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Histological and Ultrastructural Studies on the Gastric Mucosa of Rat after Treatment with Ethylene Glycol

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Abstract: The aim of the present investigation to reveal the histological and ultrastructural effects of ethylene glycol on the gastric mucosa of rats. A sublethal dose of ethylene glycol was administered orally to two groups of rats. The control group was only given distilled water. Stomach samples of both experimental and control groups were taken at 2 and 4 days after treatment. The gastric mucosa of the experimental groups, showed histological changes in the cells of the gastric mucosa included disruption of surface epithelium or mucosal erosions and the cells were swollen and disrupted accompanied by irregular in nuclear shape of the different types of the mucosal cells. On the other hand, electron microscopy revealed some changes in the Golgi apparatus, mitochondria, endoplasmic reticulum and nucleus of all gastric mucosal cells (surface mucous, chief and argentaffine).

Key words: Ethylene glycol, rat, stomach mucosa, ultrastructure.

INTRODUCTION

Ethylene glycol has many uses including antifreeze in cooling and heating systems in hydraulic brakes, industrial solvent as in the paint and plastics industries in the formulations of printer inks and is also used to de-ice airport runways and aircraft and also, it is used for improving tomatoes, apples, coffee, berries and grapes.

Ingestion of ethylene glycol may cause serious poisoning (Leth and Gregerson 2005).

Ethylene glycol is readily absorbed from the gastrointestinal tract and is rapidly and uniformly distributed to the blood and the tissues (Bachmann and Goldberg, 1971; Tyl *et al*. 1995; Giermaziak and Orkisz,1995; Giermaziak and Tosik 1996).

The animal lethal dose of ethylene glycol in dogs had been reported (Kersting and Neilson, 1966). And the death reported (Beckett and Shields, 1971).

The histopathological alterations by the effect of different substances of organophosphorus insecticides, that reported marked degenerative changes in the gastrointestinal tract (Boyd and Chen, 1968; Sastry and Malik 1979; Sastry and Sharma 1981; Alich, *et al*. 1983 and Cherner, *et al*.,1989).

Many investigators studied the toxicity of ethylene glycol in producing gastric mucosa damage Depass, *et al.* (1986) who studied the chronic toxicity of ethylene glycol in mice and rats and found many histopathological changes in soft tissue as fatty changes and hyperplasia.

2 Butyoxyethanol is a member of a family of ethylene glycol monoalkyl ethers, caused inflammation, necrosis, ulceration or epithelial hyperplasia in the fore stomach in mice after treatment (N. T. P., 2000).

Moreover, Poet, *et al.* (2003) studied the effects of oral administration of undiluted Butoxyethanol and showed that it caused irritation and regenerative hyperplasia in mice fore stomach.

Zhao, *et al*. (2002) had shown morphometeric changes in the wall thickness, wall area in rat small intestine after the effect of ethylene glycol Fluoride (EGF).

Thus, examination of stomach mucosa tissue by use of light and electron microscopy in the early days of ethylene glycol intoxication was warranted in an attempt to document such structural lesions. Primary goals of the study reported here were to characterize in individual rats, the existence and progression of gastric

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mucosa ultrastructural changes from 2 to 4 days after ethylene glycol injection and to correlate ultrastructure changes with concurrent histological examination results.

MATERIALS AND METHODS

Male albino rats weight about 100-150 gm were divided to three groups of 10 rats each. One group of rats was a normal control group and the other two groups received by oral administration of a single dose of 1/2 LD_{50} of ethylene glycol (3.3 ml/kg body weight). After 2 and 4 days of treatment the rats were scarified and the stomach samples of normal and treated rats were collected for light and electron microscopic examination.

The stomach was cut into small pieces about 1mm thick, then were fixed overnight in 3% glutraldehdye, 2% paraformaldehyde in 0.1 M phosphate buffer pH 7.4 at 4°C, after three rinses in 0.1 M phosphate buffer (10 mins. each), the tissues were post fixed in 2% aqueous osmium tetroxide for 2 hours at 4°C then washed in 0.1 M phosphate buffer (2 times) and dehydrated in ethanol series and embedded in epoxy resin.

Semithin sections (1um) were cut by RMC ultratome stained with 0.5% touldine blue in borax and examined under light microscope.

Ultrathin sections were mounted on copper grids stained with 2% aqueous uranyl acetate for 20 mins. and lead citrate for another 20 mins., then examined and photographed using Joel 1200 Ex-II electron microscopy.

