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## **Histological and Ultra Structural Study of 5-fluorouracil-induced Small Intestinal Mucosal Damage in Rats**

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### **ABSTRACT**

The fluoropyrimidine 5-fluorouracil (5-FU) is one of the most commonly used chemotherapeutic agents. It is particularly mucotoxic and causes many gastrointestinal manifestations. The aim of this study was to assess the light and electron microscopic aspects of 5-fluorouracil induced cytotoxicity on the small intestinal mucosa. Sixteen adult male albino rats divided into two groups; control and FU groups were used in this study. Control group rats received a single intraperitoneal saline injection, equivalent to the 5-fluorouracil dose. FU group rats were given a single intraperitoneal dose of 5-fluorouracil (5-FU) (150 mg kg<sup>-1</sup>) and were subdivided into 2 subgroups (FU3 and FU9). Four rats from each subgroup were sacrificed 3 and 9 days after 5-FU injection. Specimens from the jejunum were obtained and processed for transmission electron microscopic study. Paraffin sections were prepared and stained with Haematoxylin and Eosin and Periodic acid-Schiff's stains. In FU3 group, 5-fluorouracil administration resulted in detachment and loss of surface epithelium, shortening and marked exfoliation and denudation of the villi. The cell architecture of both villi and crypts was disturbed, with marked widening of the intercellular spaces. Ultrastructural degenerative changes in the form of loss of microvilli, cytoplasmic vacuolations, many lysosomes, dilated and vesiculated rER, swollen mitochondria with disintegrated cristae and degranulated goblet cells were also demonstrated. Recovery of these changes and restore of the normal structure were observed in FU9 group with only some residual ultrastructural changes. It could be concluded that 5-FU induces marked histological and ultrastructural degenerative changes in the small intestinal mucosa.

**Key words:** 5-fluorouracil, small intestine, ultrastructure

### **INTRODUCTION**

The fluoropyrimidine 5-fluorouracil (5-FU) is an antimetabolite drug and is considered one of the most commonly used chemotherapeutic agents in clinical oncology practice (Fata *et al.*, 1999). It is derived from a naturally occurring pyrimidine uracil in which a hydrogen atom at C-5 position is replaced by a fluorine atom (Longley *et al.*, 2003). It acts by blocking DNA synthesis via inhibition of thymidylate synthase enzyme (McCarthy *et al.*, 1998).

The mucotoxic effect of 5-fluorouracil has been reported. It has a long and consistent history of inducing mucosal damage. The mucositis is characterized by inflammatory and ulcerative lesions of any part of the gastrointestinal tract resulting in a range of symptoms that negatively impact

patients' quality of life and ability to tolerate chemotherapy. The most common patients' complaints are mouth and throat pain, dysphagia, nausea, vomiting, abdominal pain and diarrhea (Sonis, 2010; Lalla and Keefe, 2011).

The current study was performed to assess the light and electron microscopic aspects of 5-fluorouracil induced cytotoxicity on the small intestinal mucosa.

## **MATERIALS AND METHODS**

The study was conducted on 16 adult male albino rats weighing (200 -250) grams. The rats were housed in separate cages under constant environmental conditions and were allowed free access to food and water. The duration of the experiment was 9 days. The rats were randomly assigned into two groups and treated as follows:

- **Control group:** This group consisted of 8 rats. At day 1 of the experiment, they received saline (a single intraperitoneal injection), equivalent to the 5-fluorouracil dose
- **FU group:** This group was comprised of 8 rats which received a single intraperitoneal dose of 5-fluorouracil (5-FU) (150 mg kg<sup>-1</sup>) at day 1 of the experiment (Torres *et al.*, 2008)

The drug 5-Fluorouracil is a product of Biosyn, Arzneimittel, GmbH, Germany with a trade name; 5-fluorouracil biosyn. It is available in the form of ampoules, with a concentration of 1000 mg 20 mL<sup>-1</sup> of 5-fluorouracil.

Animals of this group were subdivided into 2 subgroups (4 rats each) according to the time of sacrifice:

- **FU3 group:** Rats sacrificed 3 days after 5-FU injection
- **FU9 group:** Rats sacrificed 9 days after 5-FU injection

At each duration of the experiment (3,9 days after 5-FU), four animals from each group (control and FU) were sacrificed.

The animals were anaesthetized intraperitoneally with thiopental sodium (10-15 mg kg<sup>-1</sup>) and then the abdomen was opened surgically by a midline incision. Specimens of the jejunum were obtained and flushed with isotonic saline.

Paraffin sections (5 um thick) were prepared and stained with:

- Haematoxylin and eosin (H and E) stain (Gamble and Wilson, 2002)
- Periodic acid-Schiff's (PAS) reaction (Totty, 2002)

Small fragments, about 1 mm<sup>3</sup> of tissues, were obtained and processed to prepare semithin and ultrathin sections for transmission electron microscopic study using JEOL-JEM-100 SX transmission electron microscope (Woods and Stirling, 2008) in the Electron Microscopy unit, Tanta University, Egypt.

## **RESULTS**

**Light microscopic results:** Light microscopic examination of the control rat small intestine (jejunum) revealed that the wall was formed of mucosa, submucosa, muscularis externa and serosa. The mucosa showed villi and crypts (Fig. 1). The intestinal villi had connective tissue cores and



Fig. 1: A photomicrograph of control rat jejunum. The wall consists of mucosa, SM: Submucosa, ME: Muscularis Externa and Arrows: Serosa, V: Intestinal Villi and C: Crypts (H and E  $\times 100$ )

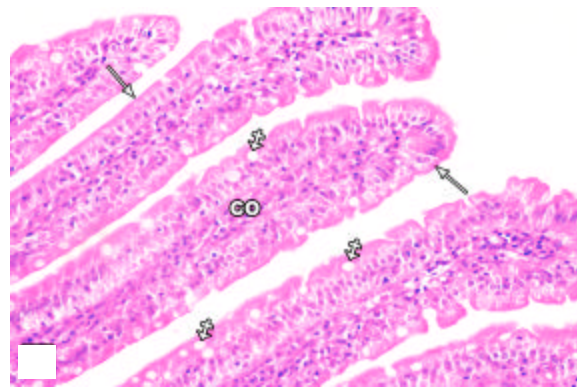


Fig. 2: A higher magnification of the previous figure showing the intestinal villi with their connective tissue cores (CO) covered by columnar absorbing cells with a brush border (arrows) and goblet cells (crossed arrows) (H and E  $\times 400$ )

were covered by columnar absorbing cells with a brush border and goblet cells (Fig. 2). The crypts were lined with columnar absorbing cells and goblet cells and showed many mitotic figures at their bases. The lamina propria surrounded the crypts and extended upward to form the core of the villi and downwards to the muscularis mucosa. The muscularis externa consisted of inner circular and outer longitudinal layers of smooth muscle fibers (Fig. 3).

The control rat small intestine showed a strong PAS positive reaction in the brush border and also in the goblet cells of the villi and crypts (Fig. 4).

