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# Histopathological Alterations In The Stomach Of Rats After Gavage Ethanol

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**Abstract**

The study aims to detect the effects of ethanol on pathological changes in the stomach according to the fed and fasting states and detect whether interferon-gamma (IFN-γ) represents an indicator measuring the impact of ethanol on induced damage grades. Twenty-five adult albino rats with average weight (230 ± 12) g, divided into five groups (5 in each), groups (A, B), and groups (C, D) administered with (30%, 50% ethanol ) respectively in fed and fasting state, and group E served as the control group. The treated groups administered (1 ml) ethanol orally every 48 hours for an entire month. at the end of the experiment, the rats anesthetized and the stomach fixed in formalin used for routine histological procedures, the serum samples obtained from blood were used to measure the level of IFN-γ by enzyme-linked Immunosorbent Assay (ELISA). The histological analysis of treated groups with 30% ethanol reveals a mild alteration in gastric mucosa and more change appeared at 50% ethanol. These changes appear more severely in the fasting state compared to the fed status for the same concentration. Results reveal a significant increase ($P<0.05$) in serum level of IFN-γ in the treated groups with increasing ethanol concentration and the fasting state. It can conclude that the severity of the damage in the gastric mucosa and the serum level of IFN-γ increase with increasing the concentration of ethanol and the fasting state.

**Keyword:** ethanol. Gastric mucosa .interferon-gamma .adult rats . fasting state

## introduction

Ethanol, often known as ethyl alcohol and grain alcohol, is a member of a class of organic compounds that is commonly referred to as alcohol. Many alcoholic beverages, such as beer, wine, and distilled spirits, contain ethanol in quantities ranging from 4% to 40% (Mitchell et al., 2014).

Ethanol is a tiny water-lift and lipids soluble molecule that penetrates all of the body's tissues and impacts essential activities, Because ethanol affects nearly all organs in the body, excessive ethanol consumption causes a range of gastrointestinal illnesses. The amount of consumption as well as the patterns of drinking, particularly irregular, heavy drinking, have been shown to determine the disease burden (Hassan et al., 2015).

Several studies reviewed the mechanism of absorption of alcohol from the stomach in the case of feeding and fasting and examined the effect of food on the absorption of alcohol in the stomach and stomach emptying. According to several studies, alcohol is removed more slowly in the fasting state than in the fed state due to lower ADH levels (Le Daré et al., 2019). According to Rajendram and Preedy, (2006), the rate of ethanol absorption is

slowed by delayed gastric emptying and the presence of food in the stomach. Alcohol consumption following meal consumption leads to substantial increases in the concentrations of intragastric alcohol and residence in comparison with fasting states (Rubbens et al., 2017).

These aspects referred to the fact that the presence of food in the stomach delays alcohol absorption and thus decreases its concentration in the blood. As a result, the damage caused by alcohol to all organs is severe when consumed on an empty stomach, which resulted in the popular concept "don't drink on an empty stomach" (Cederbaum, 2012).

on the other hand, Previous research suggests that alcohol intake is one of the numerous factors that might cause an increase in pro-inflammatory cytokines (van de Loo et al., 2020). IFN-γ is a pro-inflammatory cytokine that plays a role in the pathophysiology of alcohol-induced mucosal damage (W.-F. Li et al., 2014; Liu et al., 2012), as part of the inflammatory milieu, it is a key inducer of gastric epithelial cell death (Osaki et al., 2019).

Given the importance of food in ethanol absorption from the stomach and intestines, and the role of IFN-γ as components in an inflammatory environment, It is also necessary to investigate food role in the safety and injury of the gastric mucosa in the full and empty stomach and compared the histopathological changes in the rat's gastric mucosa between fed and fasting state and examine the relationship between ethanol's consumption impact on IFN-γ production, and the possibility to use that as a diagnostic indication that quantifies the amount of damage and inflammation.

**Materials & Methods**

Twenty-five adult albino rats with average weight (230 ± 12) g and aged between 10-14 weeks, purchased from animal's house at the Kufa university/college of science. The animals were transferred to the animal house at the Technical Institute / Middle Technical University, as they were placed in plastic cages measured as 15×35×50cm (5 in each cage), before being gavaged with ethanol, the animals were given 10 days to adjust to the circumstances of the animal home. During the experiment period, the animals were placed under the same laboratory conditions of ventilation, lighting (12 h day – 12 h night), and temperature (20-30) °C, and fed with a standard pellet diet.

The rats were split randomly into four treatment groups and a control group (5 in each). Group's A, B were administered with 30% ethanol in fed and fasting state respectively, the groups C, D administered with 50% ethanol in fed and fasting state respectively, and group E served as the control group  The treated groups were administered orally (1 ml) ethanol by intragastric gavage (the lethal dose (LD50 oral) in rats is 7 g per kg of body weight)(Criddle et al., 2019) every 48 hours for a full month. Animals in the fasting groups are deprived of food and water for 12 hours period being gavage with ethanol.

The stomach samples were carefully removed, cut into tiny pieces, fixed in 10% buffered formalin and the specimens were then dehydrated in progressively higher concentrations of alcohol, cleared in xylene, and embedded in paraffin wax. A rotatory microtome was used to obtain serial slices (4-5 m thick). Hematoxylin and eosin (H&E) and PAS stains were used to stain the deparaffinized sections. Photomicrographs were taken in the biology department of the University of Wasit using a research photography microscope.

A gel tube was used to collect blood samples, and the serum was separated in the lab by centrifugation at 3000 rpm/15 min and stored at -20 ℃, which are used for measurement of IFN-γ level by enzyme-linked Immunosorbent Assay (ELISA).