RESULTS AND DISCUSSIONS

Light Microscopic Examination:

Control Group:

The gastric mucosa represents the innermost lining coat of the stomach and comprises the largest and widest portion of the stomach wall.

The gastric mucosa is covered internally by a simple layer of columnar epithelial cells with prominent nuclei. The bulk of the gastric mucosa is occupied by the gastric glands situated at the luminal surface of the epithelial lining, opening singly or in groups with certain apertures.

The basal portions of these glands are composed of chief (zymogenic) cells with occasional parietal cells, whereas the neck of each gland contains both mucous neck cells and parietal cells.

1. The Surface Epithelial (Mucous) Cells:

These cells constitute the lining epithelium covering the inner surface of the stomach being in direct contact with the lumen. These cells are irregular shapes tend to a pyramidal with an ovoid nucleus having a basal locations surrounded by clear cytoplasmic mass. The apical potions of these cells are occupied by dense discrete granules.

2. Chief (Zymogenic) Cells:

These cells are mainly located at the bases of the gastric glands and are responsible for the secretion of propepsinogen.These cells have a conical or pyramidal outline; with a basophilic cytoplasm and basally situated spherical nuclei.

3. Parietal (Oxytic) Cells:

These cells known as acid-forming cells and are the principle secretors of hydrochloric acid in the stomach. They are scattered among the other cell types.

The parietal cells are large in size with a spherical or pyramidal outline and with acidophilic cytoplasm and centrally spherical nucleus.

4. Argentaffin (Enteroendocrine) Cells:

These cells are found at the bases of the gastric glands, they are small sizes and conical or pyramidal shapes. The cytoplasm contains secretory granules at the basal regions with spherical nuclei in the basal regions (Fig.1).

Treated Groups:

After 2 Days:

After 2 days of rat treatment with ethylene glycol, the gastric mucosa has revealed a homogenous response of the epithelial lining of the gastric mucosa. Some areas are obvious degenerative changes, whereas the remaining ones are still normal in appearance.

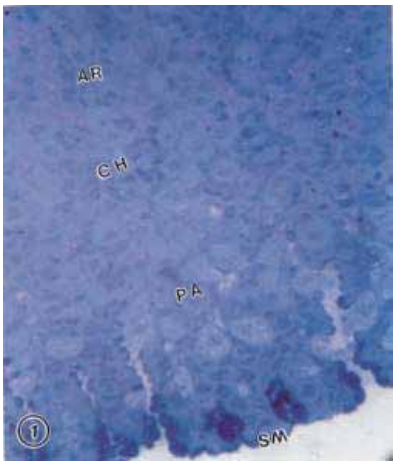


Fig. 1: Photomicrograph of a semithin section of the gastric mucosa of a normal control rat, showing the surface mucous cells (SM), parietal cells (PA), chief cells (CH) and positively stained argentaffin cells (AR) which contain zygomogen granules.Toludine stain. X1000.

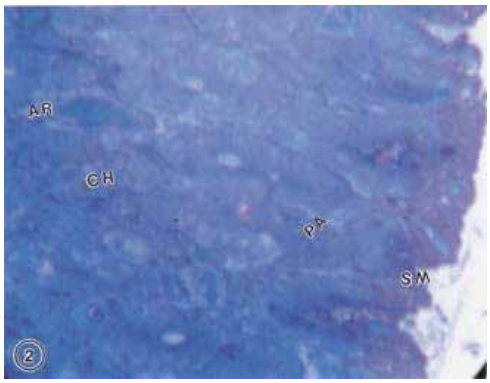


Fig. 2: Photomicrogh of a semithin section of gastric mucosa cells of a rat after 2 days of treatment with a single dose of 1/2 LD_{50} of ethylene glycol (3.3 mg/kg body weight), showing little damage of surface mucous cells (SM), parietal cells (PA), chief cells (CH) and argentaffin cells (AR). Toludine blue stain. X1000.

In the injured areas, the surface mucous cells were hypertrophied and disrupted with some alteration in their staining affinities being shifted toward a faint coloration of their cytoplasmic matrix resulting in a reduced contrast between this matrix and its cytoplasmic inclusions.

The nucleus are swelled and their chromatin became clumped and noticeably marginated at nuclear peripheries.The mucous granules acquired a lesser uniformity with an apparent tendency to massed together in certain areas in the apical portions of the surface epithelial cells (Fig.2).

After 4 Days:

After 4 days of treatment the mucosal cells were injured with more marked and showed a wider spread in regard to the surface mucous cells after 2 days of treatment.

