Effect of Starvation on Rat Gastric and jejunal Epithelium
A Histopathological and Immunohistochemical Study
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Abstract:
Starvation is a severe reduction in vitamins, nutrients, and energy intake, and is the most extreme form of malnutrition. In human, prolonged starvation causes permanent organ damage and, eventually, death. A number of factors may result in starvation as anorexia nervosa, bulimia nervosa, intentional fasting, prolonged coma, stroke, inability to obtain food or severe gastrointestinal diseases beside poverty and over-population.

The objective of this work was to determine the effects of starvation on gastric and jejunal epithelium in starved rats. Eighty normal male adult albino rats, weighing 150-180 g, were divided into four equal groups. First group served as control (group I) and had a free access to food and water, second, third and fourth groups were given access to water only for 24 hours (group II), 48 hours (group III) and 72 hours (group IV) respectively. At the end of the experimental period, the rats were sacrificed, the stomach and jejunum were subjected to histopathological and immunohistochemical examinations. The histopathological examination of the stomach of control rats (group I) and the rats starved for 24 hours (group II) showed no pathological changes while the stomach of the rats starved for 48 hours (group III) showed gastric mucosal cells swelling, ballooning, vacuolar and hydropic degeneration with necrotic changes in the form of lyses, nuclear pyknotic changes, nuclear fragmentation, and appearance of apoptotic bodies. Mononuclear inflammatory infiltration was seen, all changes were predominant and more severe in the rats starved for 72 hours (group IV). At the same time, the jejunum of the rats starved for 48 hours (group III) showed fragmentation of intestinal villi, focal glandular epithelial cells, and mononuclear inflammatory cell infiltration in submucosa with necrotic changes was seen. All changes were more severe in the rats starved for three days (group IV). No pathological changes can be detected in control rats (group I) and the rats starved for 24 hours (group II). Immunohistochemical staining of stomach and jejunum revealed that Bcl-2 was expressed in the rats starved for 24, 48 and 72 hours. At the same time, Bcl-2 was not expressed in all rats of control group. In conclusion, this study reveals that starvation can induce marked apoptotic and severe histopathological changes in the gastric and jejunal epithelium.
**Introduction:**

Starvation means deficiency of calories due to reduction of all components of a normal diet (*Symmers and Wright, 1966*). It occurs in individuals of all ages, much information about this condition was gained from postmortem studies in prisons camp during World War II (*Anderson and Kissane, 1985*). Starvation is generally the result of accident or ill treatment and also in negligence, some examples of conditions that may result in death from starvation are: inability to eat (as a result of natural diseases such as carcinoma of esophagus), voluntary refusal of food (fasting to death or hunger strike), mental disease (anorexia nervosa, Schizophrenia), deliberate withholding of food, losing way in vast desert, criminal neglect of children or infirm old person (*Madea, 2004*).

In mammals, changes in morphology of the digestive tract as well as digestive and transport function occurs with age or in response to dietary changes (*Raul et al., 1982*). The jejunal mucosa which is the site of maximal nutrient absorption exhibits a tremendous capacity to adapt in response to acute or chronic nutritional demands such as malnutrition and short fasting periods. The acute deprivation of food also results in the destruction of the intestinal villous tips, fissuring, and marked shortening of the villi (*Aldewachi et al., 1975*). The epithelium of gastrointestinal tract is continuously renewed through a balance between cell multiplication and cell death (*Potten, 1997*). Cell proliferation, which is extensive in view of the large surface area of small bowel, occurs by mitosis in the intestinal crypts (*Wolf et al., 1999*).

The epithelium of the gastrointestinal tract is continuously renewed through a balance between cell multiplication and apoptosis (programmed cell death). Apoptosis is a distinctive form of cell death that occurs predominantly under genetic control (*Michalopoulos and Defrances,*
It may occur in response to physiological situations such as during development, as homeostatic phenomenon to maintain cell populations in tissues, or as a defensive mechanism during immune reactions. Pathological apoptosis occurs when irreversible cell injury occurs (Cotran et al., 1999).

Cells undergoing apoptosis are characterized morphologically by condensation of nuclear chromatin into caps at the edge of nucleus and detachment from their neighbors. There is no mitochondrial swelling or early rupture of the plasma membrane as in necrosis. Blebs develop on the cell surface and the cell fragment (apoptotic bodies) which are recognized by macrophages and phagocytes (Taub, 1996). Finally, there is destruction of remaining cellular elements. Unlike necrosis, cells which swell, apoptotic cells characteristically shrink.