The small intestine of FU3 group showed denudation of villi and marked loss of the surface epithelium which was sloughed into the lumen (Fig. 5a). The columnar absorbing cells covering the villi showed either reduction in height, flattening, disruption or sloughing. Groups of enlarged cells with pale vacuolated cytoplasm and pale nuclei were seen over the villi (Fig. 5b). Short broad and blunt villi with disruption and sloughing of the surface epithelium were observed (Fig. 6). The

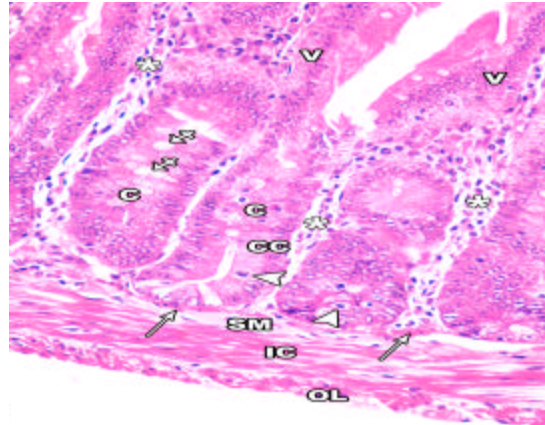


Fig. 3: A photomicrograph of control rat jejunum showing the mucosa consisting of Villi (V), Crypts (C), lamina propria (\*) and muscularis mucosa (arrows). The lamina propria contains mononuclear cells and extends into the villus core and surrounds the crypts. The crypts are lined with columnar absorbing cells (CC) and goblet cells (crossed arrows). Note the mitotic figures (arrow heads) at the base of the crypts. The muscularis mucosa separates the crypts from the submucosa (SM). The muscularis externa is formed of inner circular (IC) and Outer Longitudinal (OL) smooth muscle layers (H and E  $\times 400$ )

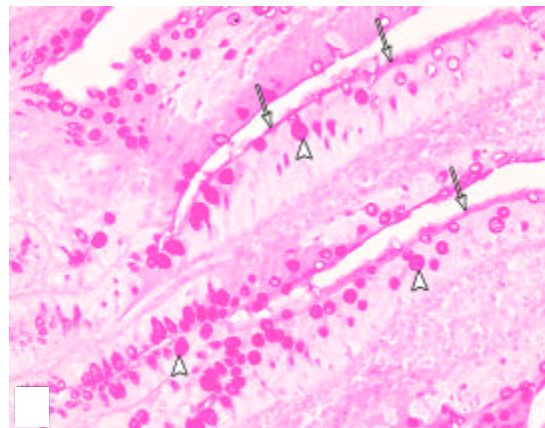


Fig. 4: A Photomicrograph of the control rat jejunum showing a strong PAS positive reaction in the brush border (arrows) and goblet cells (arrow heads) (PAS  $\times 400$ )

lamina propria in the core of the villi was detached from the surface epithelium and showed empty spaces (Fig. 6) and heavy cellular infiltration (Figs. 5, 6).

The intestinal crypts were distorted, short and few and were mostly lined by enlarged cells with pale vacuolated cytoplasm and pale disintegrated nuclei (Fig. 7).

In PAS stained sections, the brush border was interrupted and exhibited weak or moderate PAS reaction or was even lost in some areas. Marked reduction in goblet cell number over the villi and crypts was noticed. Most of the cells contained few mucous granules and others were completely depleted and appeared PAS negative (Fig. 8).

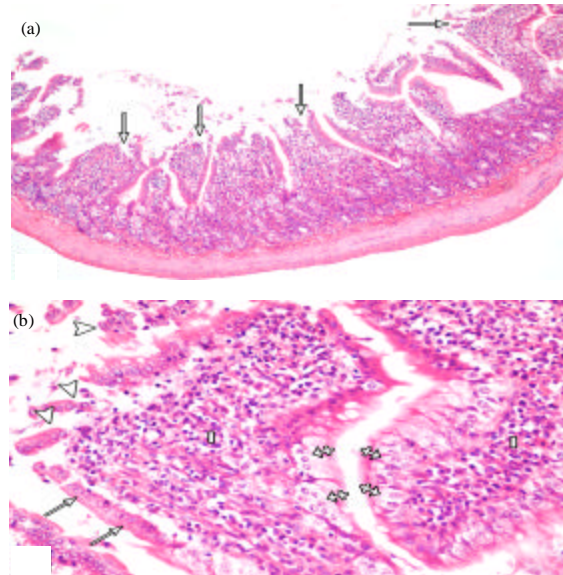


Fig. 5(a-b): Photomicrographs of the jejunum of FU3 group. 5a: Shows extensive sloughing of the surface epithelium into the lumen (arrows) and denudation of the villi. 5b: Higher magnification of Fig. 5a. The columnar absorbing cells covering the villi are either reduced in height (arrows), enlarged with pale vacuolated cytoplasm and pale nuclei (crossed arrows) or extensively sloughed into the lumen (arrow heads). Note the excessive cellular infiltration (I) in the villi cores (H and E: a  $\times$ 100, b  $\times$ 400)

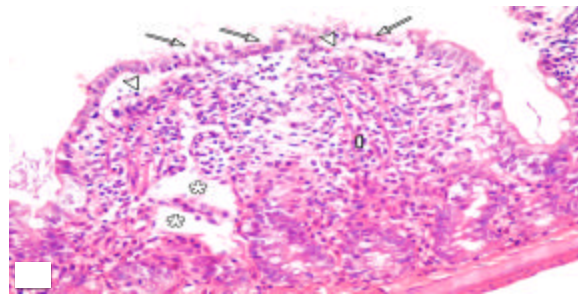


Fig. 6: A photomicrograph of the jejunum of FU3 group showing short, broad and blunt villus with disruption and sloughing of the surface epithelium (arrows). The lamina propria is detached from the surface epithelium (arrow heads) and contains empty spaces (asterisks) and heavy cellular infiltration (I) (H and E  $\times$ 400)

The FU9 group showed almost histological signs of full recovery. Almost all the intestinal villi and crypts appeared intact and displayed a normal appearance similar to that of the control (Fig. 9).

In PAS stained sections, FU9 group showed full recovery; a moderate to strong PAS positive reaction was demonstrated in the continuous, well-defined brush border. The goblet cells were apparently increased in number, especially over the villi and displayed a strong PAS positive reaction. Most cells were expanded with mucous granules (Fig. 10).