**Statistical analysis** The results of IFN-γ were expressed as (Mean ± SEM). ANOVA single factor was used to compare the difference of results between the groups and a T-test was used to compare the difference between every two groups. A value $P<0.05$ was considered to be significant. All statistical analyses were performed using Microsoft Excel 2016.

**Results**

**Histological study**

The findings of our research of the stomach's histological structure in the control group indicate the general structures of typical histology, which showed the stomach wall to be comprised of three major layers (mucosa, submucosa, and muscularis layer), as shown in figure (1 - A).

The mucosa, which is made up of rows of columnar cells, penetrates the lamina propria, producing many gastric pits that open into the lumen and are based on the lamina muscularis mucosae.

The stomach glands, which occupied the whole lamina propria, were made up of mucous neck cells, parietal cells, and chief cells, figure (1 -B).

The mucosa is lined by a thin layer of smooth muscle cells called muscularis mucosae that separates the mucosa from the submucosa; additionally, the findings indicated a lamina propria made up of loose connective tissue with many blood vessels, especially near the muscularis mucosae in the bottom portion of the mucosa, as well as vessels distributed between the stomach glands inside the mucosa.

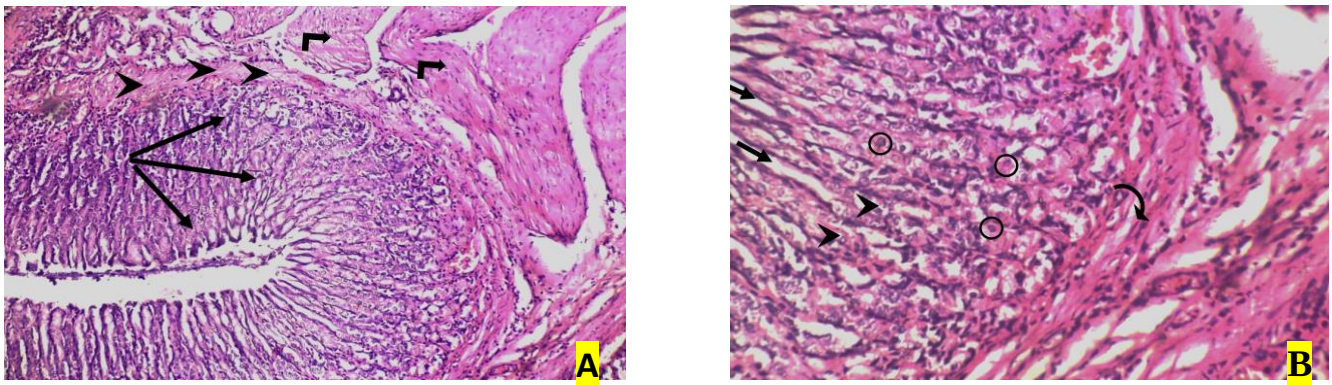



Figure 1 The histological section of the stomach from the control group showing (A) the general histological structure of the stomach wall includes mucosa (arrow), submucosa (arrowheads), and muscularis (angled arrow) H&E (10X). (B). gastric pit (arrows), parietal cells (circles), lamina propria (LP), and muscularis mucosae (MM) H&E (40X).

**groups treated with 30% ethanol**

The results of the present study in the animals of the fed group (30% ETOH) showed a mild histopathological change in the structures of the gastric mucosa it revealed capillary congestion in the lamina propria (Figures 2-A), as well as swelling and vacuolation in the cytoplasm of gland cells (mucous neck cells, chief and parietal cells). As seen in figures (2-B), ethanol intake destroyed lamina propria capillaries and bled blood cells throughout the mucosa cells. Blood vessels congestion and rupture caused injury to the submucosa, allowing blood cells to infiltrate into the interstitial tissue, as seen in the image (2-C)