Most of the cells were pushed into gastric cavity, many cells displayed a diffuse stain ability indicating

clear signs of cloudy swelling and the nuclei of these cells became irregular in shape.

However, the outer gastric mucosa cell types (chief, parietal and enteroendocrine) are affected with ethylene glycol and showed inflammation, necrosis and ulceration (Fig.3).

Electron Microscopic Examination:

Control Group: The gastric mucosa of the stomach of normal rat have four types of cells,the surface mucous cells, parietal or oxyntic cells, chief or peptic cells and argentaffin or enteroendocrine cells.

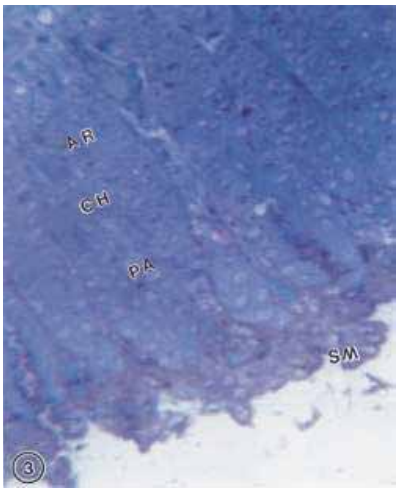


Fig. 3: Photomicrograph of semithin sections of the gastric mucosa of a rat after 4 days of treatment (3.3 mg/kg body weight) showing, most of the surface mucosa cells (SM), parietal cells (PA) are displaying of damage. The chief cells (CH) and argentaffin cells (AR) are also injured. Toludine blue stain. X800.

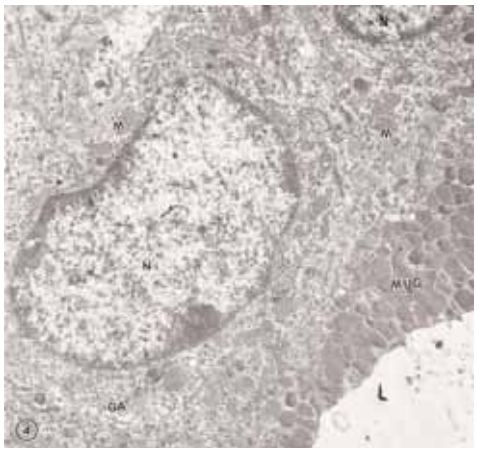


Fig. 4: Photo electron micrograph of the gastric mucosa of a control rat, showing a part of surface epithelial cells bordering the lumen (L) of stomach, Golgi apparatus (GA), mitochondria (M), nucleus (N) and mucoid granules(MUG). X20000.

The surface mucous epithelial cells are lining the gastric lumen of the stomach and have a low striated border. The Golgi complex located in the supranuclear region and consisting of small cistarnea and a number of small vesicles. The mitochondria are distributed in the cytoplasm and have transversely cristea and dense intra mitochondrial inclusions.

The rough endoplasmic reticulum is observed in cytoplasmic matrix.

The apical areas of these cells are loaded with numerous mucous granules of considerable sizes and spherical or discoidal outlines.

The nuclei of these cells are situated at the bases of the cells having double layered nuclear envelope with compact nucleolus (Fig.4).

The parietal cells are found in the neck and isthmus regions of the gastric mucosa and rarely among the chief cells being located in the deeper portion of the gland.

The parietal cells are pyramidal in shape with their pieces directed towards the lumen of the stomach. The surface area of cytoplasm of these cells is exposed by intercellular canaliculi. The Golgi apparatus is poorly developed but it is comprised of one or two hardly discerned Golgi elements of membranous structure together with some small vacuoles and a number of small vesicles.

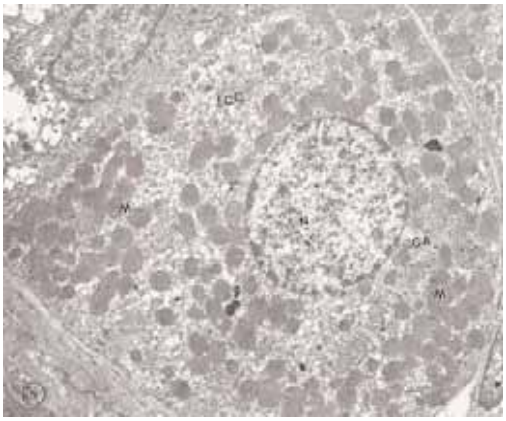


Fig. 5: Photo electron micrograph of the parietal cell from a deeper part of the gastric mucosa of the control normal rat stomach, showing Golgi apparatus (GA), mitochondria (M), intercellular canaliculi (ICC) and nucleus (N). X24000.

