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Effect of Losartan on the mucous glands of the trachea in the albino rat- a histomorphometric study

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ABSTRACT: *Losartan, an an AT_1 receptor antagonist used as an antihypertensive, is known to produce nonproductive cough, bronchoconstriction[1-4] and angioedema[5,6] in some individuals. The structural/histopathological cause has not been ascertained. It is required to know the exact mechanism of these side effects in order to improve the therapeutic effects of the drug. This fact prompted the present study.*

Inbred adult Wistar albino rats weighing 150-200 grams were used and were divided into two groups- Experimental group (Group I) and Control group (Group II) comprising of 15 animals each. The rats in group I were given the normal therapeutic dose, losartan potassium 50 mg/kg of body weight dissolved in water, orally through a thin tube daily for 15 days. Group II was given the same amount of vehicle. The animals were sacrificed on the 16th day. They were perfused by injecting 10% formal saline under phenobarbitone sodium anesthesia.

The trachea was cut into three equal parts; the upper, middle and lower and embedded in paraffin wax. Serial sections of each part were stained with Haematoxylin and Eosin, Masson's Trichome, PAS, combined Alcian blue and PAS and studied under the light microscope. The Goblet cells in the mucosal lining of the trachea increased statistically after losartan and the number of glands in the submucosa also showed a similar increase in number and amount of secretions.

Losartan should be used with caution and the patients on this therapy should be assessed for increase in postnasal secretions and resulting cough.

Keywords: *Trachea, goblet cells, losartan*

I. INTRODUCTION

Angiotensin-converting enzyme (ACE) inhibitors play a significant role in the treatment of hypertension,[7] congestive heart failure[7] and acute myocardial infarction.[7] Although these agents are well tolerated and have a low incidence of serious adverse reactions, a persistent, nonproductive cough is a well-documented side effect of this drug class [7] ACE inhibitor–induced cough is thought to result from inhibition of bradykinin degradation by ACE inhibitors, with accompanying effects of bradykinin on production of prostaglandins, leukotrienes, and substance P. [7]

Losartan is an antihypertensive, [8] nonpeptide an AT_1 receptor antagonist with high affinity and selectivity for the AT 1 receptor.[8, 9] It has been reported to produce bronchoconstriction [1-4] and angioedema[5, 6] in some patients suggesting that AT_1 receptor antagonists may not be entirely free of ACE inhibitor-related side effects. The reports are based on clinical findings and no histological studies are available to substantiate these findings. Most studies on drug induced cough are focused on the changes in the smaller airways of lung, what happens in the larger airways like the trachea in these conditions has not been given much attention.

Millar[10] reported that activation of the renin–angiotensin system with elevation of plasma renin and angiotensin II levels is observed in asthmatic patients during acute severe attacks,

Nally [11] studied the potentiating effect of angiotensin II on endothelin-1-induced contraction of bovine bronchial smooth muscle. These observations suggest that angiotensin II could be a putative mediator in asthma.

Kanazawa et al[12,13] have demonstrated that type 1 angiotensin II (AT1) receptors are involved in angiotensin II–induced bronchoconstriction in guinea pigs and peptide leukotriene (LT) production in guinea pig airways.

Myou et al [14] examined the effect of losartan on bronchial hyper responsiveness of peripheral and central airways in patients with asthma showed that bronchial hyper responsiveness to methacholine, in terms of PC35-PEF40, but not PC20-FEV1, was attenuated by losartan.

Many patients are known to complain of postnasal secretions when on losartan treatment. This could be a result of increased secretions in the upper respiratory tract. The present study was planned to evaluate the short term effect of therapeutic doses of losartan on the tracheal glands in the rat.

II. METHODOLOGY

Inbred adult Wistar albino rats weighing 150-200 grams were used in this study. The study was carried out after obtaining clearance from the ethical committee for animal experimentation of the institution.

Thirty adult Wistar albino rats weighing 150-200gms of either sex were randomly selected for the study. The animals were divided into two groups, Experimental group (Group I) & Control group (Group II) comprising of 15 animals each. The animals in the experimental group were administered losartan potassium 50mg/kg of body weight dissolved in water through a polythene tubing attached to a dropper once daily for 15 days. The control group animals were given only water/vehicle with the dropper for 15 days. The animals were sacrificed on the 16th day by perfusion with formal saline under anaesthesia with 1ml phenobarbitone sodium 60mg/ml.

