ABSTRACT
In this study, the effects of removal of the submandibular gland in Wistar albino rats has been examined by transmission electron microscope in terms of the effects on the susceptibility of gastric mucosa and mucus to the damaging actions of ethanol. The adult female Wistar albino rats were divided into four groups. The animals served as Control(C), Sialoadenectomy(SX), Ethanol(E) treated and Sialoadenectomy + Ethanol(SX+E) treated groups (n: 7). Mucus level of the gastric mucosal barrier were measured using Corne's spectrofotometric method. The difference of mucus mean levels between the groups was evaluated statistically by Kruskal-Wallis test. A significant difference was found between the groups (p<0.05). The importance of difference among the mucus levels was compared by Dunnet test with multiple comparisons. According to comparisons statistically a significant difference was found between the C-E and SX-E groups (p<0.05). Electron microscopic evaluation indicated that the stomach mucosa epithelium in control group showed a normal appearance while changes were observed in all experimental groups. However, severe basal epithelium of intercellular adhesions was destroyed, apical mucus granule was decreased, and parietal cell of intrasitoplasmic canaliculi-microvillus was increased in sialoadenectomy and ethanol treated groups. These data showed that sialoadenectomy leads to an increase in mucosal inflammation, decrease in mucus level shown electron microscope in animals subjected to sialoadenectomy and ethanol treated.

Introduction
Gastrointestinal mucosa is one of the most rapidly changing tissues in the body and the balance between cell regeneration and cell loss may lead to mucosal lesion and ulcerations. Mucosal growth is under the influence of various growth factors, among which epidermal growth factor (EGF) (13, 16).

Epidermal Growth Factor (EGF) is a polypeptide containing 53 amino acids (19). That is secreted into the gastrointestinal lumen by salivary glands, Brunner's glands of the duodenum, and pancreas (2, 15). Several reports have demonstrated that EGF protects the gastric mucosa against ethanol-induced injury. Removal of salivary glands (Sialoadenectomy) in experimental animals was associated both with the appearance of gastric ulcers in response to ethanol (3, 22, 5, 12) and an increase in the susceptibility of stomach to the stress, NSAIs, or bile salt induced mucosal damage (21, 15, 6).

The mechanisms by which EGF exerts protective actions on the mucosa are not
fully understood but certainly involve several factors: inhibition of gastric acid secretion (11), changes in mucosal blood flow (8), mucosal biosynthesis protective agent Prostaglandin E₂ (PGE₂) (20) and secretion of mucus which covering the gastrointestinal mucosa in the functional barrier against acid is well established (10, 7, 14). Salivary EGF may be the major source of gastrointestinal system (GIS) EGF, and it may be play a role in maintenance of GIS mucosa integrity in SX rats. (1, 17).

The present study was designed to investigate the possible role of SX in mucosal damage and ultrastructural changes in gastric mucosa after administration of a corrosive agent ethanol.

**Materials and Methods**

Twenty-eight adult Wistar albino female rats were obtained from University of Dicle, Medical Science and Application Center (DÜSAM). Twenty-eight adult Wistar albino female rats were maintained and fed standard pellet food ad libitum during the study. For adaptation, each rat was placed in an individual cage in the experimental period. All animals received human care according to criteria outlined in the ‘Guide for the Care And Use of Laboratory Animals’ prepared by the National Academy Of Sciences And Published by the National Institutes of Health. The ambient temperature (22°C) and relative humidity (45%) were maintained throughout the experiments. The rats were divided randomly into four groups:

**Group I:** The animals served as control (n: 7).

**Group II:** Under Ketamine HCl (50mg/kg) and Xylazine (10 mg/kg) anaesthesia the sublingual-submandibular complexes were removed after the ducts were ligated. (Sialoadenectomy: SX) (n: 7).

**Group III:** Ethanol treated (n: 7), the third group of rats were anesthetized an incision of skin on neck was made as for sialoadenectomy but submandibular salivary glands were left intact.

**Group IV:** Sialoadenectomy+Ethanol treated (n: 7).

After 30 days of recovery 1ml/300g body weight dose absolute ethanol, (Aldrich-99.5%) administered via oro-gastric feeding to group III and IV. One day after ethanol administration, the rats were sacrificed under Ketamine HCl anaesthesia.

**The Measurement of Gastric Mucus**

The measurement of gastric barrier mucus level was performed using Corne’s method (4). The stomach was dissected out and opened along the greater curvature, and gently cleansed with 0.9% cold saline. A portion of the stomach were excised and immersed for 2 h in 0.1 % Alcian Blue in a 0.16 mol/L sucrose solution buffered with 0.05 mol/l natrium acetate (pH adjusted to 5.8 with HCl). The unbound dye was removed by two subsequent washing of 15 and 45 min in 0.25mol/L sucrose; the mucus-bound dye was eluted by immersing the stomach in a 0.5 mol/L MgCl₂ solution for 2 h. The solution thus was shaken with diethyl ether and the optical density of aqueous phase was measured at 605 nm in a spectrophotometer (Shimadzu-1601). The quantity of Alcian Blue, extracted per g of wet stomach tissue, was then calculated from standard curves.