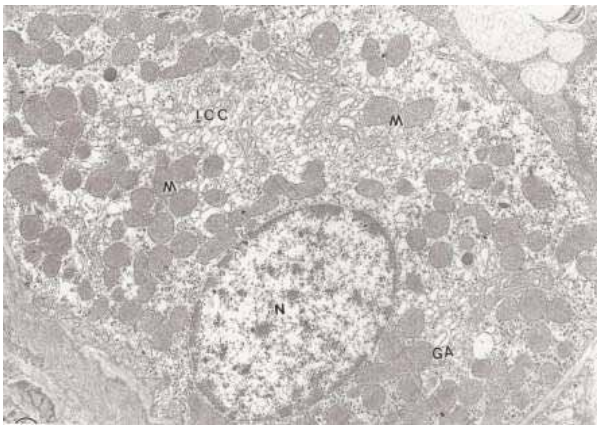


Fig. 6: Photo electron micrograph of the parietal cell located at the base of the gastric pit of control rat stomach, showing Golgi apparatus (GA), mitochondria (M), intercellular canaliculi (ICC) and nucleus (N). X24000.

The mitochondria tend to distributed in lateral and basal borders of the cells and near to the nuclei and are oval or short rod-like structures with cristea arranged in long axial mode of arrangement. These ridges or cristea have curved outline.

The cytoplasmic matrix contain small vesicles and tubular strands of smooth endoplasmic reticulum and the rest contains numerous ribosomes, some attached with the endoplasmic reticulum.

The nuclei of the parietal cells are centrally placed with patches of chromatin material (Fig.5).

The neck parietal cells are located in the region of junction of the gastric pits and the gastric glands.These cells differ from those (isthmus regions) described above. The cytoplasmic vacuoles are lesser in number but the granular membranes are more prominent.The mitochondria appear less numerous and more widely spaced than those in the isthmus regions. The intercellular canaliculi are closed and less extensive than those in the deeper cells (Fig.6).

The Chief Cells:

The chief cells are frequently observed in contact with the neck cells. Their basal cytoplasm is highly basophilic and there is an accumulation of zymogenic granules at their apices. The basal cytoplasm contains numerous lamellae arranged in straight parallel rows. The Golgi apparatus located beside the nucleus and consists of a small number of parallel membranes arranged together with a few vacuoles and vesicles. The mitochondria are numerous and are generally similar in structure to those of the mucous cells. The rough

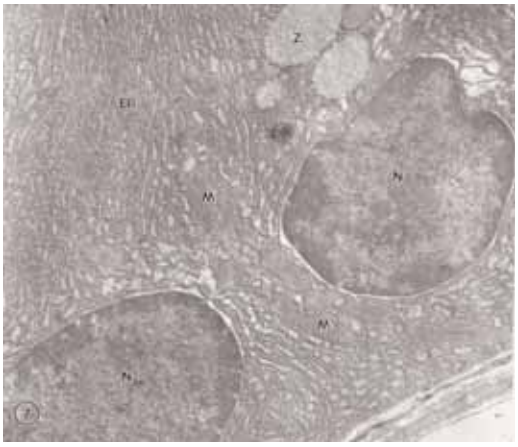


Fig. 7: Photo electron micrograph of the chief cell of the control rat stomach mucosa revealing abundant of granular endoplasmic reticulum (ER) in the form of aggregation of packed cisternae, mitochondria (M), nucleus (N) and zymogen granules (Z). X38500.

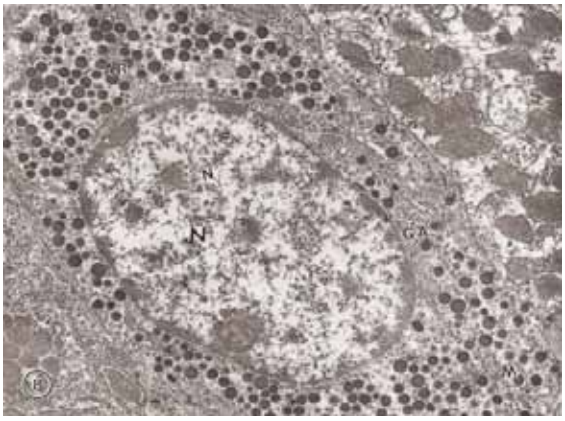


Fig. 8: Photo electron micrograph of argenttafin cell from a control rat stomach mucosa, showing dense granules (argentiffin) (AR), Golgi apparatus (GA), mitochondria (M) and nucleus (N). X20000.

endoplasmic reticulum is much more abundant in the chief cells than in any other cell types in the gastric mucosa and there are great numbers of free ribosome distributed throughout the cytoplasmic matrix (Fig.7).