The trachea was cut into three equal parts; the upper, middle and lower and embedded in paraffin wax. Every fifth slide of the serial sections of each part were stained with Haematoxylin and Eosin, Masson's Trichome, PAS and combined Alcian blue and PAS and studied under the light microscope and Image Pro Express Analyser for the quantitative and qualitative observations.

The number of goblet cells in the mucous membrane and cross sections of the glands in the submucosa were counted in each section.. The findings of the experimental and control group were compared and statistically analyzed using student t-test to ascertain the changes produced by losartan on the glands.

III. RESULTS

The number of goblet cells were significantly increased ($p < 0.001$) in the experimental animals in all the three parts. They were 193.45± 9.46 in the upper part, 165± 8.1009 in the middle part and 146± 15.615 in the lower part as compared to control where the cells were 123.10±19.775, 90.1±21.84 and 76.517±9.44.in the upper, middle, & lower parts respectively (Table1, Figure 1).

There was a significant increase ($p < 0.001$) in total number of glands of the submucosa in the experimental group in the upper part (113.57± 40.987) and middle (17.866±8,116) part as compared to the control group upper (33.58± 14.097) and middle (0.0000± 0.0000) part. There were no glands in the submucosa of lower part of trachea in both the groups (Table 2, Figure 2).

The secretion of the goblet cells increased remarkably and the epithelial membrane showed papilla like appearance at some sites and the secretions could be seen discharging and collecting on the surface in the experimental group (Fig. 3). Many nuclei were entangled in the secretions suggestive of a holocrine discharge. Some round cells with magenta cytoplasm appeared to be migrating from the lamina propria and entering between the columnar cells and then discharging into the lumen (Fig 3). The enlarged glands with PAS positive material was markedly increased in the upper part of the trachea of the experimental group (Fig 4).

In the lower and middle parts of the trachea the goblet cells and the glands were much less than that in the upper part of the trachea.

IV. DISCUSSION

In the present study, after giving losartan for 15 days, the goblet cells and glands in the submucosa of the trachea had increased significantly in number. Reid [15] demonstrated hypertrophy of the goblet cells and the mucous glands, in the bronchial epithelium of laboratory animals by subjecting it to repeated and prolonged exposure to an irritant mainly the sulphur dioxide. They suggested that the increase in these secretary glands is physiological way of washing away the irritants from the tracheobronchial tree. This increase was similar to that seen in patient suffering from chronic bronchitis**[16].** There is a possibility of multiplication of the cells of this category but no dividing cells were observed in this layer. It is required to confirm the source of the increase in number of the goblet cells by further auto radiographic studies.

Many large round cells were seen to be migrating from the lamina propria through the epithelium into the lumen (Fig 3). It appears that the tracheal secretions comprise of serous, mucous and cellular components. The secretions were seen forming a thick layer on the surface of the mucosa where many cells were seen entangled in the secretions. This phenomenon would definitely damage the cilia and their function of mucociliary clearance (Fig 3) .The appearance of lymphoid cells in the mucous membrane were more in most of the control rat trachea than in the experimental group (Fig 5). There is a possibility that the mucous layer on the surface prevents the exposure of foreign material of the inhaled air to the tracheal surface resulting in decrease of lymphocytic or inflammatory cells in the experimental group. Lamb [17] also demonstrated increase in the number of goblet cells and histochemical changes in mucus in rat bronchial epithelium subsequent to the exposure to sulphur dioxide. Jones at el [18] reported an increase in goblet cell number in the mucosal lining after tobacco exposure.

The above mentioned studies clearly show that losartan does act on the lung. The bronchoconstriction produced by losartan in some cases definitely show that losartan affects the respiratory tract. The increase in the mucous secreting glands seen in this study indicates that there is some irritative phenomenon in this organ which

nature is trying to relieve by hyper secretion of mucous. So losartan should be cautiously used in patients with severe asthma and bronchitis and should be periodically assessed for increased mucous production.

The mechanism of action of losartan is not clear from the present study and needs to be further investigated.