Another cardinal feature of apoptosis is cleavage of DNA fragments which are multiples of 180-200 basepairs in size, by cleavage between nuclosomes by endonucleases (Watson et al., 1995). This produces a characteristic ladder appearance when DNA is subjected to agarose gel electrophoresis (Brown et al., 1993).

Molecules that regulate apoptosis include members of Bcl-2 family which either promote apoptosis (Bcl-xs, Bad, Bax) or suppress apoptosis (Bcl-xl, Bcl-2) (Cao et al., 2000). Cell death occurs in small bowel epithelial cells by two distinctly different mechanisms; cell necrosis and apoptosis (Steller, 1995). Necrosis in gastrointestinal tract is characterized morphologically by cellular swelling, dilatation of mitochondria and endoplasmic reticulum, and flocculation of nuclear chromatin. Later, there is rupture of plasma, nuclear and organelle membranes, which leads to destruction of the cell. Large contiguous population of cells undergoes necrosis simultaneously and an inflammatory response is evoked (Wyllie et al., 1980). The Bcl-2 proto-oncogene encodes a 25-KDa protein that is involved in the regulation of cell death by inhibiting apoptosis through
mechanisms which are not yet defined. Its over expression provides a growth advantage to tumor cells by blocking apoptosis. Bcl-2 does not act alone but forms heterodimers with other family members including Bax to regulate apoptosis system (Mcleod and Murry, 1999). In Forensic science, starvation is of great importance. So, histopathological and immunohistochemical study of gastric and jejunal epithelium will help to give an idea about the changes that occur in stomach and jejunum and whether apoptosis enhanced by starvation.

**Material and Methods:**

This study is performed upon eighty adult male albino rats weighing 180-200 grams each, aged around three months. They were acclimatized to laboratory conditions for one week where they housed in a special stainless steel cages. They were fed the basic laboratory suitable commercial diet until beginning of the experiment. The animals were divided randomly into four equal groups, each contained 20 rats. The first group served as a control group and given free access to food and water through the period of the experiment up to the sacrifice, while the second, third and fourth groups were given access to water only for 24, 48, 72 hours respectively. At the end of the experimental period, the animals were sacrificed by cervical dislocation; the stomach and jejunum of each rat were dissected, fixed in 10% formalin and prepared in paraffin sections. Three sections of 4 micron-thickness were obtained for every rat and subjected to the following stains:

**I. Histological stains:**

One section for each animal is stained with hematoxylin and eosin (H&E) (Drury and Wallington, 1980).
II. Immunohistochemical technique:

The other two paraffin-embedded tissue sections were mounted on poly-lysine coated slides and heated at 60°C for 30 minutes then deparaffinized and rehydrated through a series of xylene and alcohol before staining. Antigen retrieval was done using microwave treatment in 10 mM citrate buffer (Neo-markers, cat"AP-9003), PH 6, endogenous peroxidase was blocked with 3% hydrogen peroxide for 20 minutes. These sections were washed three times with cold 0.01 phosphate buffered saline (PBS). After blocking with 10% normal rabbit serum, the sections were incubated with rabbit anticow polyclonal antibody against Bcl-2 protein (Santacruz Biochemicals, Santa Cruz CA, dilution 1:50). The immunohistochemical stain was performed using the streptavidin / biotin complex technique (ABC kit – Vector Laboratories, Burlinguine, CA). The primary antibody was incubated for 2 hours for Bcl-2 protein. The ABC reaction was developed in the presence of Diamino Benzedine (DAB) supplement with hydrogen peroxide. Lastly, sections were counterstained with Mayer's Hematoxylin (Brown, 1997).

Interpretation of immunohistochemical staining results:

The stained slides were then microscopically examined by two observers using the parameters and the semi-quantitative criteria for Bcl-2 expression. The pattern of expression was nuclear membrane and cytoplasmic staining of glandular epithelial cell cytoplasm as dark brown staining. According to Zirbes et al. (1998):

- 0 : negative cases (no cells stained).
- Low staining : weak- intensity brown cytoplasmic staining.
- High expression : strong- intensity brown cytoplasmic staining.
III- Counting the apoptotic bodies:

Applying the distinctive morphological features of apoptotic cells, with marked condensation of the chromatin and cytoplasm with or without nuclear fragments, as described by Zirbes et al. (1998), their incidence was examined in hematoxylin and eosin stained sections under high power magnification (x40 objective and x10 ocular). Apoptotic index (AI) values were calculated after examining at least 1000 nuclei in randomly selected fields from both gastric and intestinal components for each case. The frequencies were recorded as the number of positive cells (n) per 10 high power field (HPF).