The Argentaffin Cells:

These cells have a pyramidal or ovoid configuration with their broad bases resting on the basement membranes. Numerous dense, spherical granules are basally located in these cells each being enveloped with

a loose fitting membrane. The Golgi complex is located beside the nucleus, consisting of a small number of membranes arranged in pairs,as well as a number of some rounded vacuoles and narrow vesicles. The mitochondria are small in size and nearly spherical in shape with hardly visible mitochondrial ridges. The granular or rough endoplasmic reticulum and free ribosomes are present in the cytoplasmic matrix. The nuclei of these cell types are usually located in deeply infolded envelops (Fig. 8).

Treated Groups:

After 2 Days:

After 2 days of treatment, the gastric mucosa revealed cellular damage in the cells than those of control groups.

The surface mucous cells showed severe damage and inflammation. And also, disruption of the surface epithelium as mucosal injury. Some areas of these cells showed breaks in the plasmalemea and inflammation of the mucus released from the mucoid body.

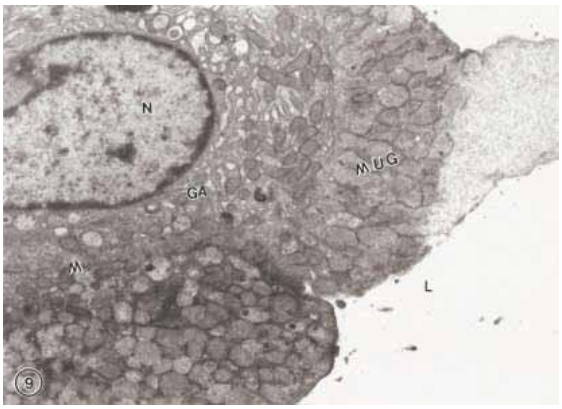


Fig. 9: Photo electron micrograph of surface mucous cells from treated rat stomach after 2 days of the treatment,illustrating the injuries in the lumen (L), mucous granules (MUG),Golgi apparatus (GA),mitochondria (M) and nucleus (N). X 18000.

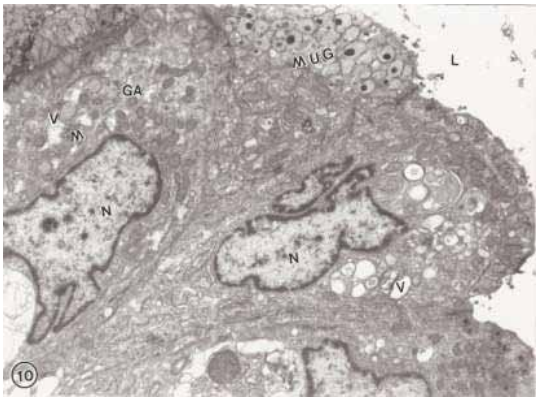


Fig. 10: Photo electron micrograph of the treated rat stomach mucosa after 2 days of treatment, showing damaged surface mucous cells, dilated Golgi apparatus (GA), mitochondria (M), cytoplasmic vacuoles (V), nucleus (N) with irregular nuclear membrane and lumen (L). X 12000.

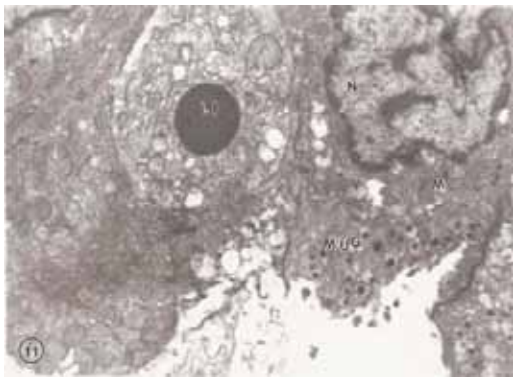


Fig. 11: Photo electron micrograph of the treated rat stomach mucosa after 2 days of treatment, showing more damaged surface mucous cells with mucoid granules (MUG), lipid droplet (Li) mitochondria (M) and nucleus (N). X18000.

The cytoplasmic organelles showed some alterations as in Golgi apparatus are dilated and broken down cisternae and vacuoles. The mitochondria were hypertrophied with some rupture of their internal ridges and limiting membrane and the cytoplasm are vacuolated and the nucleus had irregular and complete fusion of the two nuclear membranes. The chromatin are together as indicating initial stages of nuclear damage.