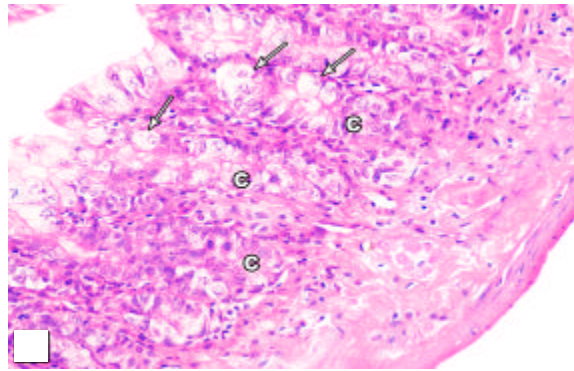


Fig. 7: A Photomicrograph of the jejunum of FU3 group. The Crypts (C) are few and ill-defined. The crypt lining cells (arrows) are enlarged with pale vacuolated cytoplasm and pale stained nuclei (H and E  $\times 400$ )

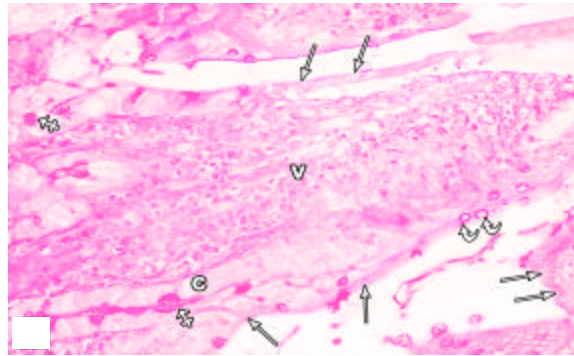


Fig. 8: A Photomicrograph of the jejunum of FU3 group showing loss of the brush border (arrows) over the Villi (V). The goblet cells (crossed arrows) are markedly decreased in number over the villi and Crypts (C). Some goblet cells appear completely depleted of mucus and are PAS negative (curved arrows) (PAS  $\times 400$ )

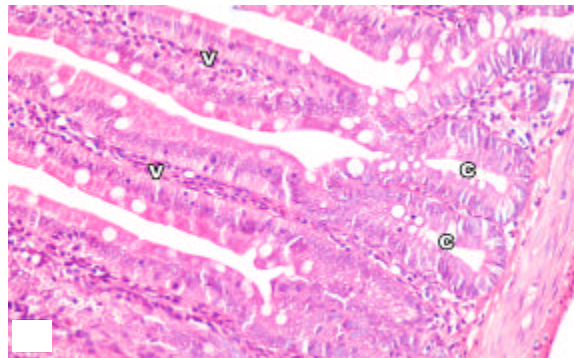


Fig. 9: A Photomicrograph of the jejunum of FU9 group. The Villi (V) are intact and completely covered with epithelium. Intact Crypts (C) with normal epithelial lining are also observed (H and E  $\times 400$ )

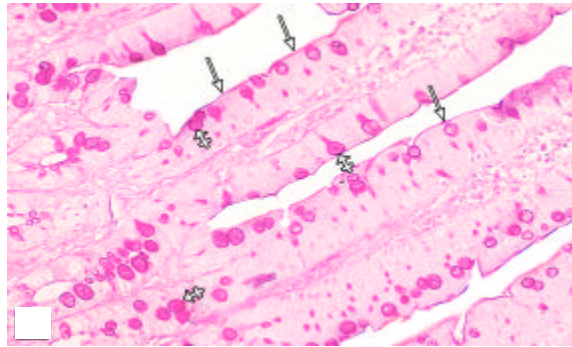


Fig. 10: A Photomicrograph of the jejunum of FU9 group. A moderate to strong PAS positive reaction is observed in the well-defined brush border (arrows). The goblet cells of the villi and crypts (crossed arrows) are strongly positive and distended with mucous granules. The number of goblet cells is apparently increased. (Compare versus Figs. 4, 8) (PAS  $\times 400$ )

### **Electron microscopic results**

**Control group:** Ultrastructural examination of the control rat small intestine revealed that the epithelium covering the villi and crypts was formed mainly of columnar absorbing cells and fewer goblet cells (Figs. 11, 12). Intraepithelial lymphocytes were also seen (Fig. 12). The columnar absorbing cells possessed microvilli on their luminal surface and basal oval vesicular nuclei with prominent nucleoli. The cytoplasm was rich in mitochondria, rER cisternae, free ribosomes and contained few lysosomes (Fig. 11). The neighboring cells were firmly attached near the luminal

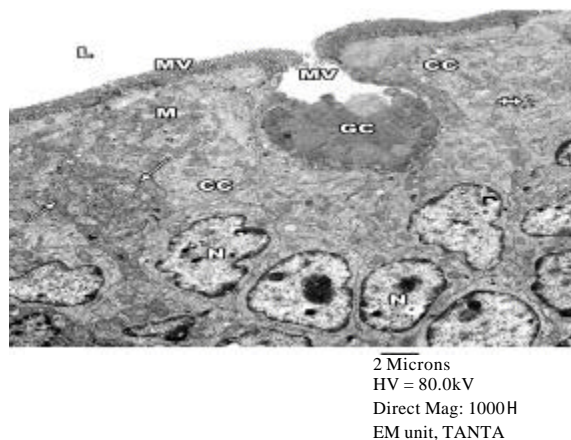
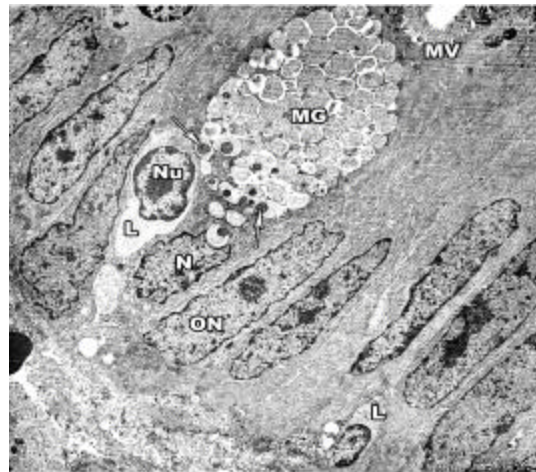


Fig. 11: An electron micrograph of the control rat jejunum showing the villus epithelium. The columnar absorbing cells (CC) have Microvilli (MV) on their luminal surface and basal oval Nuclei (N). The cytoplasm of the columnar cells is rich in Mitochondria (M), rER profiles (arrows) and few lysosomes (crossed arrows). A Goblet Cell (GC) containing mucous globules and bearing few Microvilli (MV) is located between the columnar cells and opens into the Lumen (L) (TEM  $\times 1000$ )





2 microns  
HV = 80.0kV  
Direct Mag: 1000H  
EM unit, TANTA

Fig. 12: An electron micrograph of the control rat jejunum showing the epithelial lining of the crypt. The columnar absorbing cells have apical Microvilli (MV) and basal Oval Nuclei (ON). The goblet cell has an apical part distended with large rounded mucous globules (MG) of moderate electron density and a thin basal part containing the Nucleus (N). Some of the mucous globules have electron dense cores (arrows). Migrating Lymphocytes (L) are seen between the cells. The lymphocyte had a vesicular nucleus with prominent nucleolus (Nu) and its cytoplasm appeared of low electron density (TEM  $\times 1000$ )

surfaces by junctional complexes that consisted of zonula occludens, zonula adherens and desmosomes. A prominent terminal web was seen under the microvilli and lateral interdigitations were seen between the cells (Fig. 13).