V. CONCLUSION

Losartan has an irritant effect on the trachea which leads to an increase in goblet cells and gland in the trachea. It should be prescribed with caution.

Table 1: Comparison of number of goblet cells between control and experimental group.

Density	Control		Experimental		P value (f test)	Significance Tukey's
	Mean	**S.D. ±**	**Mean**	**S.D. ±**		
Upper	**123.10**	**19.775**	**193.45**	**9.496**	**<0.001**	**Significant**
Middle	**90.10**	**21.8425**	**165.467**	**8.1009**	**<0.001**	**Significant**
Lower	**76.517**	**9.4479**	**146.033**	**15.6150**	**<0.001**	**Significant**
P value (f test)	**<0.001**		**<0.001**			
Significance (Tukey's)	**Significant**		**Significant**			

Table 2: Comparison of number of glands and ducts between control and experimental group.

Glands	Control		Experimental		P value (f test)	Significance (Tukey's)
	Mean	S.D. ±	Mean	S.D. ±		
Upper	33.58	14.097	113.57	40.987	<0.001	**Significant**
Middle	0.0000	0.00000	17.8667	8.11682	<0.001	**Significant**
Lower	0.0000	0.0000	0.00	0.0000	-	
P value (f test)	<0.001		<0.001			
Significance Tukey's	**Significant**		**Significant**			

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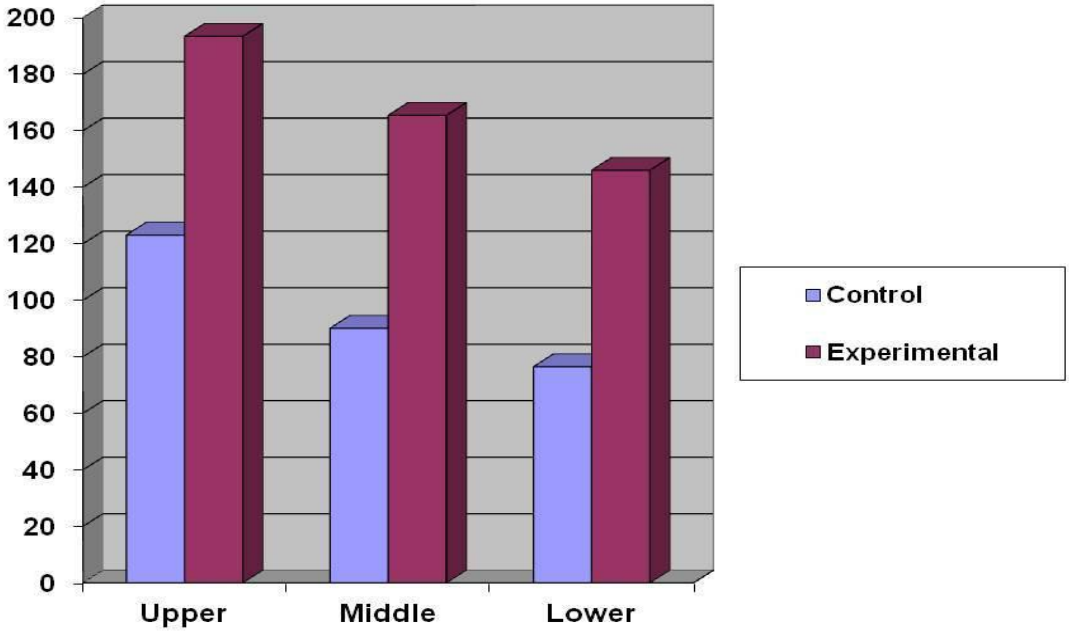

Figure 1: Comparison of number of goblet cells between control and experimental group