The data of the present study were statistically analyzed by using Z- and t-tests

**Results:**

A- Histopathological results for the examined groups:

- Changes in gastric mucosal cells:

  Microscopical examination of the stomach of the control rats (group I) showed relatively thick mucosa that consists of three layers, the surface epithelium which is simple columnar epithelium; corium (lamina propria) which is connective tissue layer between the epithelium and muscularis mucosa and contains reticular fibers, lymphocytes, plasma cells, gastric glands, blood vessels, lymphatics, and nerves; and lastly, muscularis mucosa which consists of an inner circular and outer longitudinal muscle layers. There is no histopathological changes can be detected in the stomach of the control rats (group I) and the rats starved for 24 hours (group II) (fig.1). While the microscopical examination of the stomach of the rats starved for 48 hours (group III) showed ballooning, vacuolar and hydropic degeneration of gastric mucosal cells with necrotic changes in the form of lyses, nuclear pyknotic changes, nuclear fragmentation, and appearance of apoptotic bodies. At the same
time, inflammatory foci formed of mononuclear cells mainly lymphocytes, histiocytes, eosinophils were detected (fig.2-A). These changes were predominant in the rats starved for 72 hours (group IV) than in the rats starved for 48 hours (group III) (fig.2-B).

Table (1) shows the statistical analysis of histopathological finding in the stomach. Starvation resulted in histopathological changes in the gastric epithelium that were significantly increased in groups III, IV as compared to group I and a non significant increase in group II as compared to group I as regarding ballooning of cells and hydropic degeneration. As regarding necrotic changes and mononuclear cellular infiltration, there was a significant correlation in groups II, III as compared to group I. At the same time, the correlation was non-significant in group II as compared to group I.
Table (1): Histopathological changes affecting gastric epithelium of the rats starved for 24 hours (group II), 48 hours (group III), 72 hours (group IV) and control group (group I).

<table>
<thead>
<tr>
<th></th>
<th>Group I</th>
<th>Group II</th>
<th>Group III</th>
<th>Group IV</th>
<th>Z</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No</td>
<td>%</td>
<td>No</td>
<td>%</td>
<td>No</td>
<td>%</td>
</tr>
<tr>
<td>Ballooning of cells</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>5%</td>
<td>14</td>
<td>70%</td>
</tr>
<tr>
<td>Hydropic degeneration</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>10%</td>
<td>11</td>
<td>55%</td>
</tr>
<tr>
<td>Necrotic changes</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>5%</td>
<td>12</td>
<td>60%</td>
</tr>
<tr>
<td>Mononuclear cellular infiltration</td>
<td>1</td>
<td>5%</td>
<td>5</td>
<td>25%</td>
<td>13</td>
<td>65%</td>
</tr>
</tbody>
</table>

Z\(_1\): For group II (control group) versus group I.
Z\(_2\): For group III (control group) versus group I.
Z\(_3\): For group IV (control group) versus group I.
P \(>0.05\): the difference is non-significance.
P \(<0.05\): the difference is significant.

N.B. Number of rats in each group equals 20 rats.

- Changes in intestinal mucosal cells:

Examination of jejunal mucosa of (group I) showed villi and crypts, the mucosa consists of intestinal epithelium which covers the villi and lines the crypts and formed of simple columnar, corium of connective tissue that contain blood vessels, lymphocytes, nerves, lymphatic, macrophages and plasma cells; and lastly, muscularis mucosa which consists of an inner circular and outer longitudinal smooth muscle layers underlying the bottoms of the crypts. Goblet cells are present between the columnar cells over the villi and in the superficial parts of
the crypts. There is no histopathological changes can be detected in the jejunum of the control rats (group I) and those starved for 24 hours (group II)(fig.3). Examination of intestinal villi of group III revealed cloudy swelling, fragmentation, with focally detached glandular epithelial cells. Submucosa showed mononuclear inflammatory cell infiltrate mainly lymphocytes, histiocytes, and occasional plasma cells. Necrotic changes were seen in the form of diffuse homogenous pink areas surrounded by inflammatory cells, apoptotic body formation were detected (fig.4-A). All previous changes were more severe in group IV than group III (fig. 4-B).

Table (2) shows the statistical analysis of histopathological changes of the jejunum of the rats . All pathological changes including cloudy swelling, fragmented villi, detached glandular cells, inflammatory cellular infiltration and necrotic changes showed a significant correlation in groups III, IV as compared to group I while the correlation was non-significant in group II when compared with group I.
Table (2): Histopathological changes affecting jejunal epithelium of the rats starved for 24 hours (group II), 48 hours (group III), 72 hours (group IV) and control group (group I).