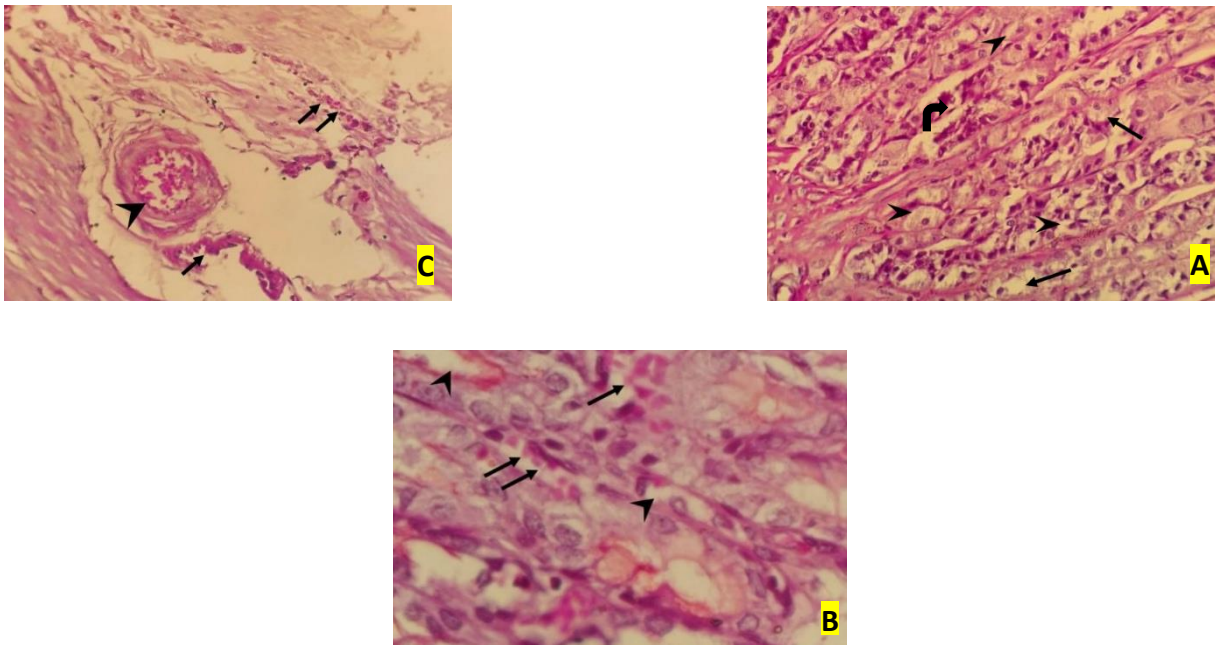



Figure 2-A Histological section of the stomach from the fed group (30% ethanol) showing the presence of congestion ( arrow angle ) of capillaries in the lamina propria and swelling (arrows) and vacuolation (arrowheads) in the cytoplasm of gland cells (arrows). PAS stain (20X).(B). destruction of capillary in the lamina propria (arrowheads) and bleeding of RBCs (arrows), PAS stain (100X). (C). blood vessel destruction (arrowheads) and bleeding of blood cells (arrows) in the interstitial tissue. PAS stain (40X).

The rats administered 30% ethanol while fasting had more severe abnormalities in the stomach mucosa and cells, and other changes in the gastric glands. Hemolysis, erythrocyte extravasation, and vascular congestion were found, the hemorrhage progressed across the mucosa layer, causing epithelial lifting and rupture. Surface epithelium exfoliation, shedding of the surface epithelium into the stomach lumen as shown in figure (3-A), and widespread vacuolation in the cytoplasm of various kinds of mucosal cells. These groups' deep gastric glands exhibit shrinkage or atrophy in certain cells and a gap between cells and basement membrane, as illustrated in (3-B). figures (3-C) show a filtration of inflammatory macrophages and leukocytes, a large lymphocyte and eosinophil population in the interstitial tissue and between mucosa cells in the fasting groups (30 % ethanol).

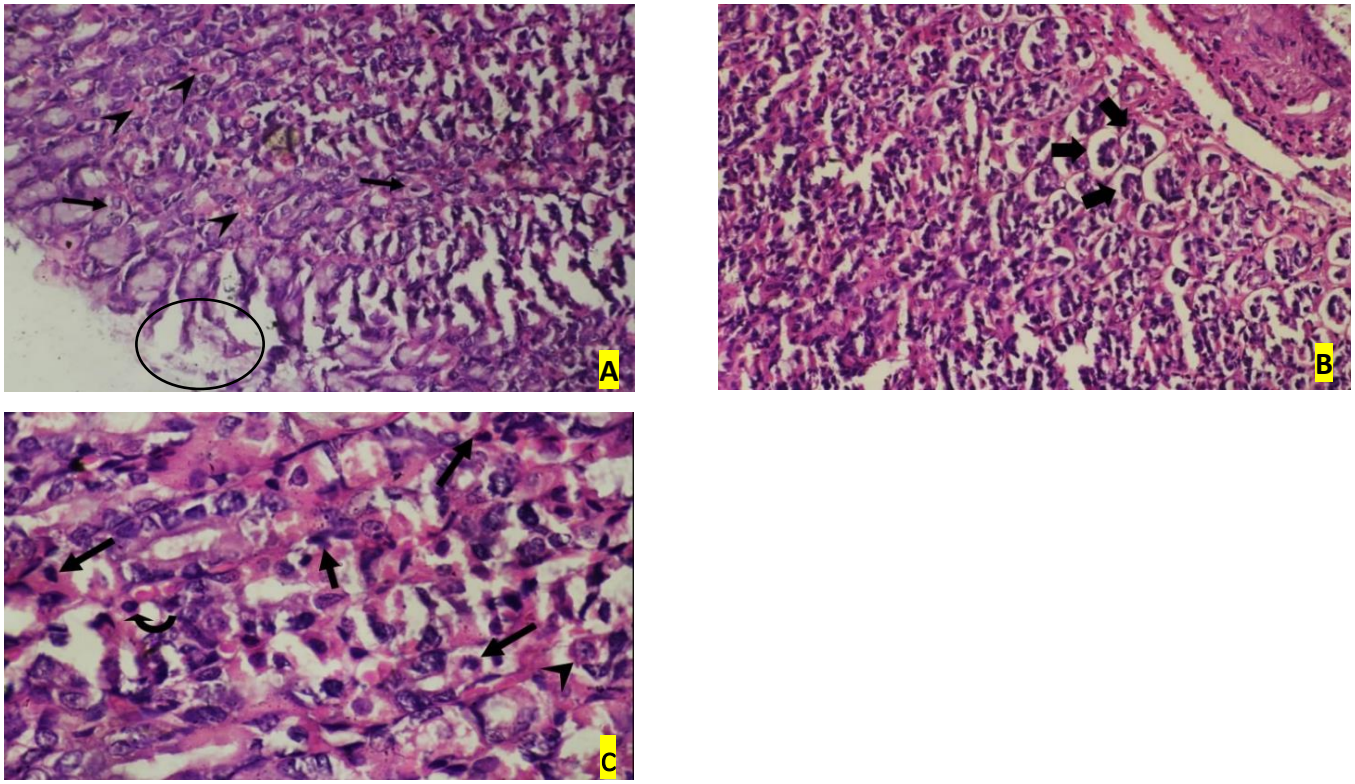



Figure 3. Histological section of the stomach from the fasting group (30% ETOH) showing (A) congestion and disruption of capillaries (arrowheads) near to surface epithelium with vacuoles in mucosa cells(arrows), shedding of the surface epithelium into the stomach lumen. H&E stain (40X). (B) the atrophy of gastric gland (arrows) and the abnormal space between the cells and basement membrane, H&E stain (40X). (C) the infiltration of inflammatory macrophages (arrowhead), eosinophil (arrows), lymphocytes (curved arrow) in the interstitial tissue of mucosa, H&E stain (100X).