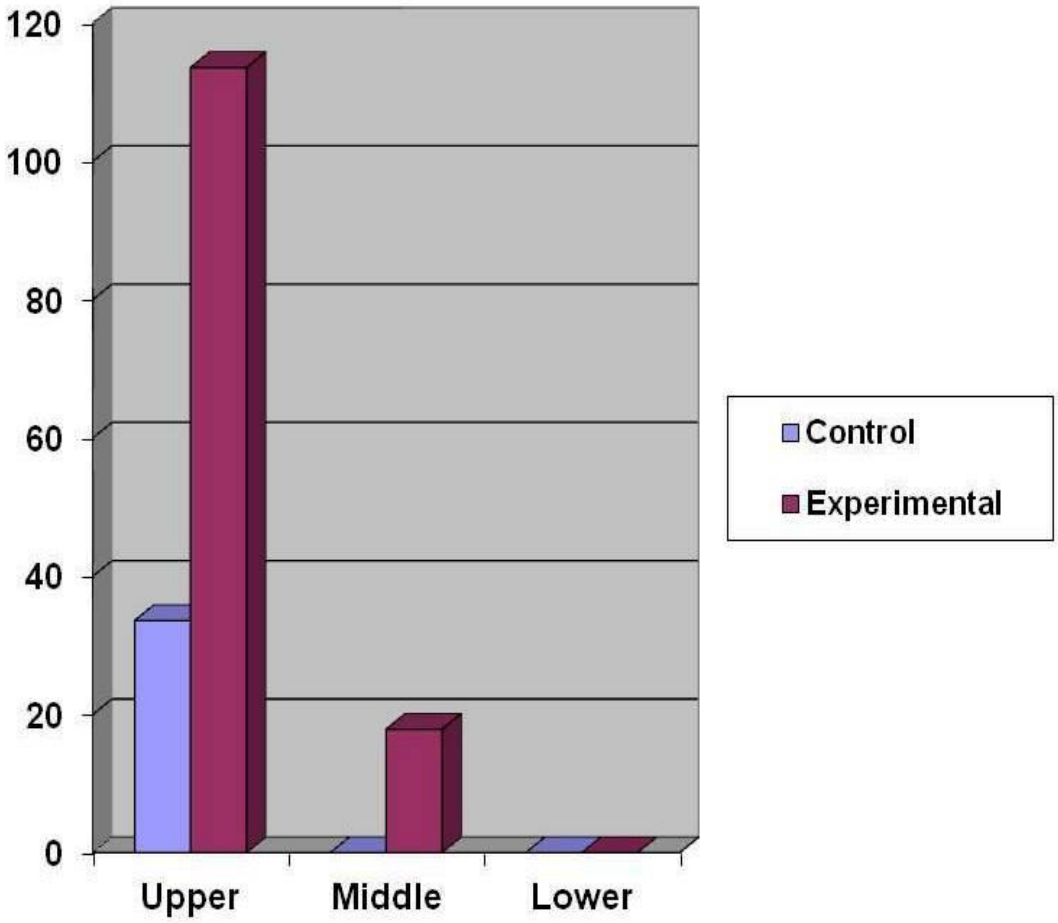

Figure 2: Comparison of number of glands and ducts between control and experimental group.