Large lipid droplets appeared the cytoplasm of the damaged epithelial cells (Figs. 9, 10 and 11).

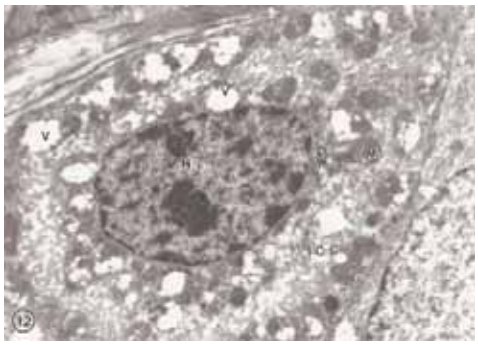


Fig. 12: Photo electron micrograph of the treated rat stomach mucosa after 2 days of treatment,showing parietal cells with cytoplasmic vacuoles (V) , swollen Golgi apparatus (GA), mitochondria (M), intercellular canaliculi (ICC) and nucleus (N). X 18000.

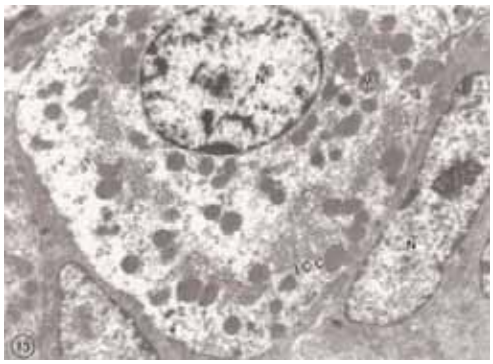


Fig. 13: Photo electron micrograph of the treated rat stomach mucosa after 2 days of treatment, showing deeply situated parietal cell, aggregation of intercellular canaliculi (ICC), mitochondria (M) and nucleus (N). X 19000.

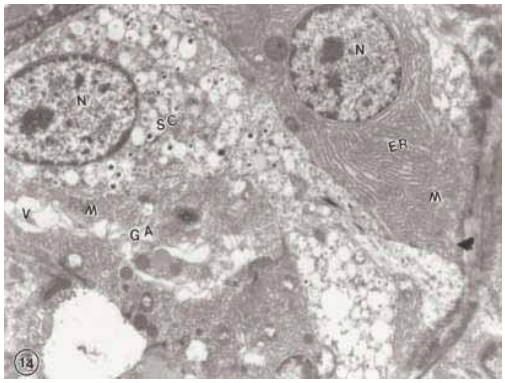


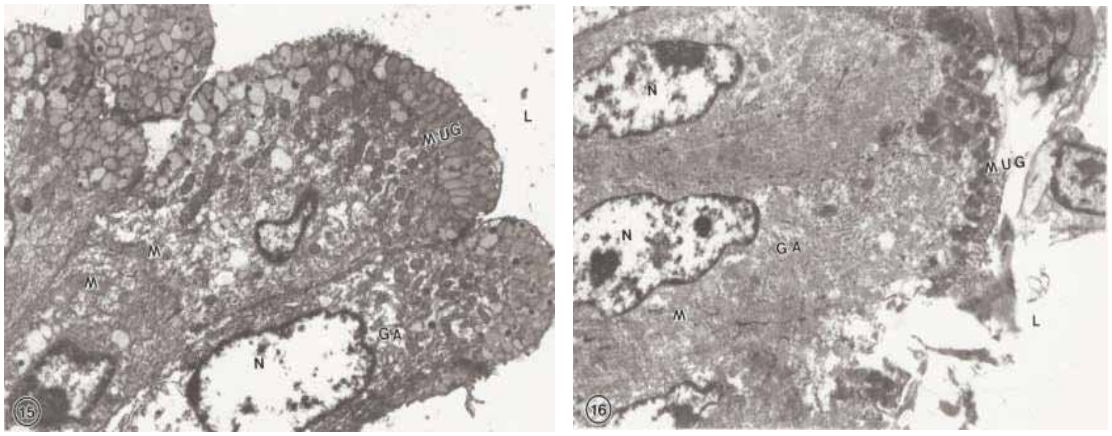
Fig. 14: Photo electron micrograph of the treated rat stomach mucosa after 2 days of treatment, showing the chief cell with dilation of the rough endoplasmic reticulum (ER), Golgi apparatus (GA) and argentiffin cells with less broken secretory granules (SC), mitochondria (M), the nucleus of both cells (N) are affected and cytoplasmic vacuoles (V). X 15000.