<table>
<thead>
<tr>
<th></th>
<th>Group I</th>
<th>Group II</th>
<th>Group III</th>
<th>Group IV</th>
<th>Z</th>
<th>P</th>
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<tbody>
<tr>
<td></td>
<td>No</td>
<td>%</td>
<td>No</td>
<td>%</td>
<td>No</td>
<td>%</td>
</tr>
<tr>
<td>Cloudy swelling</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>5%</td>
<td>12</td>
<td>60%</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Z2 4.0</td>
<td></td>
</tr>
<tr>
<td>Fragmented villi</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>10%</td>
<td>13</td>
<td>65%</td>
</tr>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Z2 4.4</td>
<td></td>
</tr>
<tr>
<td>Detached glandular cells</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>5%</td>
<td>12</td>
<td>60%</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Z2 4.0</td>
<td></td>
</tr>
<tr>
<td>Inflammatory cellular infiltration</td>
<td>1</td>
<td>5%</td>
<td>2</td>
<td>10%</td>
<td>14</td>
<td>70%</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Z2 4.2</td>
<td></td>
</tr>
<tr>
<td>Necrotic changes</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>5%</td>
<td>11</td>
<td>55%</td>
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<td></td>
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<td></td>
<td></td>
<td>Z2 3.9</td>
<td></td>
</tr>
</tbody>
</table>

N.B. Number of rats in each group equals 20 rats.
Z1: For group II (control group) versus group I.
Z2: For group III (control group) versus group I.
Z3: For group IV (control group) versus group I.
P>0.05: the difference is non-significance.
P<0.05: the difference is significant

**B- Immunohistochemical results:**

In parallel with hematoxylin and eosin staining, stomach and jejunal sections were examined immunohistochemically using the avidin-peroxidase complex technique.
- Immunohistochemical staining results of the stomach:

Examination of the stomach of the control rats (group I) showed that Bcl-2 was not expressed (fig. 5), while the rats starved for 24 hours (group II) revealed that the expression of Bcl-2 in all rats examined (100%) with low intensity stain 8 out of 20 rats examined (40%) and high intensity stain 12 out of 20 rats (60%). At the same time, Bcl-2 was expressed in all rats starved for 48 hours (group III) with low intensity stain 4 out of 20 (20%) and high intensity stain 16 out of 20 rats (80%). In the rats starved for 72 hours (group IV), Bcl-2 was expressed in all rats (100%) with low expression in 3 out of 20 (15%) and high expression in 17 out of 20 rats (85%). These results revealed that the relationship was statistically significant (P < 0.05) (table 3, figs 6- A,B).

Table 3: Bcl-2 expression in stomach of the rats starved for 24 hours (group II), 48 hours (group III), 72 hours (group IV) and control group (group I).

<table>
<thead>
<tr>
<th></th>
<th>Group I</th>
<th>Group II</th>
<th>Group III</th>
<th>Group IV</th>
<th>Z</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No</td>
<td>%</td>
<td>No</td>
<td>%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative</td>
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<td>0</td>
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<td>0</td>
<td>0</td>
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</tr>
<tr>
<td>Low Intensity</td>
<td>0</td>
<td>0</td>
<td>8</td>
<td>40%</td>
<td>4</td>
<td>20%</td>
</tr>
<tr>
<td>High intensity</td>
<td>0</td>
<td>0</td>
<td>12</td>
<td>60%</td>
<td>16</td>
<td>80%</td>
</tr>
<tr>
<td></td>
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</tr>
<tr>
<td></td>
<td>Z1</td>
<td>6.3</td>
<td>Z2</td>
<td>6.3</td>
<td>Z3</td>
<td>6.3</td>
</tr>
<tr>
<td></td>
<td>P&lt;0.05</td>
<td>P&lt;0.05</td>
<td>P&lt;0.05</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Z1</td>
<td>3.20</td>
<td>Z2</td>
<td>2.11</td>
<td>Z3</td>
<td>1.38</td>
</tr>
<tr>
<td></td>
<td>P&lt;0.05</td>
<td>P&lt;0.05</td>
<td>P&gt;0.05</td>
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<td></td>
</tr>
<tr>
<td></td>
<td>Z1</td>
<td>4.1</td>
<td>Z2</td>
<td>5.1</td>
<td>Z3</td>
<td>5.3</td>
</tr>
<tr>
<td></td>
<td>P&lt;0.05</td>
<td>P&lt;0.05</td>
<td>P&lt;0.05</td>
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</tbody>
</table>