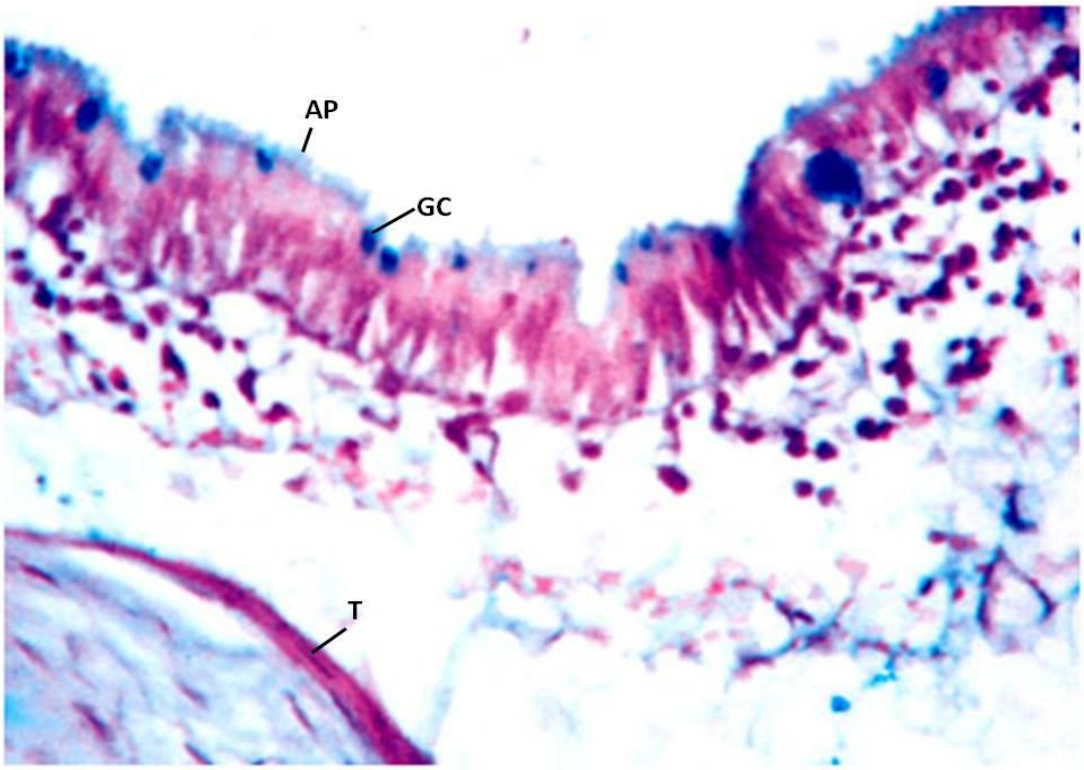

Figure 3: Photomicrograph of the upper part of trachea from the experimental group showing epithelial cells with interspersed increased Goblet Cells (GC). Alcian blue and PAS stain (400 X). Acid mucosubstance (AP), Trachealis muscle (T).

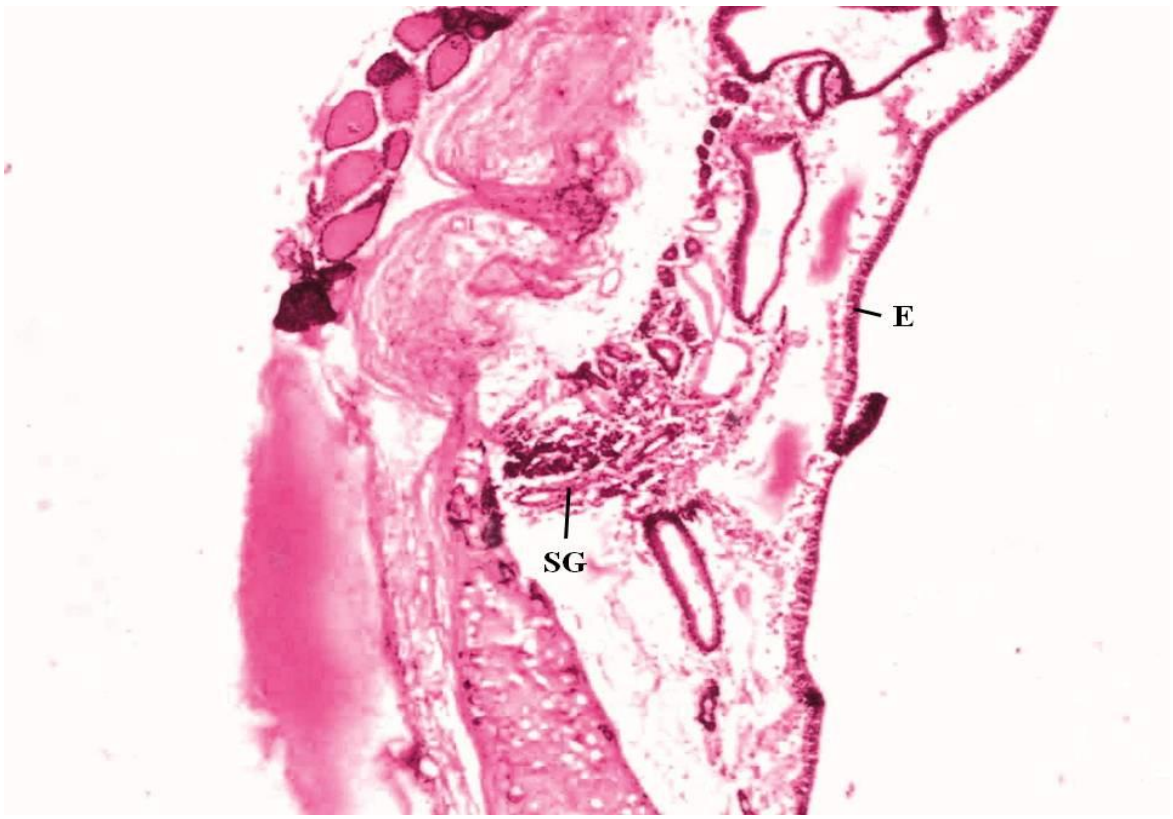

Figure 4: Photomicrograph of the upper part of the trachea from the experimental group showing the collection of glands in the submucosa(SG) extending into the lamina propia. PAS stain (200X). Epithelium (E)