The goblet cells had few microvilli and were formed of an apical part distended with mucous globules of variable electron density and a constricted basal part containing a nucleus with prominent nucleolus. Some mucous globules had electron dense cores (Figs. 11, 12).

Enteroendocrine cells were seen among the intestinal epithelial cells. The nucleus appeared vesicular with prominent nucleolus. The cytoplasm was of lower electron density than the surrounding cells and contained small rounded granules that were mostly concentrated at the basal region (Fig. 14).

Paneth cells were seen in groups at the base of the intestinal crypts. Paneth cell had apical microvilli and the cytoplasm was filled with large spherical granules surrounded with clear halos. Most of the granules were electron dense. The basal part contained a vesicular nucleus indented by the secretory granules, rER cisternae, mitochondria and lysosomes (Fig.15).

The lamina propria was seen under the villus epithelium and around the crypts and contained collagenous fibers, fibrocytes, lymphocytes, plasma cells and eosinophils. The plasma cell had a characteristic cart wheel nucleus, mitochondria and parallel rER profiles. The eosinophil had a bilobed nucleus and contained characteristic ellipsoid granules with coarse crystalloid cores (Fig. 16).

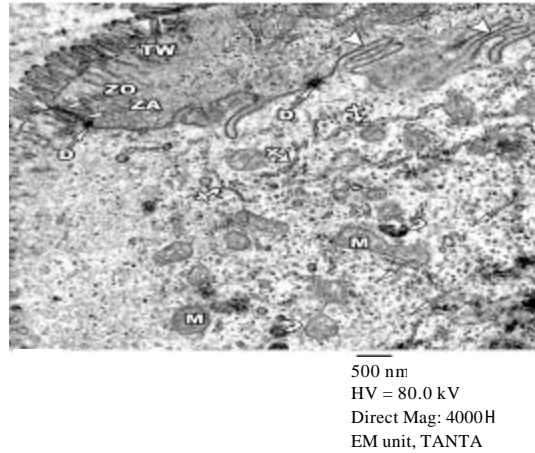


Fig. 13: An electron micrograph of the control rat jejunum showing the apical region of the columnar absorbing cells. The neighboring cells are firmly attached near the luminal surface by junctional complexes consisting of Zonula Occludens (ZO), Zonula Adherens (ZA) and Desmosomes (D). Lateral interdigitations (arrow heads) are also seen between the cells. rER cisternae (crossed arrows), Mitochondria (M), few lysosomes (curved arrows) and free ribosomes (arrows) are seen in the cytoplasm. Note the Terminal Web (TW) under the microvilli (TEM  $\times 4000$ )

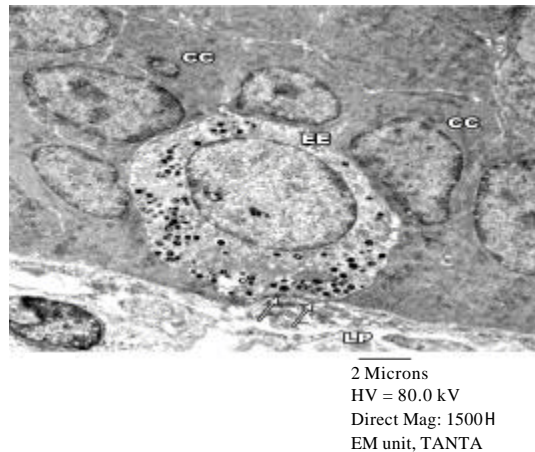


Fig.14: An electron micrograph of the control rat jejunum demonstrating an Enteroendocrine cell (EE). The cell rests on the basal lamina (arrows) and is surrounded by columnar absorbing cells (CC). The cytoplasm is of lower electron density than the surrounding cells and contains electron dense granules which are mainly concentrated in the basal region. Part of the Lamina Propria (LP) is seen under the epithelium (TEM  $\times 1500$ )

**FU group:** Electron microscopic examination of the intestinal villi of FU3 group revealed disturbed cell architecture with marked widening of the intercellular space; however, the cells were still bound to each other by cytoplasmic extensions (Fig. 17). The columnar absorbing cells demonstrated

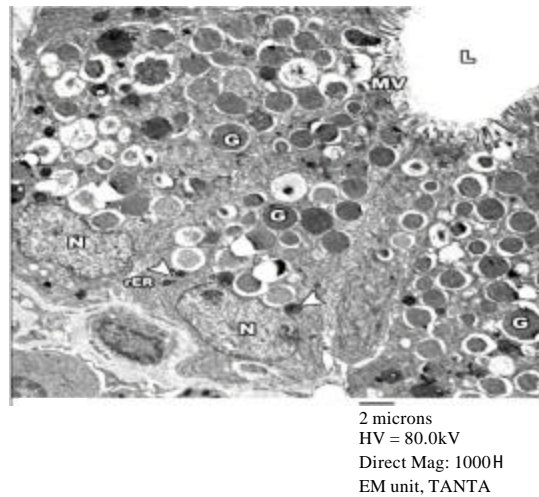


Fig.15: An electron micrograph of a crypt base of the control rat jejunum demonstrating a group of Paneth cells. The cells have apical Microvilli (MV) extending towards the Lumen (L) and basal Nuclei (N). Their cytoplasm is filled with large spherical Granules (G) surrounded by clear halos. Most of the granules are electron dense. rER cisternae and lysosomes (arrow heads) are also seen (TEM  $\times 1000$ )

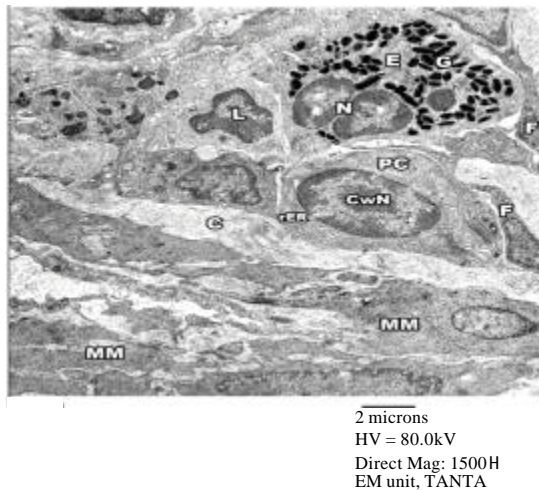
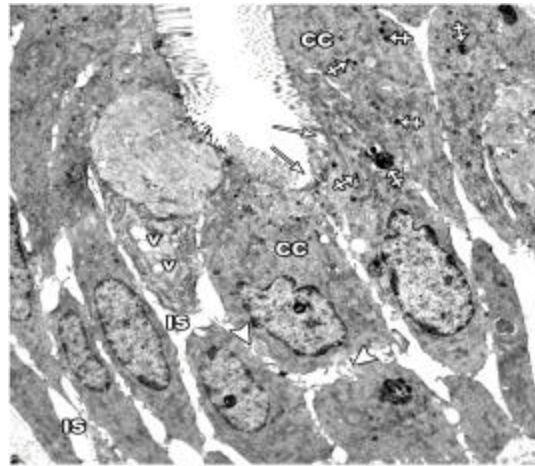