N.B. Number of rats in each group equals 20 rats.
Z1: For group II (control group) versus group I.
Z2: For group III (control group) versus group I.
Z3: For group IV (control group) versus group I.
P>0.05: the difference is non significance.
P<0.05: the difference is significant
• Immunohistochemical staining results of the jejunum:

Bcl-2 was not expressed in the jejunum of the control rats (group I) (fig.7) but it was expressed in all rats of group II (100 %) with low intensity stain in 15 out of 20 (75%) and high intensity in 5 out of 20 rats (25%). At the same time, group III showed expression of Bcl-2 in all examined rats (100%) with low intensity stain of 7 out of 20 (35 %) and with high intensity of 13 out of 20 rats (65%). The same results was found in group IV, Bcl-2 was expressed in all examined rats (100%) with low expression in 2 out of 20 (10%) and with high expression of 18 out of 20 rats (90%). This relationship was statistically significant (P < 0.05) (table 4 , figs 8-A,B).

Table 4:Bcl-2 expression in jejunum the rats starved for 24 hours (group II), 48 hours (group III), 72 hours (group IV) and control group (group I).

<table>
<thead>
<tr>
<th></th>
<th>Group I</th>
<th>Group II</th>
<th>Group III</th>
<th>Group IV</th>
<th>Z</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No</td>
<td>%</td>
<td>No</td>
<td>%</td>
<td>No</td>
<td>%</td>
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<tr>
<td>Negative</td>
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<tr>
<td></td>
<td>Z_1</td>
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<td>Z_2</td>
<td>6.3</td>
<td>Z_3</td>
<td>6.3</td>
</tr>
<tr>
<td></td>
<td>P&lt;0.05</td>
<td>P&lt;0.05</td>
<td>P&lt;0.05</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low intensity</td>
<td>0</td>
<td>0</td>
<td>15</td>
<td>75%</td>
<td>7</td>
<td>35%</td>
</tr>
<tr>
<td></td>
<td>Z_1</td>
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<td>2.9</td>
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<td>1.4</td>
</tr>
<tr>
<td></td>
<td>P&lt;0.05</td>
<td>P&lt;0.05</td>
<td>P&gt;0.05</td>
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<tr>
<td>High intensity</td>
<td>0</td>
<td>0</td>
<td>5</td>
<td>25%</td>
<td>13</td>
<td>65%</td>
</tr>
<tr>
<td></td>
<td>Z_1</td>
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<td>4.4</td>
<td>Z_3</td>
<td>5.7</td>
</tr>
<tr>
<td></td>
<td>P&lt;0.05</td>
<td>P&lt;0.05</td>
<td>P&lt;0.05</td>
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</table>

N.B. Number of rats in each group equals 20 rats.
Z_1: For group II (control group) versus group I.
Z_2: For group III (control group) versus group I.
Z_3: For group IV (control group) versus group I.
P>0.05: the difference is non significance.
P<0.05: the difference is significant
III- Counting the apoptotic bodies results:-

Table (5) shows the incidence of apoptotic bodies in both the gastric and jejunal epithelial cells of the starved rats. Examination of gastric epithelium revealed a significant increase in group II (152.1), group III (273) and group IV (395.75) as compared to group I (31.75). (figs 9 A-B).

Table (5): Incidence of apoptotic bodies in stomach and jejunum of the rats starved for 24 hours (group II), 48 hours (group III), 72 hours (group IV) and control group (group I).

<table>
<thead>
<tr>
<th></th>
<th>Group I</th>
<th>Group II</th>
<th>Group III</th>
<th>Group IV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>31.75</td>
<td>152.1</td>
<td>273</td>
<td>395.75</td>
</tr>
<tr>
<td>± S.D.</td>
<td>19.3</td>
<td>47.4</td>
<td>41.8</td>
<td>24.9</td>
</tr>
<tr>
<td>t</td>
<td>-</td>
<td>10.5257</td>
<td>23.4312</td>
<td>51.6579</td>
</tr>
<tr>
<td>p</td>
<td>-</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

N.B. Number of rats in each group equals 20 rats.
P<0.0001: the difference is significant.
Fig.(1): A photomicrograph of a stomach section of control group (H&E x 200).

Fig.(2): A photomicrographs of a stomach sections of rats starved for 48 hours (A) and for 72 hours (B) showing hydropic degeneration of gastric mucosal cells, inflammatory cellular infiltration and necrotic changes. (H&E x 200).
Fig.(3): A photomicrograph of a jejunal section of control group (H&E x 200).