The parietal cells especially those lying at the apices of the lumen or near the surface mucous cells showed more damage in the Golgi apparatus; the cisternea were particularly broken down into small vacuolar structure.

The mitochondria were swollen into irregular bodies, their ridges were broken. Some cytoplamic vacuoles were found.

The endoplamic reticulum are dilated and broken, the nucleus had clumped chromatin (Fig.12). But in the deeper parietal cells, lesser impairments were seen except some marked changes in the mitochondrial shape which became condensed and dark and intercellular canaliculi were accumulated together (Fig.13).

The chief cells have alterations after 2 days of treatment with ethylene glycol. The rough endoplasmic reticulum were dilated, condensed mitochondria and chromatin are clumped in the nucleus (Fig.14).



Figs. 15,16: Photo electron micrographs of the treated rat stomach mucosa after 4 days of treatment, showing severe damaged in surface mucous cells in cytoplasmic organelles in lumen (L), mucoid granules (MUG), degeneration in Golgi apparatus (GA), mitochondria (M) and nucleus (N). X1800 and X1200 respectively.

The argentaffin cells severe more damage after 2 days of treatment than those of the control groups.

The secretory granules are damaged and most of it appeared empty and vacuolated, the cytoplasm have many vacuoles, the mitochondria are condensed and appeared dark in shape (Fig.14).

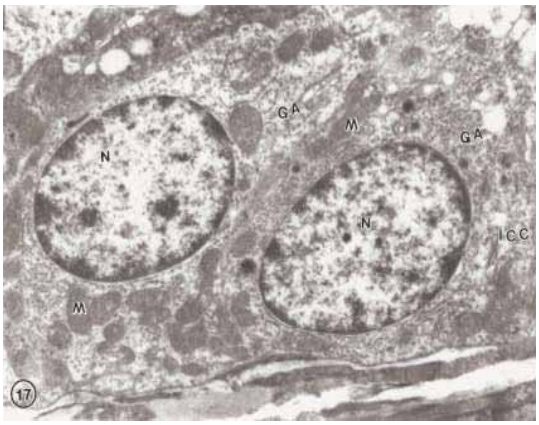


Fig. 17: Photo electron micrograph of the treated rat stomach mucosa after 4 days of treatment, showing parietal cells with more destruction in Golgi apparatus (GA), mitochondria (M), intercellular canaculi (ICC) and cytoplasmic vacuoles (V). X15000.

After 4 Days:

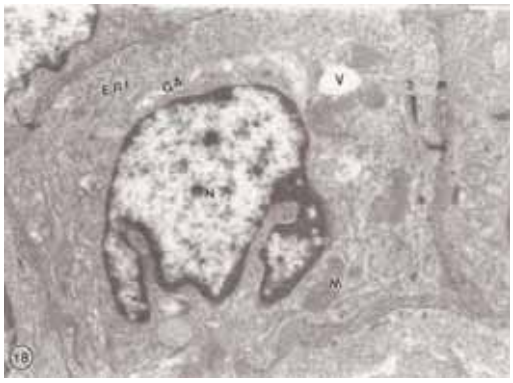
The surface mucous cells revealed more damage than those inspected after 2 days of ethylene glycol treatment.

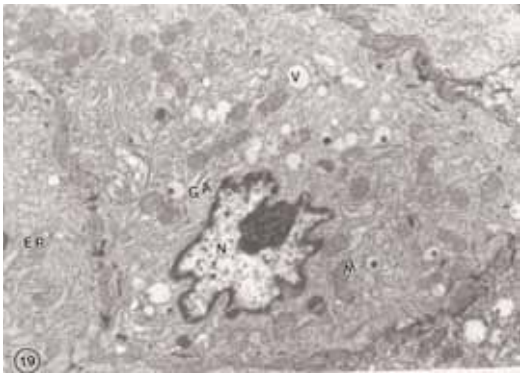
The cytoplasm and the nucleus showed severe degeneration and most of cytoplasmic organelles showed degeneration as in the Golgi apparatus and the mitochondria are condensed in shape (Figs.15 and 16).

The parietal cells after 4 days of treatment appeared more degenerative changes in comparison with those obtained in 2 days treated rat.

However such lesions were less in the deeper parietal cells which almost mildly affected. The Golgi apparatus was completely broken down and the mitochondria were swollen and dense appearance. The cytoplasm was vacuolated. The nuclei contained aggregation of intensely stained chromatin. The endoplasmic reticulum are dilated and fragmented (Fig.17).

