies on aging.[16]

A number of methodological issues limit generalizations of our findings. First, our histologic study was limited to qualitative morphologic assessment of the submandibular and the parotid glands using H & E stain. Molecular studies using other techniques may produce better insight to the pathophysiology of age related changes in salivary glands morphology and function in rats. Secondly, our study involved only three groups, and further studies are needed to investigate the effects of aging on older groups of animals.

In conclusion, aging is associated with histopathological changes in the salivary glands without diminishing changes in salivary secretion after stimulation and biochemical composition in rats. Further studies are needed to understand the pathophysiology of unhindered function of aging salivary glands despite morphological changes both in human and animals.

**Conflict of interest statement**: The authors declare no conflict of interest.

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