**groups treated with 50% ethanol**

The current study found that used at 50% ethanol produced greater damage and severe histological alterations in the stomach than animal groups that administered at 30% ethanol. The fed group's stomach sections had more damage, with extensive vacuolations occupying a large area of the gastric mucosa, a clear case of ballooning degeneration of mucosa cells, as well as necrotic lesions of the stomach mucosa characterized by cell nucleus lysis, as seen in figures (4 -A). The gastric glands showed cytoplasmic vacuolations and necrosis, cellular atrophy, and bleeding owing to rupture of the blood vessel while RBCs diffused between the gland cells (figure 4-B). The submucosal blood vessels were congested and had severe bleeding, and the blood cells flooded the surrounding connective tissue, indicating an acute inflammatory response (Figures 4-C). The findings showed severe bleeding and acute inflammation in the gastric mucosa owing to leukocyte infiltration at the site of mucosal cell injury. the bleeding was severe due to the high destruction of numerous blood vessels of lamina propria, which appeared near the surface, as shown in figures (4-D) (4-E).

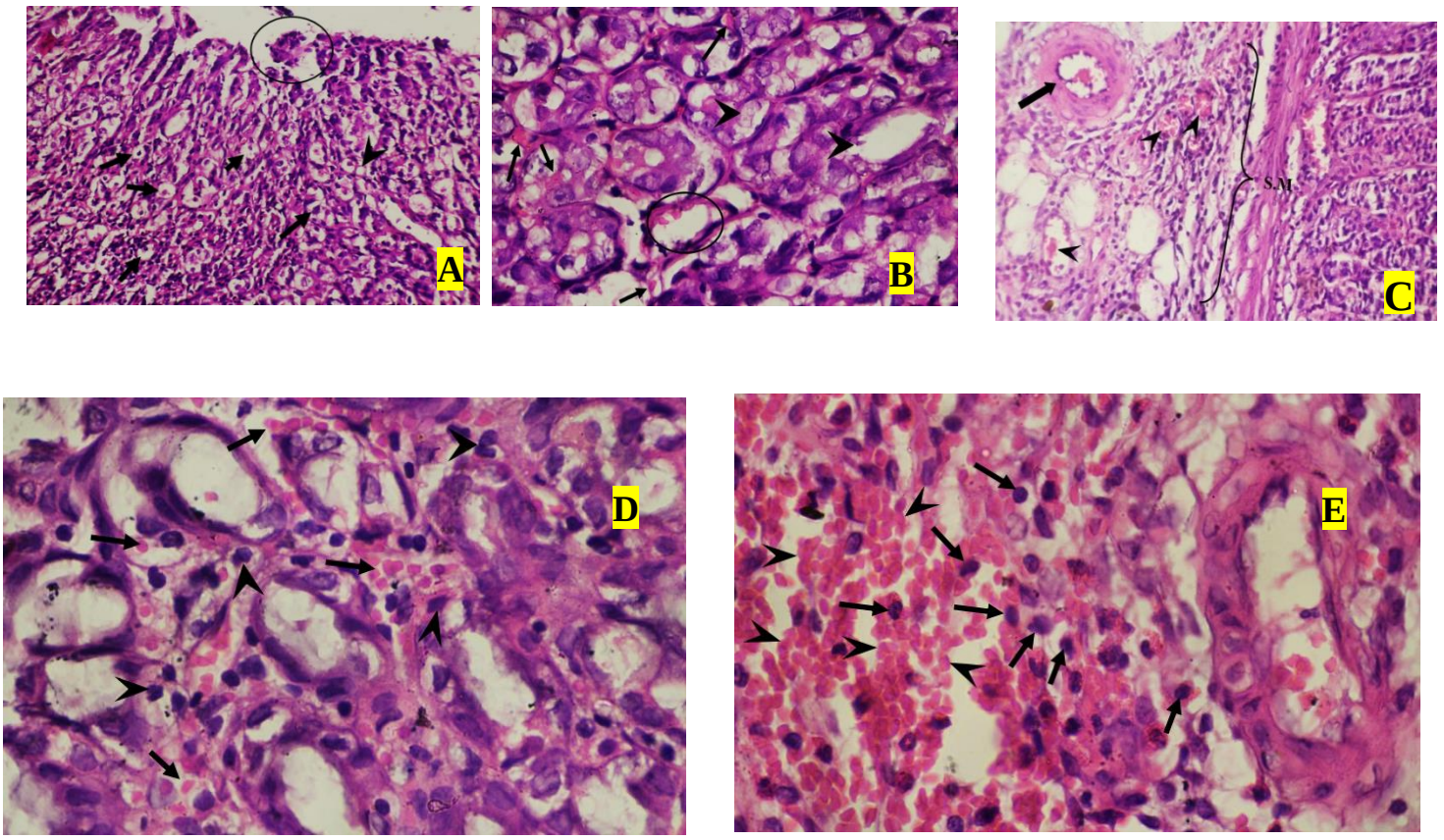



Figure 4. Histological section of the stomach from the fed group (50% ethanol) showing (A) damage of some surface epithelial cells (circle) and balloon degeneration (arrows) and vacuoles of mucosa cells (arrowheads), H&E (40X). (B) cytoplasmic vacuolations (arrowheads) and necrosis (circle), also hemorrhage (arrows) due to rupture of the blood vessel while the RBCs spread between the gland cells. H&E (100 X). (C) blood vessels in the submucosa exhibit high congestion (arrow) and severe bleeding (arrowheads); the blood cells appeared widely distributed and fill the surrounding connective tissue with infiltration of inflammatory cells. submucosa (S.M), H&E (40X). (D) severe hemorrhage (arrowheads) ;(E) a highly acute inflammation case seen in the mucosa layer due to infiltration of leukocyte (arrows) at the site of damage of mucosal cells and RBCs (arrows), H&E (100 X).