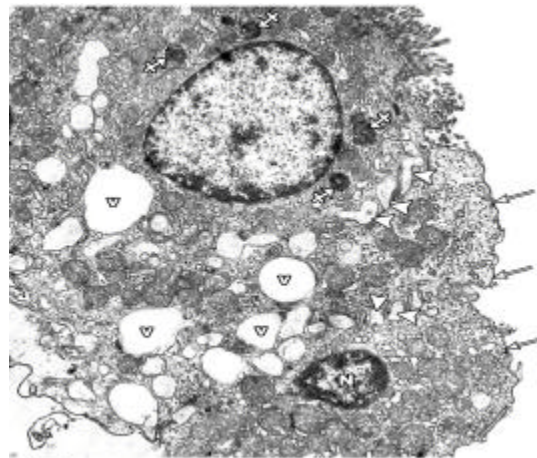
Fig. 16: An electron micrograph of the control rat jejunum showing the lamina propria formed of collagenous fibers (C) and cellular content. Fibrocytes (F), a Lymphocyte (L), a Plasma Cell (PC) and an Eosinophil (E) are seen. The plasma cell has a Cart wheel Nucleus (CwN) and numerous rER profiles. The eosinophil has a bilobed Nucleus (N) and characteristic ellipsoid Granules (G) with coarse crystalloid cores. The smooth muscle fibers of the Muscularis Mucosa (MM) are seen below the lamina propria (TEM  $\times 1500$ )

partial or even complete loss of microvilli. Some cells had pyknotic nuclei (Figs. 17, 18). The columnar absorbing cells also showed extensive lateral interdigitations (Fig. 19) and numerous



2 Microns  
HV = 80.0 kV  
Direct Mag: 1000H  
EM unit, TANTA

Fig.17: An electron micrograph of the jejunum of FU3 group showing disturbed cell architecture. The Intercellular Spaces (IS) between the villus cells are markedly widened but the cells are still bound to each other by cytoplasmic extensions (arrow heads). Areas of partial or complete loss of microvilli (arrows) and increased lysosomes (crossed arrows) are observed in the columnar absorbing cells (CC). Large Vacuoles (V) are seen in goblet cell cytoplasm (TEM  $\times 1000$ )



2 Microns  
HV = 80.0 kV  
Direct Mag: 1500H  
EM unit, TANTA

Fig.18: An electron micrograph of the jejunum of FU3 group showing the columnar absorbing cells of the villus. Two cells appear completely devoid of microvilli (arrows). Cytoplasmic Vacuolations (V), increased lysosomes (crossed arrows), pyknotic Nucleus (N) and widened intercellular spaces (arrow heads) are also observed (TEM  $\times 1500$ )

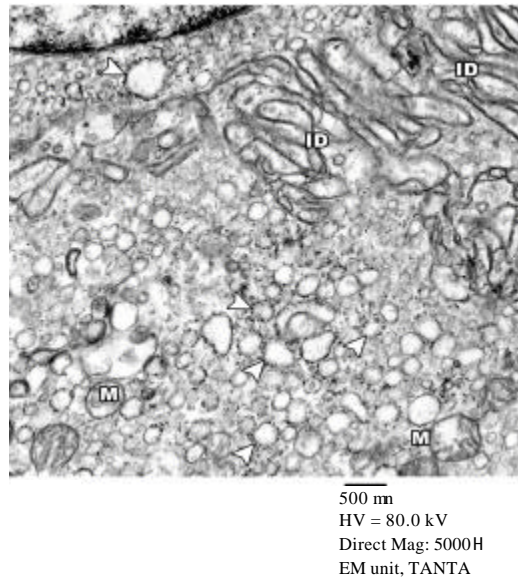


Fig. 19: An electron micrograph of the jejunum of FU3 group showing extensive lateral Interdigitations (ID) between two adjacent columnar absorbing cells. Vesiculated rER (arrow heads) are observed; some are partly degranulated. The Mitochondria (M) show disintegrated cristae (TEM  $\times 5000$ )

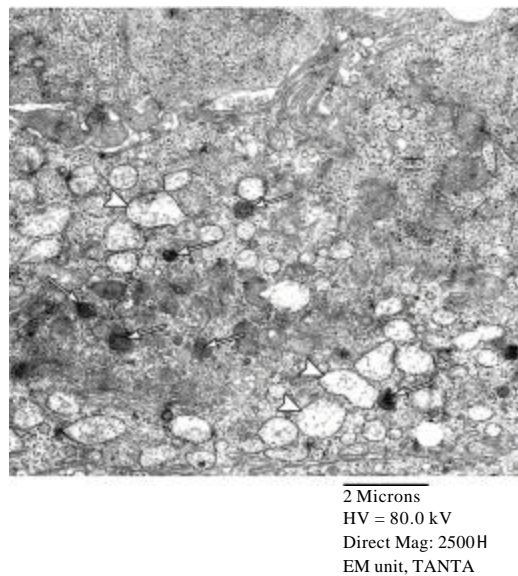


Fig. 20: An electron micrograph of the jejunum of FU3 group showing some columnar absorbing cells. The cytoplasm contains increased lysosomes (arrows) and swollen mitochondria with few, short, disintegrating cristae (arrow heads) (TEM  $\times 2500$ )

cytoplasmic vacuolations of different sizes (Fig. 18). Their cytoplasm contained increased lysosomes (Figs. 17, 18, 20), dilated and vesiculated rER; which may be partially degranulated and swollen mitochondria with few short disintegrated cristae (Fig. 20).

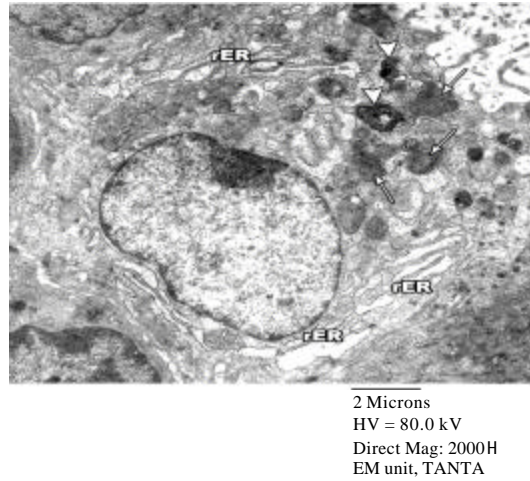


Fig. 21: An electron micrograph of the jejunum of FU3 group showing a goblet cell opening into the lumen. The cell is almost depleted of mucus; only few electron dense mucous granules are seen (arrows). The cytoplasm contains dilated rER cisternae and some lysosomes (arrow heads) (TEM  $\times 2000$ )