Fig.(4): A photomicrographs of a jejunal sections of rats starved for 48 hours (A) and for 72 hours (B) showing cloudy swelling, detached glandular epithelial cells, mononuclear inflammatory cellular infiltration with necrotic changes. (H&E x 200).
**Fig. (5):** Immuno-stained stomach of the control rats showing negative expression of Bcl-2 antibody (absent brown nuclear membrane/cytoplasmic staining) in glandular epithelial cells (Streptavidin – biotin DAB X 200).

**Fig. (6):** Immuno-stained rat stomach showing low Bcl-2 expression (A) and high Bcl-2 expression (B) of Bcl-2 antibody (brown nuclear membrane/cytoplasmic staining) in glandular epithelial cells (Streptavidin – biotin DAB X 200).
**Fig. (7):** Immuno-stained jejunum of the control rats showing negative expression of Bcl-2 antibody (absent brown nuclear membrane/ cytoplasmic staining ) in glandular epithelial cells (Streptavidin – biotin DAB X 200).

**Fig. (8):** Immuno- stained rat jejunum showing low Bcl-2 (A) expression and high Bcl-2 expression (B) of Bcl-2 antibody (brown nuclear membrane /cytoplasmic staining) in glandular epithelial cells (Streptavidin–biotin DAB X 200).
Fig. (9): A photomicrographs of a stomach (A) and jejunal (B) sections of rats starved for 24, 48 or 72 hours in the form of shrunked nuclei with crumpled chromatin surrounded by clear perinuclear halo showing apoptotic bodies. (H&E x 200).

**Discussion:**

It is well known that the status of feeding versus fasting has important consequences upon the structure and function of the digestive tract ([Secor and Diamond, 1997](#)). The effects of food deprivation on gut morphology and function have been described in many animals ([Secor et al., 1994](#)) and in humans ([Groos et al., 2003](#)).

This study was performed to detect the histopathological changes in the gastric and jejunal epithelium of the starved rats and counting the apoptotic bodied as well as to detect if apoptosis enhanced by starvation or not with an immunohistochemical study. Starvation resulted in histopathological changes in the gastric epithelium in the form of ballooning of cells and hydropic degeneration. Moreover, necrotic changes and mononuclear cellular infiltration. These results were consistent with the results done by [Bengmark and Jeppsson (1995)](#) who found that dietary restriction results in severe metabolic impairment and gastrointestinal hypofunction in animals and humans. In addition, [Núñez](#)
et al. (1996) reported that starvation may evoke a massive inflammatory as well as necrotic changes in gastrointestinal glandular epithelial cells and explained their results on a bases that malnutrition induced by dietary restriction and severe starvation produces a series of metabolic changes that lead to reduction in body weight, depression of immunocompetence and altered function of the digestive system, particularly of the liver and small intestine. These changes have a profound effect on variables such as brush border enzymatic activity, mucosal mass, protein and DNA contents and mucosal integrity (Firmansyah et al., 1989; Núñez et al., 1996; Ortega et al., 1996).

Furthermore, Bengmark and Jeppsson (1995) provided evidence that starvation may provoke a massive translocation of microbes, microbial products or microbial residues. This could initiate or maintain clinical sepsis that could ultimately lead to severe inflammatory reactions and multiple organ failure. Moreover, increased intestinal permeability to macromolecules facilitates the movement of antigens into the bloodstream, thus increasing the risk of provoking local or systemic immune sensitization (Uhnoo et al., 1990; Boza et al., 1996). Similarly, this increased permeability alters the plasma amino acid profile (Roosouw and Pettifor, 1990). Depletion of systemic glutathione levels has been reported in a number of stress conditions, including short-term food deprivation and chronic dietary protein deficiency (Hum et al., 1991; Grimble et al., 1992). The fall in glutathione levels under these conditions implies that a persistent oxidative load leads to the net consumption of reduced glutathione in excess of the body’s ability to resynthesize the molecule (Jahoor et al., 1995).

The histopathological changes of the jejunum of the rats showed that there was a detached glandular cells and necrotic changes, cloudy swelling, fragmented villi and inflammatory cellular infiltration. These results were coincide with the results done by Stevens (1958) who
reported variable results regarding histologic intestinal mucosal changes after starvation as decreased cell numbers in both the crypts and villi, hydropic changes, inflammatory and necrotic foci in rats starved for 5 days. In addition, Clarke (1975) demonstrated decreased villous height in rats fasted for 3 days. However, Holt et al. (1986) showed that, after 3 days of starvation, villous height decreased in the duodenum and was unchanged in the ileum of one strain of rats while villous height increased in the ileum of another strain. Although average crypt depth, villous height, and cells per villous were unchanged in mice, the number of villi per millimeter of bowel decreased in the small bowel of fasted mice, indicating a decrease in villous density as a possible cause for the decrease in bowel weight (Chappell et al., 2003).