The effects of 50% ethanol in fasting animals were similar to the effects of 50% ethanol in fed groups with some pathological alterations and higher degrees of gastric mucosa injury. The results also showed significant damage and distortion in the stomach mucosa, with shedding or loss of surface epithelium in certain areas. The expansive gaps appeared in the gastric mucosa with some necrosis that extends from the deep layer of gastric mucosa toward the surface epithelium that appears in figure (5-A). The results revealed atrophy, distortion, irregularity, necrosis, and detachment of certain gland cells from the basement membrane in the stomach glands, figure (5-B). The findings indicated that mucous neck cells encroached on the lumen, with a weakening of the basement membrane. The cytoplasm of certain parietal cells was pale and granular, while others had vacuolated cytoplasm with pyknotic nuclei, which exhibit in figure (5-C). Acute inflammation and hemorrhage were also seen in the gastric mucosa, dilation of blood vessels down the length of the mucosa extend to the surface, and infiltration of leukocytes and erythrocytes into the gastric mucosal cells. Surface epithelium distortion showed early signs of gastric ulcer with

deep mucosal necrosis. There is a marked reduction of the height of the mucosa due to cell sloughing. These changes are illustrated in Figures (5-D) and figure (5-E).

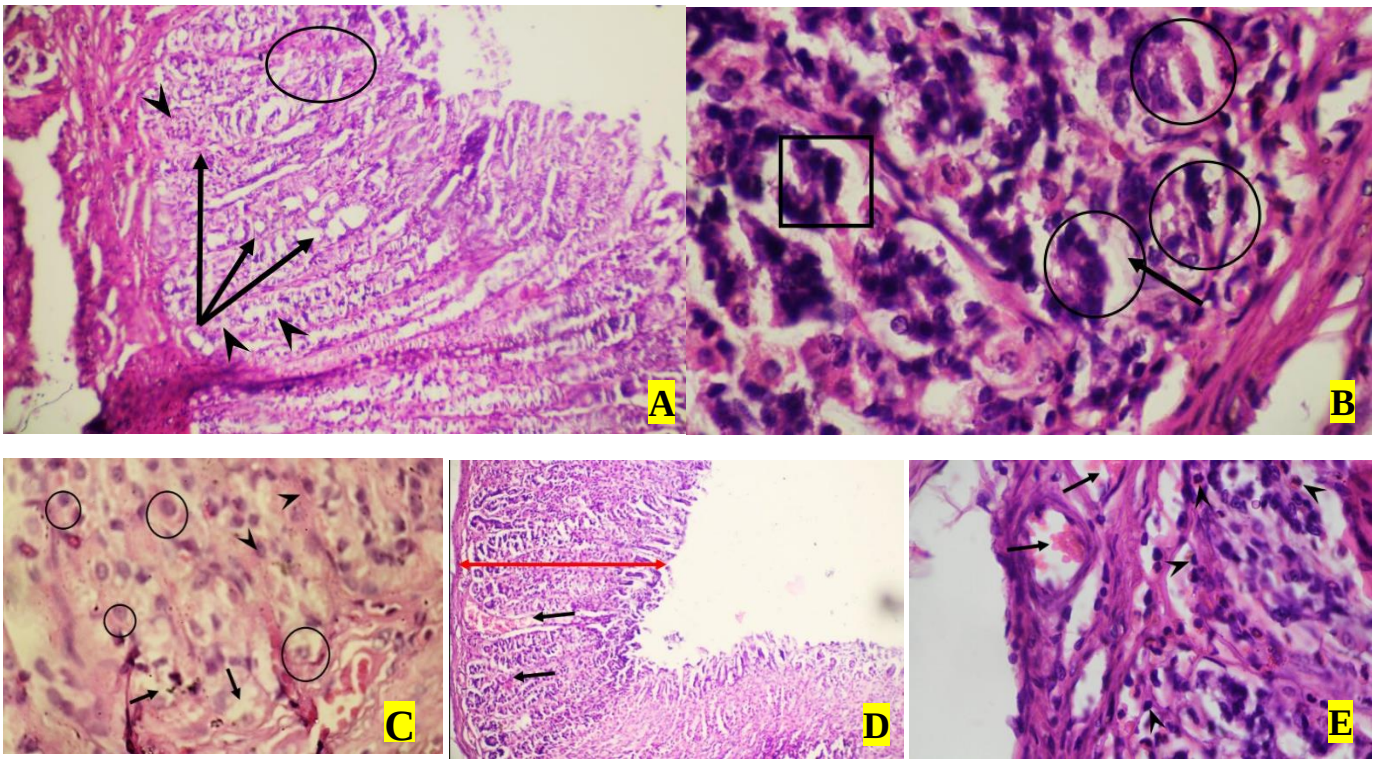



Figure 5. Histological section of the stomach from the fasting group (50% ETOH) showing (A) Sloughing or loss of the surface epithelium in some regions of the mucosa (circle) and the extensive holes (arrows) appeared in the gastric mucosa with some necrosis (arrowheads) extend from the deep layer of gastric mucosa toward the surface epithelium, H&E (40 X). (B) Disorganization in the shape of atrophy can be seen in some regions of the gastric glands (circles), distorted, and irregular glands with necrosis (Square) of some of the gland cells with detached of some gland cells from the basement membrane (arrow), H&E (100 X). (C) mucous neck cells (circles), expanded and invaded by the lumen, some of the parietal cells (arrowheads) inflated with pale and granular cytoplasm, numerous vacuolated cytoplasm (arrow) with pyknotic nuclei, H&E (100 X). (D) severe damage of blood vessels with hemorrhage (arrows) in the gastric mucosa, dilatation on blood vessels along the length of mucosa reach the surface. There is a marked reduction of the height of the mucosa (arrow red) due to cell sloughing, distortion of surface epithelium revealed the early symptoms of gastric ulcer with necrosis of deep structures of the gastric mucosa, H&E (20 X). (E) signs of acute inflammation (arrowheads) and hemorrhage (arrows), H&E (100 X).