**Histological Examination**

Half of stomachs were pinned out on dental wax to their length and placed immediately in a 2.5% phosphate-buffered glutaraldehyde solution at pH 7.4 for 4 hours. Tissue pieces were chosen at random, then split along the long axis into 2 or 3 pieces and placed in fresh fixative for a further 40 min, post fixation was performed in 2% osmium tetroxide, and washed in three changes of phosphate buffer (pH:7,4) and dehydrated in graded Ethanol series. Sample tissues were embedded Araldyte-Cy 212. Semi-thin sections were stained with Toludine Blue. Thin sections were stained with Uranyl Acetate & Lead Citrate. The specimens were examined and photographed using transmission electron micro-
Findings
The difference of gastric mucus mean levels between the groups was evaluated statistically by Kruskal-Wallis test. A significant difference was found between the groups (p<0.05). (Table 1)

Multiple comparisons between groups were performed by Dunnet test. A value of p<0.05 was considered statistically significant. Multiple comparison revealed a statistically significant difference among mean values of (C-E) and (C-SX+E) groups (Table 2).

Ultrastructural Findings

Control Group (C)
We observed that the gastric mucosal cells of the rats of the control group were in the normal appearance. It was seen that the junctional complex between the gastric surface and neck mucous cells was normal appearance and apical cytoplasm of the cells were full with large and dense secretory granules containing mucus (Fig. 1).

Sialoadenectomy Group (SX)
Comparing with the other experimental groups the changes occurred in the gastric mucosal surface epithelial cells of the SX group observed that were lighter while SX group was performing a nearer view of to the control group, but we observed partly degeneration of the apical cells junction. In this group surface neck mucous cells contain dense and not full with large secretory granules (Fig. 2).

Ethanol Treated Group (E)
Comparing with the control group it was seen that in the epithelial cells of the gastric mucosa of the Ethanol group changes occurred.

Ethanol effects negatively the proliferation of the basal cells and renews mucous cells, destroying the structure of the junction zone among the cells in the surface mucous neck cells. Although both in the surface mucous neck cells granules were completely full of mucus secretion (Fig. 3).

<table>
<thead>
<tr>
<th>Groups</th>
<th>Mean ranks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (C)</td>
<td>24.43</td>
</tr>
<tr>
<td>Sialoadenectomy (SX)</td>
<td>18.57</td>
</tr>
<tr>
<td>Ethanol (E)</td>
<td>11.00</td>
</tr>
<tr>
<td>Sialoadenectomy+Ethanol (SX+E)</td>
<td>4.00</td>
</tr>
<tr>
<td>Total</td>
<td>28</td>
</tr>
</tbody>
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Table 1: The differences between mucus mean levels of control and experimental groups.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Control-SX</th>
<th>Control-E</th>
<th>Control-(SX+E)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>41 NS</td>
<td>94*</td>
<td>145*</td>
</tr>
</tbody>
</table>

Table 2: Multiple comparisons between control group and experimental groups.

NS: Non-significant, * : p<0.05
Fig. 2. Electron micrograph of surface cells from the rat stomach of the SX group. It has been noted that a slight degeneration in the junctional complex of the basal region of the surface epithelial cells (arrow) (Uranyl acetate-lead citrate, X3000).

Fig. 3. Electron micrograph of a surface cell from the rat stomach of the ethanol group. It has been noted that the apical of the surface epithelia cells is full of large mucous granules (arrow). (Uranyl acetate-lead citrate, X3000).

Fig. 4. Electron micrograph of surface cell from the rat stomach of the SX+Ethanol group. At the low magnification intracellular apical and basal junction complex are in the completely degenerated view (thick arrow). Apical cell membrane in a destroyed situation and significantly numerical decrease in the secretion granules is seen (thin arrow) (Uranyl acetate-lead citrate, X3000).

Results and Discussion

This study showed that salivary glands play an important role in gastric mucosal integrity and secretion of mucus in rats administered of ethanol. The removal of salivary glands greatly increased the susceptibility of gastric mucosa to the formation of lesions and this accompanied by marked reduction of mucus secretion granules and additionally occurred ultrastructural changes.

The relevance of the mucus layer covering the gastrointestinal mucosa in the functional barrier against noxious agents is well established and is well known that salivary EGF stimulates secretion of mucin and essential for the maintenance of mucus coat along the gastrointestinal tract (18, 10, 7, 14).

According to our findings in SX, E and SX+E groups, it was found with spectro-measurement that mucus level was decrease in these cells the greatly decreasing of secretion granules was also sign of the significantly mucus contain decrease (Fig. 4).
creased. But in statically evaluation the observed decrease in mucus level in SX group was found unimportant. By TEM evaluation in SX group partly in SX+E group the mucus content was marked to be decreased considerably.

We didn’t encounter any TEM study related to the effect of sialoadenectomy on gastric mucosa. In this study, TEM evaluation indicated that the stomach mucosa epithelium in control group showed a normal appearance while changes were observed in all experimental groups.

The distribution of EGF receptors in the normal adult human gastrointestinal tract showed it to be present only on basolateral membranes and not on the luminal surfaces (16). Evidence in favour of this EGF receptors localisation include the finding that rats that have had their salivary glands removed may not develop visible damage of the gastric epithelium compared with control animals. Our study also approves the previous study that in TEM findings in SX and SX+E groups intercellular adhesion spoiling is present. Severe basal epithelium of intercellular adhesions was destroyed.

EGF has distinct effects on mucus synthesis in specific layer of the rat gastric mucosa. The EGF induced stimulation of mucin biosynthesis occur in the surface mucus cells, and not in the gland mucus cells, of rat gastric mucosa (9). According to our TEM findings it has been seen that comparing with control group mucus content in surface mucus cells in SX and SX+E groups has decreased. In our study we didn’t examined glanduler and surface mucus cells.

These data indicated that sialoadenectomy leads to an increase in mucosal inflammation, decrease in mucus level and ultrastructural changes in ethanol induced sialoadenectomized rats.

Acknowledgements
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REFERENCES