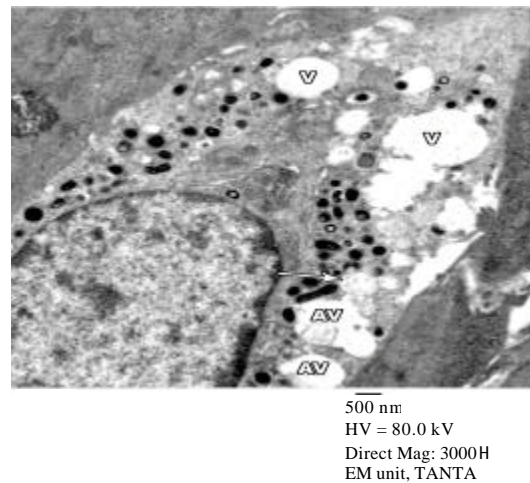


Fig. 22: An electron micrograph of an enteroendocrine cell of the jejunum of FU3 group. A swollen degenerated mitochondrion with disintegrated cristae (arrow), cytoplasmic Vacuolations (V) and Autophagic Vacuoles (AV) are seen (TEM  $\times 3000$ )

The goblet cells appeared almost depleted of mucus with only few cytoplasmic granules. Their cytoplasm contained dilated rER and some lysosomes (Fig. 21). Enteroendocrine cells demonstrated cytoplasmic vacuolations, autophagic vacuoles and swollen degenerated mitochondria with disintegrated cristae (Fig. 22). Paneth cells demonstrated dilated and vesiculated rER, large lysosomes and swollen degenerated mitochondria (Fig. 23). The lamina propria showed increased number of the eosinophils (Fig. 24).

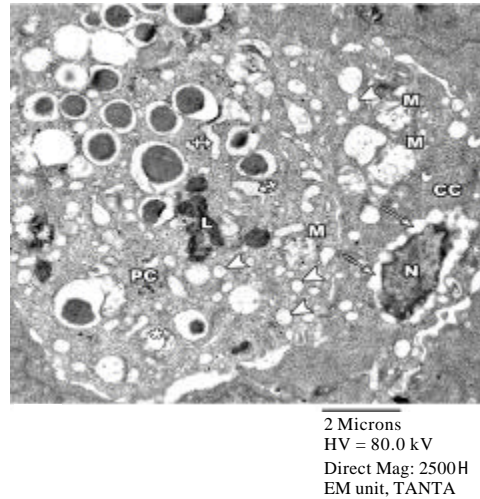


Fig. 23: An electron micrograph of the crypt base in the jejunum of FU3 group showing parts of a Paneth Cell (PC) and adjacent Columnar Cell (CC). The Paneth cell shows dilated (crossed arrows) and vesiculated rER (arrow heads), large Lysosomes (L) and swollen degenerated Mitochondria (M). One of the mitochondria shows a central cavity (asterisk). The columnar cell has a pyknotic Nucleus (N) with a very wide perinuclear space (arrows), vesiculated rER (arrow heads) and swollen Mitochondria (M) with disintegrated cristae (TEM  $\times 2500$ )

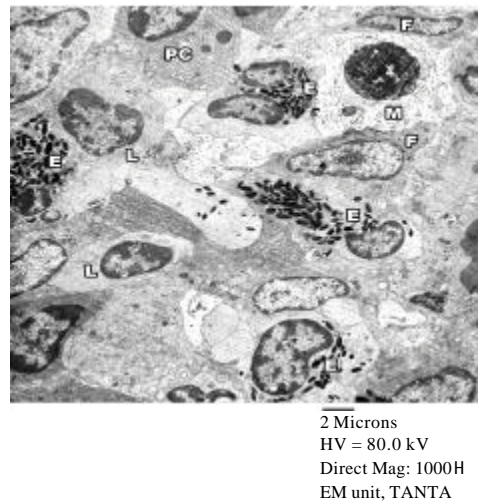


Fig. 24: An electron micrograph of the lamina propria of the jejunum of FU3 group showing an increased number of Eosinophils (E). A Plasma Cell (PC), Lymphocytes (L), a Macrophage (M) and Fibroblasts (F) are also seen in the lamina propria (TEM  $\times 1000$ )

The FU9 group revealed an intestinal structure, more or less, similar to that of the control. The luminal surface of the columnar absorbing cells was completely covered with regular, long

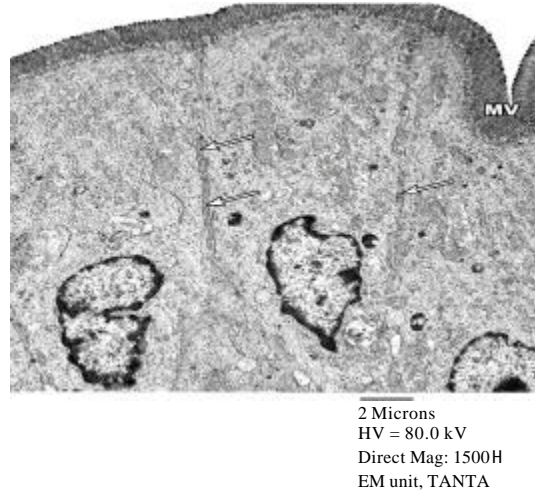


Fig. 25: An electron micrograph of the villus epithelium of the jejunum of FU9 group. The absorbing cells are more or less similar to the control and contain, more or less, intact organelles. The luminal surface is completely covered with regular long Microvilli (MV) and the lateral interdigitations (arrows) present between adjacent cells are similar to those of the control (TEM  $\times 1500$ )

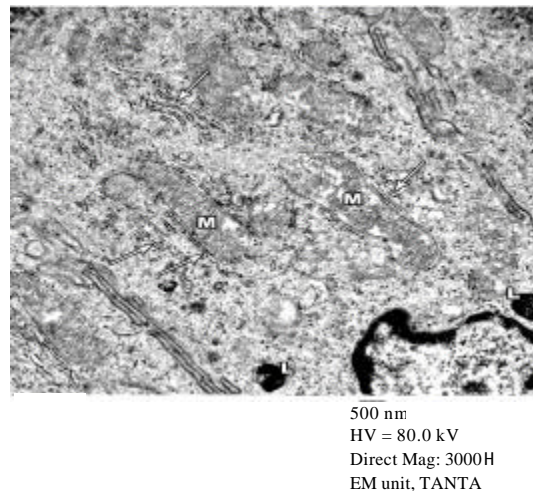


Fig. 26: A higher magnification of the previous figure. The apical cytoplasm of the absorbing cells contains Mitochondria (M) with intact cristae, intact rER cisternae (arrows) covered with attached ribosomes and some Lysosomes (L) (TEM  $\times 3000$ )

microvilli. The lateral cell interdigitations appeared similar to those seen in the control (Fig. 25). Intact organelles were seen in the cytoplasm (Fig. 26); however, swollen mitochondria with disintegrated cristae were still observed in some cells (Fig. 27). Goblet cells appeared normal and were distended with mucous globules (Fig. 27). The cytoplasm of the enteroendocrine cells showed some swollen, degenerated mitochondria (Fig. 28). Paneth cells contained intact rER, mitochondria and many lysosomes (Fig. 29).