**Results of Interferon-gamma (IFN-γ)**

The results of IFN-γ that done using the ELISA method of the serum samples of the rats presented by mean ± S.E. with (ng/L) unit illustrated in the table (3 – 2) and figure (3 – 29).

The concentration of IFN-γ in the control group's blood samples was measured (11.213 ± 0.48). Oral ethanol administration resulted in a significant rise in IFN- levels as ethanol concentrations increased.

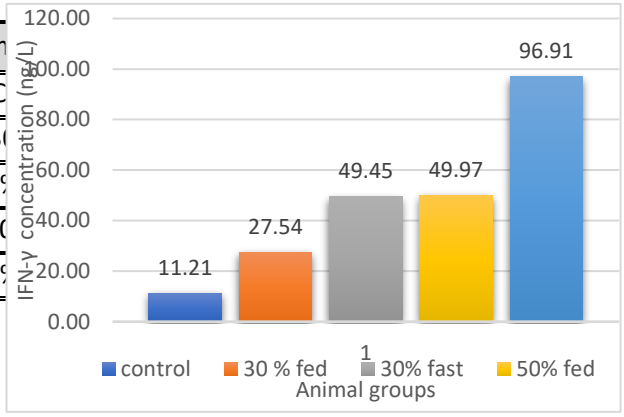
The statistical analysis revealed the recorded increasing difference of experimental groups was significantly $P< 0.05$ compared with levels recorded in the control group.

The statistical analysis of the increase in IFN-γ levels between every two groups was done using a t-Test that showed a significant increase P<0.05. The highest increase was in group D, which increased significantly more than the other groups.the analysis was not significant P>0.05 between groups (B,C), which recorded (49.45 ± 3.70 and 49.97 ± 4.33)

**Table 1.** Showing the concentrations of

IFN-γ (ng/L) of experimental groups

Figure 6. Illustrate the concentration of IFN-γ in experimental groups (ng/L)




**Discussions.**

**Histopathological study**

In the current study, ethanol administered by intra-gastric gavage to rats at two concentrations (30%, 50%, 1ml each 48h) in both fed and fasted states caused nonuniform histopathologic changes in the gastric mucosa. The magnitude of the damage is closely connected to the ethanol concentration and the animal's state, whether fasting or feeding.

Histological alterations were nearly equivalent in all treated animals but differed in degree and depth. The study found that animals given 50 % ethanol experienced greater damage than animals given 30 % ethanol; the findings demonstrate that fasting animals' stomach mucosa is more damaged than fed animals'.

When compared to a fasted state, alcohol ingestion after a meal resulted in a substantial rise in intragastric alcohol concentrations and residence duration (Rubbens et al., 2017), and ethanol is rapidly passed into the duodenum from the stomach in the fasted state (Cederbaum, 2012), but the present study revealed more damage in the fasting state compared to fed status for the same of ethanol concentration used.

The reason behind this discrepancy is perhaps because eating food coats the stomach and slows alcohol absorption, reducing direct contact of alcohol with the gastric mucosa, leading to the conclusion that the empty stomach influenced the severity of damage seen in the fasting state, or perhaps due to alcohol dilution with residual fluids of the stomach and the liquid meal when consuming an alcoholic beverage in the presence of food in the stomach (Rubbens et al., 2017)

The processes that produce stomach mucosal damage caused by luminal ethanol are complex and multifaceted; we can say that The pathophysiology of gastric mucosal damage by ethanol is confining into two

major pathways: direct and indirect. In the direct path: direct reactions of alcohol with molecular components influence physiological function.

Through its interaction with cell membranes, alcohol has been demonstrated to alter signal transduction at various locations (Dolganiuc et al., 2006; Szabo et al., 2007) and signaling proteins and ion channels. Alteration in the function of receptors and other signaling molecules leads to changes in the operation of many signaling pathways that mediate many important processes, culminating in tissue-specific damage as a result of Alcohol's modification of fundamental signaling pathways (Jung et al., 2011).

The impact of ethanol on the gastric mucosa may be linked to a disruption in the balance between defensive and offensive components in the gastric mucosa. Gastric slime layer, mucosal blood flow, HCO3-, prostaglandins, epidermal growth factor, and epidermal cell renewing factors are found in the gastric mucosa, whereas defensive factors include gastric slime layer, mucosal blood flow, HCO3-, prostaglandins, epidermal growth factor, and epithelial cell renewing (Preedy, 2014).

Ethanol damages the stomach mucosal barrier in a dose-dependent manner, disrupting the mucosal barrier, exfoliation of the surface epithelium, and microcirculatory abnormalities. Whereas in the indirect pathway, multiple pathogenic factors and mediators will be produced in response to the initial damage of ethanol causes such as inflammatory and vasoactive substances, ischemia, and oxidative stress. In addition to interfering and interaction between many of these agents, all participate in the damage caused by ethanol.

Group A showed capillary congestion in the lamina propria and blood cells distributed throughout the mucosal cells as well as blood congestion in the submucosa. furthermore swelling and vacuolation in the cytoplasm of mucous neck cells and gland cells (chief and parietal cells), indicating an early stage of mucosa cell degeneration.