The chief cells after 4 days of treatment showed severe damage in both the cytoplasmic organelles and the nucleus which are completely folded membrane and clumped chromatin together in some area. The rough endoplasmic reticulum became vesicular and dilated. It looses their ribosomes and the cytoplasm are vacuolated.





Figs. 18,19: Photo electron micrographs of the treated rat stomach mucosa after 4 days of treatment, showing affected chief cells with dilated endoplasmic reticulum (ER) with less ribosomes, Golgi apparatus (GA), mitochondria (M), nucleus (N) and cytoplasmic vacuoles (V). X15000 and X1800 respectively.

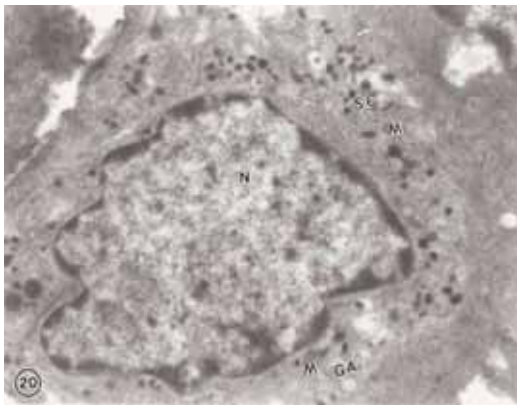


Fig. 20: Photo electron micrograph of the treated rat stomach mucosa after 4 days of treatment,showing another affected argentiffin cells, the secretory granules (SC), Golgi apparatus (GA),mitochondria (M) and nucleus (N). X22500.

The Golgi apparatus are dilated and broken and the mitochondria appeared swollen and darkly stained (Figs. 18 and 19).

The Argentaffin cells in the gastric mucosa after 4 days of treatment were also observed fewer secretory granules than in normal cells. The nucleus showed clear irregular in membrane and accumulation of chromatin near the membrane periphery. The Golgi apparatus are affected and broken down to small clusters and the mitochondria appeared darkly stain. The cytoplasm contains large number of vacuoles (Fig.20).

Our results obtained in treated rat stomach mucosa indicated that ethylene glycol (EG) had adverse effects on the histology and ultrastructure.

The dosage administered in this study was 3.3 mg/kg body weight is an attempt to maximize the likehood of gastric mucosa structural change, this agree with others authors that reported that the minimal lethal dose of ethylene glycol in dogs in 6.6 ml/kg, but death has been reported with dosage as low as 4.2 ml/kg (Kersting and Neilson 1966 and Sanyer *et al*., 1973).

The types, distribution and progression of light microscopic and electron microscopic lesions observed in the rats of this study, were similar to those that have been reported for ethylene glycol intoxication in domestic animals and human beings.

Depass, *et al.* (1986) studied the effect of ethylene on diet to rats and mice and observed that the ethylene glycol induced fatty changes and hyperplasia in liver cells.

Anton*, et al.* (2004) who reported that, the ingestion of a potential food contaminant (diquat) caused gastric and intestinal inflammation with mastocytosis.

Zhoa, *et al.* (2002) studied the inhalation of ethylene glycol butyl ether in mice stomach and found that the chronic contact irritation (cytotoxicity) and regenerative hyperplasia are hypothesized to result in fore stomach tumor development.

Chan, et al. (2000) reported the same resulted after the effect of cholecytokinin receptor on rat stomach cells and found that the oxyntic mucosal histamine and pancreastatin concentrations were reduced gradually and affected on shape and size of these cells.

And also, the national toxicology program (2000) reported similar studied on the mice stomach after the effect of 2-butoxyethanol that are ethylene glycol monoalkyl ethers inhalation and found inflammation, necrosis and ulceration in the fore stomach.

Furthermore, Cruzan *et al.* (2004) reported that the subchronic toxicity of ethylene glycol in rats was related to metabolism and found that the treatment of acute ethylene glycol poisoning was designed to prevent metabolism to treat acidosis.

Leth and Gregerson (2005) studied the ethylene glycol poisoning and found that ethylene glycol has itself a low toxicity,but in vivo broken down to four organic acids: glycoaldehyde, glycolic acid, glyoxylic acid and oxalic acid. The metabolites are cell toxins and causes severe acidosis.

In summary, the results of this study demonstrated that the oral administration of ethylene glycol in rat is stomach toxic as indicated by histological and ultrastructural changes in the gastric mucosa cells.

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