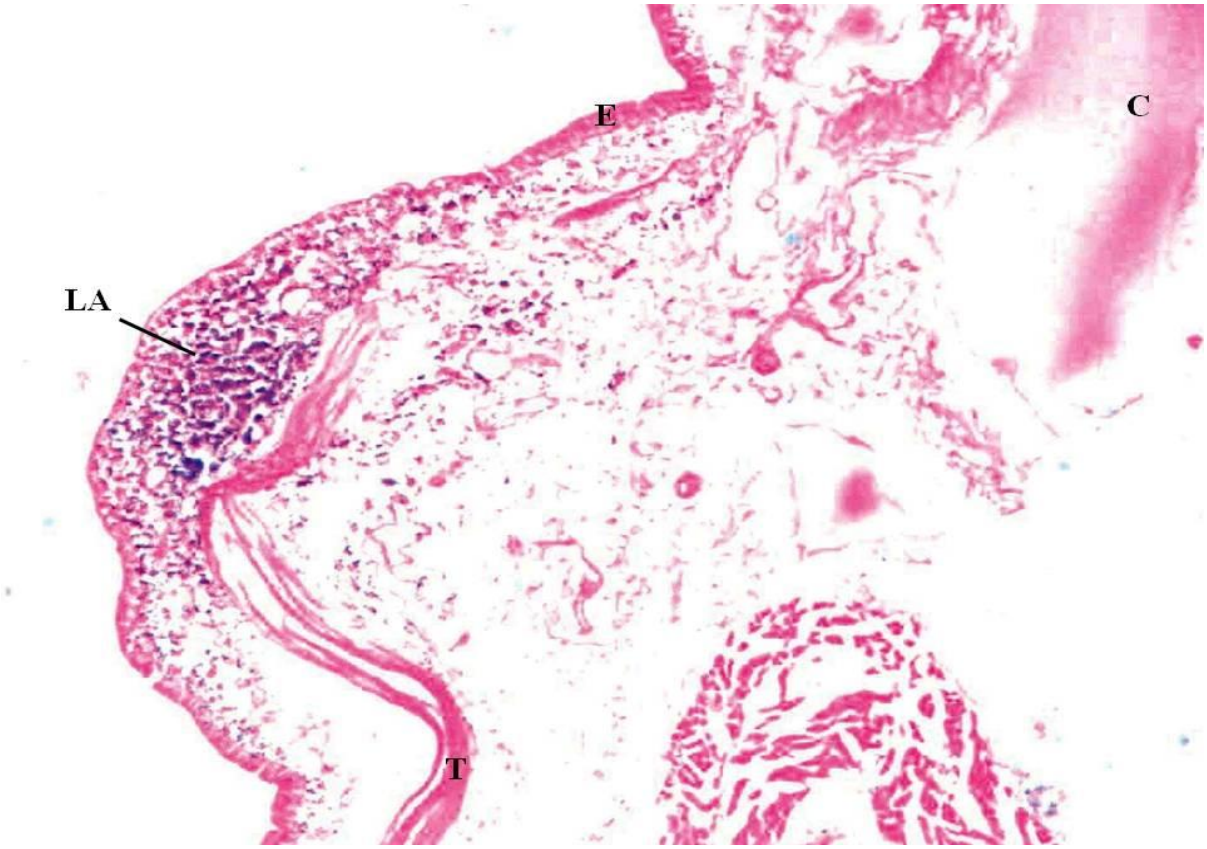

Figure 5: Photomicrograph of the transverse section of trachea showing collection of lymphoid tissue (LA) from the control group in the posterior part of the trachea. Trachealis (T), Cartilage (C). Hematoxylin and Eosin stain (200X magnification)