Findings are in line with several researchers' histology findings. Tarnawski and colleagues (2012) found that exposing the gastric mucosa to 40% ethanol causes severe injury to the microvascular endothelium, resulting in microvascular damage and microvessel rupture, erythrocyte extravasation, blood flow cessation, and fibrin deposition within the microvessels-all of which result in microvascular stasis and extensive microvessel rupture. Also, the experiments of Laine and Weinstein in 1988 on humans demonstrate a sub-epithelial hemorrhage of the gastric mucosa in chronic heavy drinkers (Haber & Kortt, 2021). the finding is also consistent with Yang et al., (2017) were indicated that ethanol caused severe hyperemia, epithelial cell loss, lamina propria mucosa lesions, and inflammatory cell infiltration. Furthermore, Szabo and colleagues (1988) showed that microvascular damage occurs as early as 1 minute following ethanol exposure in rats. After intragastric injection of concentrated Ethanol (Tarnawski et al., 2012).

The fact that microvascular damage occurs in areas where glandular and surface epithelial cells are not significantly impacted suggests that alcohol-induced gastric mucosal damage is largely directed at the microvascular endothelium (Tarnawski et al., 1988). similarly, our findings demonstrated capillary vessel congestion in the lamina propria and blood cell hemorrhage throughout the mucosal cells; damage also reached the submucosa via blood vessel congestion and rupture, leading blood cells to infiltrate into the interstitial tissue, as shown in figure (2-A), (2-B), (2-C).

The pathogenesis of the vascular lesion may be due in part to the direct effect of ethanol and its metabolites on microvascular endothelial cells, and a major proportion may be due to endogenous mediators. An important question that needs to be answered to explain these findings is how endothelial cells are injured before surface epithelial cells that are in direct touch with ethanol?

The existence of a protein called survivin, which mediates stomach epithelial cell cytoprotection against alcohol-induced damage, might be the solution to this issue. It was noticed that the expression levels of survivin in

gastric epithelial cells being higher (~ 8-fold) than the gastric endothelial cells (A. W. Jones, 2008; M. K. Jones et al., 2010).

It has been shown that exposing gastric epithelial cells to a sub-cytotoxic dose of ethanol promotes the accumulation of survivin protein, which lowers the cells' sensitivity to damage and apoptosis induced by repeated ethanol exposure at cytotoxic concentrations (M. K. Jones et al., 2010), the expression of these proteins is linked to mucosal protection (Yandrapu & Sarosiek, 2015). Survivin's expression pattern inside the gastric mucosa, along with its known anti-apoptotic activity, indicates that survivin is critical for gastric mucosal integrity and protection against damage (Chiou, Jones, et al., 2003; Chiou, Moon, et al., 2003). survivin has also been shown to be an important factor in angiogenesis and vascular injury and repair (Conte & Altieri, 2006; Simosa et al., 2005).

Due to the critical function of the stomach microvascular endothelium in transporting and supplying oxygen and nutrients to all mucosal components, its damage has severe repercussions for the whole mucosa. Thus, we can say the damage to the surface epithelium is a subsequent event that follows the rupture of the microvascular endothelium and results from its severe consequences that may influence the entire mucosa.

The fasting groups (30% ETOH) showed severe and deep damage compared to the fed groups. In addition to the microvascular damage mentioned earlier, the fed group (30% ETOH) showed varying degrees of epithelial lifting and rupture, shedding of the surface epithelium into the gastric lumen, extensive vacuolation in the cytoplasm of different types of mucosal cells, and ballooned degeneration in the deep part of the gastric gland.

In addition, the presence of neutrophils and mononuclear cells in the gastric mucosa, as well as many lymphocytes and eosinophils in the interstitial tissue and between the mucosa cells, showed acute inflammation in specific stomach regions.

Many experts concur with these findings; histological examination of Amirshahrokhi and Khalili., (2015) revealed that ethanol-induced stomach damage was characterized by gastric submucosal edema, extensive bleeding, neutrophil infiltration, and epithelial cell loss. According to Afroz et al., (2020), ethanol damages the gastric mucosa by disrupting the epithelial linings of the stomach, the surface epithelium was severely disrupted, and necrotic lesions penetrated deep into the mucosa, resulting in significant submucosal edemas and leukocyte infiltration.

Gazzieri et al., (2007) demonstrated similar results with absolute ethanol dosed orally to rats, resulting in classic alcohol damage features such as linear hemorrhagic lesions, significant submucosal edema, inflammatory cell infiltration, and epithelium loss in the stomach tissue. Raju et al., (2009) described a similar histopathological change in the surface epithelium, in which rapid ethanol penetration caused cell and plasma membrane damage, increased intracellular membrane permeability to sodium and water, and massive intracellular calcium accumulation, leading to cell death and exfoliation.

inflammation, oxidative stress, and nitric oxide are the most important elements that contribute to gastric mucosa damage resulting from ethanol consumption.

It is widely understood that the most indirect processes that cause stomach mucosal injury during alcohol consumption are the recruitment of leukocytes and the induction of inflammation. Both acute and chronic inflammatory diseases of the gastrointestinal system include leukocyte accumulation as a prominent histopathologic characteristic. The adhesion of circulating leukocytes to the endothelial lining of small blood vessels (mostly postcapillary venules), followed by leukocyte migration to the interstitial compartment, is the first step in the recruitment of leukocytes to sites of inflammation (Barrachina et al., 2005). Acute gastric mucosal

lesions have been related to neutrophil infiltration into the mucosal tissues of the stomach. Myeloperoxidase (MPO) is used to determine the amount of neutrophil infiltration into stomach mucosal tissues (W. Li et al., 2013).