The previously identified mechanism of small bowel atrophy involved decreased proliferation in the intestinal crypts. One study demonstrated a decreased crypt cell production rate in the small bowel of mice starved 24 hr (Goodlad and Wright, 1984), while another showed decreased cell division and rate of cell migration in mice starved 54–96 hr (Brown et al., 1963). In addition, starved rats had decreased mitosis in the crypts of the duodenum (Stevens, 1958). Chappell et al. (2003) reported decrease proliferation in all diet-restricted mice, even with only a 25% decrease in intake and suggested that only a minor decrement in intake is sufficient to cause a decrease in intestinal epithelial cell proliferation. They speculated that the relative absence of atrophic luminal agent leads to cell cycle arrest. The mechanism by which atrophy occurs appears to be an initial decrease in proliferation, to which is added an increase in apoptosis at more extreme starvation.

Martins et al. (2001) found that at the light microscopic level, both the duodenal and jejunal mucosa from fasted rats were apparently similar to those of fed controls. When analyzed under the electron microscope,
however, important differences could be observed between the duodenal and jejunal mucosa from fasted and fed animals. Epithelial cells with morphological signs of degeneration (increased density of cytoplasm, occasional vacuolation, and fragmentation of microvilli) were observed at a significantly higher frequency in duodenal and jejunal mucosa of plain fasted rats by comparison with normal fed controls. Morphological changes of microvilli were especially conspicuous, by fragmentation and the appearance of a huge number of variously shaped and sized vesicles. The villous surface covered by these fragmented microvilli was impressively extensive in the small intestine of fasted rats.

A short period of fasting increases apoptosis at the intestinal villous tips \((Iwakiri \ et \ al., \ 2001)\) and cell shedding into the intestinal lumen \((Clarke, \ 1975)\). Both an increase in epithelial cell death and a decrease in crypt cell proliferation contribute to the shortening of the villi, these phenomena resulting in a decrease of the jejunal mucosal mass by one half after 5 days of fasting \((Dunel-Erb \ et \ al., \ 2001)\).

Programmed cell death or apoptosis is the most common form of eukaryotic cell death and is the way in which the body eliminates unwanted cells and helps maintain homeostasis. Under normal situations, this process is not accompanied by an inflammatory response, but dysregulation may lead to pathologic conditions. The turnover of intestinal epithelial cells is a well-regulated process with immature crypt cells moving up to the villous to become terminally differentiated cells at the villous tips 2–3 days later. These differentiated cells ultimately undergo apoptosis and are either extruded from the tips or phagocytosed by cells within the villous. Thus, the number of cells undergoing apoptosis at any time will determine the level of mature, differentiated cells on the crypt-villous axis and will give an indication of repair mechanisms after injury or stress. Under normal conditions, apoptotic
cells are very few and are located at the very tips of intestinal villi and, occasionally, in the crypts (Boza et al., 1999).

In this study, immunohistochemical examination of the epithelial cells (stomach and jejunum), revealed that cells stained by the monoclonal antibody Bcl-2 for immunohistochemical study showed variable degree of apoptotic changes. These results were coinciding with the results done by Holt et al. (1986) who found that apoptosis increased in the aged, calorie-restricted rats. Also, Elmes (1977) reported that fasted rats for 24, 48, and 72 hr showed an increase in jejunal apoptosis. The clinical relevance of starvation in inducing programmed cell death has been delineated in several studies (Holt et al., 1998; Noda et al., 1998). The apoptotic process has been shown to play a clinical role in the pathogenesis of intestinal barrier dysfunction and increased small bowel epithelial apoptosis. It has been associated with simultaneously increased bidirectional permeability of the intestinal barrier, resulting in a reduced uptake of intraluminal nutrients, increased permeability to macromolecules and most important, increase permeability for bacteria translocation. Translocation of enteric bacteria, toxin, and gut-derived factors carried in the mesenteric lymph can lead to multiorgan failure and increased mortality (Carter et al., 1990; Sun et al., 1998).