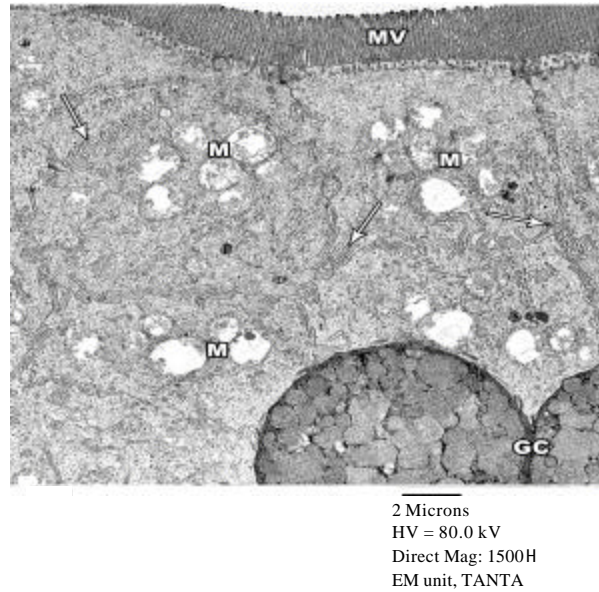


Fig. 27: An electron micrograph of the villus epithelium of the jejunum of FU9 group. Swollen Mitochondria (M) with disintegrated cristae are still observed in the cytoplasm of the columnar absorbing cells. Note the intact Microvilli (MV) and the lateral interdigitations between the cells (arrows). Parts of two Goblet Cells (GC) distended with mucous globules are seen (TEM  $\times 1500$ )

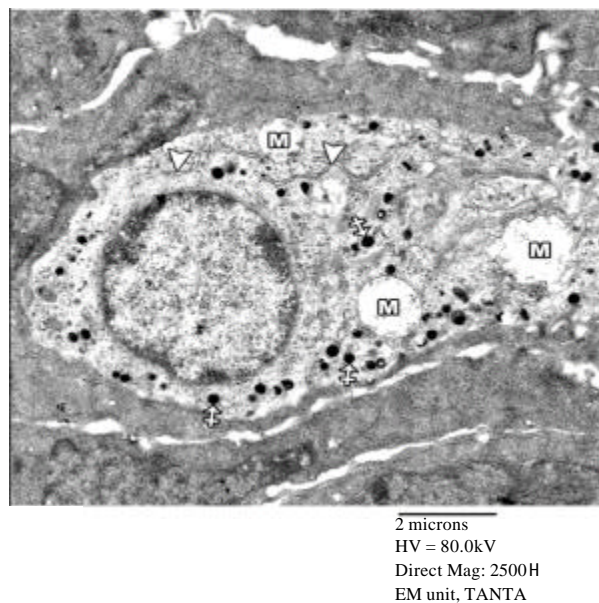


Fig. 28: An electron micrograph of an enteroendocrine cell of the small intestine (jejunum) of FU9 group. The cytoplasm of contains small electron dense granules (crossed arrows), rER (arrow heads) and degenerating swollen Mitochondria (M) with disintegrated cristae (TEM  $\times 2500$ )

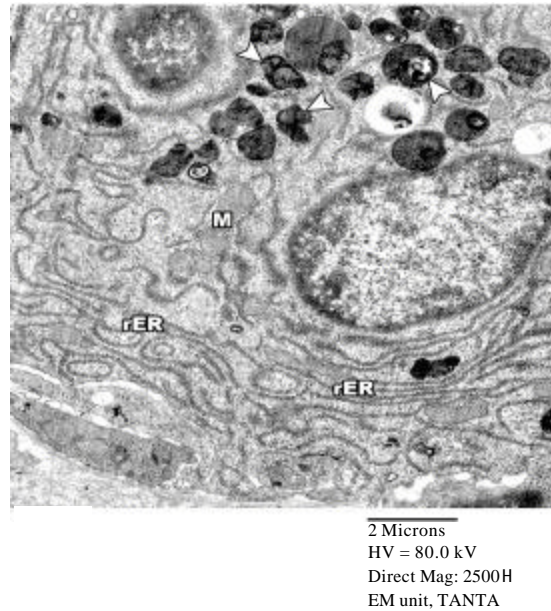


Fig. 29: An electron micrograph of a Paneth cell of the small intestine (jejunum) of FU9 group showing intact rER cisternae and Mitochondria (M). Many lysosomes (arrow heads) are observed (TEM  $\times 2500$ )

## DISCUSSION

Administration of chemotherapy in a wide variety of cancers is associated with various side effects, with toxicity to the gastrointestinal tract being a major clinical concern and a major cause of cancer treatment-related morbidity (Magne *et al.*, 2001). As the intestinal epithelium has a rapid cell turnover, it is vulnerable to chemotherapy (Yanez *et al.*, 2003). The chemotherapeutic agent 5-fluorouracil (5-FU) is an anti-metabolite drug that is widely used for anticancer therapy. This drug induces an intestinal damage known as mucositis (Soares *et al.*, 2011).

The current study has been performed to assess the light and electron microscopic aspects of 5-fluorouracil induced cytotoxicity on the small intestinal (jejunal) mucosa.

The present study revealed marked histological changes in rat small intestinal mucosa following fluorouracil administration. Different signs of mucosal damage were observed in FU3 group. The intestinal villi in the jejunum showed distortion, shortening, blunting and broadening. Apparent reduction in the number and depth of the crypts was also observed. Soares *et al.* (2008) reported significant villus shortening and blunting in the rat duodenum, jejunum and ileum by the third day after injection of a single dose of 5-FU. Logan *et al.* (2009) reported that 5-FU causes blunting and fusion of the rat jejunal villi and reduction in crypt length within 12-72 hour time period. Villus clubbing and atrophy were also observed within the first 24 hours after 5-FU injection (Stringer *et al.*, 2009).

Loss of surface epithelium, marked exfoliation and denudation of the villi were markedly observed in FU3 group. Similarly, Kolli *et al.* (2008) noticed destruction, exfoliation of the villi tips and degeneration of surface epithelium following methotrexate injection in rats. Light microscopic examination in the current study also revealed morphological alterations of the columnar absorbing cells in FU3 group. Some cells were reduced in height; others appeared pale and vacuolated with

pale nuclei. The presence of flattened and vacuolated enterocytes was previously observed in 5-FU induced mucositis (Soares *et al.*, 2008). Such pale vacuolated cells may have undergone hydropic degeneration as a consequence of 5-FU injury. Similar morphologic features of hydropic cells have been described by Levine and Saltzman (2004). Areas of hydropic epithelial degeneration were also found in the cheek pouches of hamsters following induction of oral mucositis by 5-fluorouracil injection (Leitao *et al.*, 2008).

In FU3 group, the lamina propria in the core of the villi was detached from the surface epithelium and showed empty spaces. A similar finding was observed in the rat small intestine after direct irradiation and was suggested to be due to the presence of edema between the villus columnar absorbing cells and the stroma (Cameron *et al.*, 2012).