The MPO activity is a biochemical marker of neutrophil infiltration in the damaged tissue (Keyvan Amirshahrokhi & Khalili, 2015). Other studies have discovered that ethanol increases acute gastric mucosal injury and neutrophil infiltration into injured gastric mucosal tissue, increasing MPO activity that is proportional to the degree of gastric mucosal damage. As a result, they proposed that MPO is a neutrophil indication (J. Yang et al., 2018). During neutrophil activation and degranulation, MPO catalyzes the interaction of the chloride ion with hydrogen peroxide (H2O2) to create hypochlorous acid (HOCL), which is harmful to cells. (K Amirshahrokhi & Ghazi-Khansari, 2012; Keyvan Amirshahrokhi, 2013) This finally results in stagnation of stomach blood flow and microvascular disruption, leading in bleeding and necrotic tissue damage (W. Li et al., 2013). This acid and all the events mentioned above may be responsible for a large percentage of the damage shown in our results, as evidenced by widespread vacuolation in the cytoplasm of different types of mucosal cells and degenerative ballooning in figures (3 -A), (3 -B), (3-C).

Regarding oxidative stress, ethanol's biological effects are directly linked to its metabolism; the conversion of ethanol to acetaldehyde creates reactive oxygen species and reactive nitrogen species (ROS and RNS), which, among other things, activate an immunological response and contribute to alcohol's toxicity (Hernández et al., 2016; Le Daré et al., 2019). The major source of ROS in chronic inflammation is phagocytic leukocytes; during inflammation, huge numbers of neutrophils and/or macrophages infiltrate the stomach mucosa, creating substantial levels of ROS (Bhattacharyya et al., 2014). The impact of various antioxidants on ethanol-induced stomach injuries in rats was investigated by Ligumskys and colleagues. They proposed that ethanol-induced stomach injury is linked to the production of oxygen-derived radicals that are not dependent on the xanthine oxidase system. Other research suggests that using Centella Asiatica plant extracts protects ethanol-induced stomach mucosal lesions by strengthening the mucosal barrier and decreasing the harmful effects of reactive oxygen species (ROS) (Simões et al., 2019).

During an inflammatory response, oxidative stress caused by polymorphonuclear neutrophils (PMNs) causes inter-endothelial junctions to open, allowing inflammatory cells to breach the microvascular endothelial barrier. The inflammatory cells that have moved not only aid in the clearing of infections and foreign particles, but also cause tissue damage (Mittal et al., 2014).

Excessive amounts of ROS damage cellular proteins, particularly cytoskeletal proteins, and alter the mucosal barrier, contributing to inflammation, as seen in our findings. Furthermore, excessive ROS causes inflammation by activating PMNs, resulting in further tissue damage. (Bhattacharyya et al., 2014). The presence of reactive oxygen species and the creation of an "oxidative environment" might hasten microvasculature failure as well as reduced microvascular dilator ability and flow regulation. These actions can alter the tissue's oxygen demand and supply, triggering a viscous inflammatory loop that aids inflammation growth ( Christopher, 2016).

**serological study**

**ethanol effect on the level of interferon-gamma**

In animal groups treated with (50 % ETOH), the histological changes became more prominent and reveal further damage, the finding showed extensive vacuolations that occupy a large area of the gastric mucosa, a clear case of ballooned degeneration of mucosa cells, and necrotic lesions of the gastric mucosa that appears as the lysis of the cell nucleus, with damage of some surface epithelial cells shown in figures (4-A) and (4-B). the degeneration and

necrosis extend deeply in the mucosa arrived the gastric glands that revealed cytoplasmic vacuolations and necrosis and the cellular atrophy of the gastric glands, also hemorrhage due to rupture of the blood vessel while the RBCs spread between the gland cells (figure 3 – 18). Besides the deleterious effects of ethanol in the preceding groups of animals, the severity of such damages may be related to the substantial increase in IFN-γ level, The current study's findings demonstrate a considerable increase in the serum level of IFN-γ in ethanol-treated rats, which increases gradually with increasing ethanol concentration, fasting state, and tissue damage described earlier.

For the same ethanol concentration, the fasting groups demonstrated a substantial rise in IFN-γ level compared to the fed groups, when these findings are compared to the severity of the histological alterations in the animal groups, we discover that the increased IFN-γ level is linked to the severity of the histological alterations. Thus, the severity of inflammation in the treated groups develops concurrently with an increase of interferon level is considered,

The finding that alcohol intake is one of several variables which might trigger an increase in pro-inflammatory cytokines is in line with a number of prior research. Other studies show substantial and strong increases in cytokine, notably IFN-γ, following alcohol intake (van de Loo et al., 2020).

During inflammation, neutrophils and mononuclear cells infiltrate the stomach mucosa, stimulating the production and release of numerous pro-inflammatory mediators (Keyvan Amirshahrokhi & Khalili, 2015); IFN-γ represents a crucial event in the onset of a variety of inflammatory illnesses (Javanmard & Dana, 2012). Chronic alcohol exposure induced systemic pro-inflammatory IFN- and IL-17 responses in mice, according to Frank et al., (2021), and these responses are likely important in the development of alcohol-related illnesses.

Previous research has linked inflammatory diseases to the release of pro-inflammatory cytokines and inflammatory mediators such as NO. (Golab et al., 2009; Kadkhodaee et al., 2009; Pacher et al., 2007). IFN-**γ** may contribute to gastric mucosal damage by excessively increasing its concentration as Kak et al., (2018) mentioned, " Over-activity of IFN-γ cause excessive tissue damage, necrosis, and inflammation and may contribute to disease pathology" or by triggering the expression of iNOS in vascular endothelial cells, which produces nitric oxide. other studies indicate that cytokines can induce the expression of a calcium-independent NO synthase isoform, which is critical in inflammation, immune injury, and pathophysiologic control of local blood flow (Lamas et al., 1992)

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