In the present study, there was an apparent increase in the number of apoptotic bodies after starvation and this is in agreement with a study done by Holt et al. (1998) who demonstrated an increase in apoptotic bodies during starvation and chronic caloric restriction. Also, Chappell et al. (2003) found that apoptosis occurs randomly along the crypt–villous axis in the mucosa of unstressed proximal small bowel, but with the stress of complete starvation, apoptotic cells increase toward the luminal end of the villous. Possibly, the signal for death may target the more differentiated cells at the villous tip. Cao et al. (2000) had
demonstrated that the frequency of apoptosis in the antropyloric region of
the stomach varies with the prandial state and that Nitric Oxide synthesis
is required for the increased apoptosis seen after fasting. On starvation,
the apoptotic index of the general epithelium and of the gastrin but not of
the somatostatin cells increased significantly.

Jeschke et al. (2000) demonstrated increase gut epithelial apoptosis
in response to both starvation and burn, which was associated with
decreased mucosal weight and protein content and they suggested that the
loss of mucosal mass and protein content is augmented by lack of
stimulation in gut epithelial cell proliferation. This is a different finding
from the mouse model, where increased apoptosis was accompanied by
increased epithelial cell proliferation (Wolf et al., 1999).

In conclusion, this study revealed that starvation of rats can induce
marked detectable apoptotic changes associated with severe
histopathological abnormalities in the gastric and jejunal epithelium.
More studies, clinical and experimental, are needed to learn more about
the hazards effects of starvation on different body organs by using other
different methods and more advanced techniques such as transmission
and scanning electron microscopy.

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References:-


الملخص العربي

بحث أثر التجويع على معدة و أمعاء فئران التجارب البيضاء
دراسة هستوباثولوجية و هستوكيميائية مناعية

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هناك العديد من العوامل التي تؤدي إلى النقص الحاد في الفيتامينات و المغذيات و الطاقة المتناولة مما قد يؤثر سلبا على صحة الإنسان و سلامة أعضاءه و أحياه وقته. وذكر هنا بعض هذه العوامل التي قد تؤدي إلى الموت جوعا: الحزايل أو الشرب العمري، الامتناع عن تناول الغذاء، السكوثة الدماغية و الغذوية الطويلة، عدم القدرة على الحصول على الغذاء، الإصابة بالأمراض المعدية المميتة، هذا إلى جانب الفقر الشديد و الاكتظاظ السكاني الذي قد يؤدي أحيانا لحداث المجاعات.

الهدف من هذا البحث هو دراسة آثار التجويع على معدة و أمعاء الفئران، و قد أجريت هذه الدراسة على ثمانين فأرا من فئران التجارب البيضاء البالغة التي تتراوح أوزانهم من 180-150 جرام، و كانت مقسمة إلى أربعة مجموعات متساوية العدد: المجموعة الأولى بثبات المجموعة الضابطة و كان لها حرية الوصول للماء و الطعام طوال مدة الدراسة، أما المجموعات الثانية و الثالثة و الرابعة كانت لها حرية الوصول إلى الماء فقط لمدة 42، 28 و 84 ساعة على التوالي؛ و في نهاية مدة الدراسة تم ذبح الفئران و أخذ العينات من المعدة و الأمعاء الطويلة (الجزء الثاني- الجيوجينم) و ذلك لفحصهم عن طريق الدراسات التالية: الهستوباثولوجية و الهستوكيميائية المناعية. و قد أوضحت نتائج فحص المجموعة الأولى عدم وجود أي تغيرات باثولوجية في الأنسجة المعدية و المعوية و أظهرت المجموعة الثانية نفس النتائج.

أما بفحص خلايا معدة المجموعة الثالثة فقد أظهرت وجود عدة تغيرات هستوباثولوجية مثل: تورم و انتفاخ و تكزز بالخلايا و أخرى تغيرات في نواة الخلايا. هذا و قد أظهرت الدراسة نفس التغيرات السابقة قد أصابت فئران المجموعة الرابعة و لكنها كانت أكثر شدة. و بفحص نسيج أمعاء فئران المجموعة الثالثة و جدنا تجذب بذوب الأجسام مع وجود خلايا التهابية و تكزز بالخلايا. و هذه التغيرات المرضية كانت أكثر شدة عند فحص أمعاء فئران المجموعة الرابعة.

أما بالنسبة لنتائج الدراسة الهستوكيميائية المناعية لخلايا معدة و أمعاء الفئران فقد أوضحت بدرجات متفاوتة في كل من المجموعة الثانية و الثالثة و الرابعة. و قد انتهت هذه الدراسة إلى أن التجويع يسبب تغيرات مرضية شديدة في أمعاء و معدة الفئران.