Heavy cellular infiltration was observed in the lamina propria of FU3 group. Recently, Soares *et al.* (2011) reported an increase in the proinflammatory cytokines; tumor necrosis factor alpha (TNF $\alpha$ ) and interleukin 1-b (IL-1b) concentrations in the duodenum of 5-FU treated mice, resulting in inflammatory cell infiltration in the lamina propria. Granulocytic infiltration in the lamina propria was also reported as an early event of small intestinal irradiation mucositis (Cameron *et al.*, 2012).

Pritchard *et al.* (1998) stated that, following 5-FU, the stem cells in the crypts are prevented from subsequent replication, due to loss by apoptosis as well as cytostasis due to mitotic inhibition. The end results are lowered villi and crypts cellularity which is accompanied with shortening of the villi and crypts, loss of villous and crypt area and disappearance of the crypts over the first 2-3 days post treatment. Also, Duncan and Grant (2003) mentioned that following chemotherapy, the stem cells are damaged and no longer divide or differentiate into specific cell lineages. Cell renewal is affected by this process and the villus mucosa is not replaced, leading to a rapid loss of structure and function. This may explain the signs of mucosal damage observed in the present study.

The brush border of the small intestine exhibited a progressive decrease of PAS reaction in FU3 group. A lost or interrupted brush border with weak or moderate PAS reaction was noticed and it might be due to the damaging effect of 5-FU on the brush border. This finding was confirmed by ultrastructural examination that revealed loss of microvilli on the surface of the columnar absorbing cells. These observations are consistent with those of Carneiro-Filho *et al.* (2004) who mentioned that the chemotherapy causes marked disruption of the brush border membranes and absorptive dysfunction.

The number of goblet cells was apparently markedly reduced in FU3 group both over the villi and in the crypts. Most of the cells contained little mucus or were depleted and demonstrated a negative PAS reaction. The electron microscopic examination confirmed this finding as goblet cells appeared depleted or contained few numbers of mucous globules in FU3 group. These findings come in agreement with Stringer *et al.* (2009) who observed that the goblet cells decreased significantly in the rat jejunum 24-72 hours after 5-FU injection with crypt goblet cells being affected the most. The authors explained that 5-FU affects mucous secretion and the decreased goblet cell number indicates rapid release of mucus. The rapid secretion of mucus is thought to be caused by enteric neurotransmitters acting on goblet cells, which suggests that 5-FU absorption by the intestine may cause an up-regulation of neurotransmitter release from enteric neurons, resulting in increased mucin secretion. Therefore, the protective capacity of the mucous barrier could be altered after depletion of goblet cells stored mucus. In addition, this reduction of goblet cell number observed in FU3 group in the present study may be a consequence of early stem cell death and the reflection of this on the renewal of all cell lineages including goblet cells.

Electron microscopic examination of the small intestine of FU3 group revealed marked ultrastructural changes. The cell architecture of both villi and crypts was disturbed with marked widening of the intercellular spaces, intercellular cytoplasmic extensions and extensive lateral cell interdigitations. Similar findings were previously described in the small intestine of patients exposed to methotrexate chemotherapy (Gwavava *et al.*, 1981) and rats exposed to irradiation (Labejof *et al.*, 2010). It was explained that ionizing irradiation causes breakdown of tight junctions and loosens the contact of intestinal epithelial cells with each other. As these cells possess many lateral and basal projections, their extensions penetrate into the basal lamina trying to establish contact with the underlying mesenchymal cells (Somosy *et al.*, 2002). The current ultrastructural study revealed that FU seems to have the same impact on intestinal cell junctions.

The columnar absorbing cells of FU3 group showed areas of partial or even complete loss of microvilli. Cytoplasmic vacuolations and ultrastructural degenerative changes involving cell organelles were also detected. Goblet cells appeared depleted of mucus and exhibited similar ultrastructural changes. Enteroendocrine cells and Paneth cells showed degenerative changes, cytoplasmic vacuolations and autophagic vacuoles.

In accordance with the present results, previous workers observed marked ultrastructural changes in the absorbing cells of the villi in patients' jejunal biopsy specimens, 24-72 hours following methotrexate (Gwavava *et al.*, 1981) and irradiation (Somosy *et al.*, 2002; Labejof *et al.*, 2010). These changes included patchy degeneration and fragmentation of the microvilli, swollen mitochondria and some pyknotic electron dense nuclei. They also observed cytoplasmic vacuolations, focal dilatation of endoplasmic reticulum and Golgi apparatus. They stated that the resulting cystic spaces from the dilated organelles gave rise to the degenerative vacuolar appearance. Gwavava *et al.* (1981) suggested two mechanisms to be involved in such changes: an early direct toxic effect on the mature enterocyte, coupled with interference with crypt cell generation, possibly causing aging of cells.

Mitochondrial swelling and degeneration after irradiation was attributed to the increased concentration of reactive oxygen species that leads to accumulation of the products of lipid peroxidation. As a consequence, alterations in the structure and function of mitochondria are commonly observed in irradiated cells (Somosy, 2000). Since the increase in reactive oxygen species has been documented as an early response following chemotherapy (Duncan and Grant, 2003), the same mechanism can explain the mitochondrial damage in the epithelial cells observed in the present study.

The cytoplasmic vacuolations seen in the cells of the intestinal epithelium of FU3 group in this study may be due to dilated organelles (rER, mitochondria and Golgi), autophagic vacuoles as demonstrated with electron microscope, or hydropic degeneration. Hydropic degeneration is a form of cell degeneration in which the cells accumulate much water in response to damage by a preceding injury. It occurs because of marked mitochondrial damage, cessation of ATP production and failure of Na pump. Failure of Na pump leads to influx of sodium and increase the cytoplasmic osmotic pressure leading to attraction of water. The affected cells microscopically show vacuoles in the cytoplasm with no distinct borders (Cabana, 1999).

An increased number of eosinophils were noticed in the lamina propria in FU3 group. This result comes in agreement with that previously observed by Stringer *et al.* (2009) in the rat's small intestine, 72 h after 5-FU injection. Even, chemotherapy-induced eosinophilic pneumonia has been reported (Hapani *et al.*, 2010). Eosinophilia occurs in various clinical conditions; its mechanisms are poorly understood. It may represent an idiosyncratic-allergic drug reaction (Hapani *et al.*, 2010) or may be part of the total increase in the cellular infiltration observed by the light microscope.

The current study has demonstrated signs of almost full intestinal recovery in FU9 group. The histological structure of the small intestinal mucosa was more or less similar to the control. Duncan and Grant (2003) stated that the structure and functionality of the villi and absorptive surfaces of the gut can return to normal after around 1 week from the onset of chemotherapy. At the ultrastructural level, FU9 group appeared almost similar to the control; however some residual effect as swollen mitochondria in the absorbing and enteroendocrine cells were still observed.

In PAS-stained sections, the FU9 group demonstrated signs of almost full recovery. The brush border was continuous and well-defined and demonstrated a moderate to strong PAS positive reaction. Moreover, most goblet cells appeared distended with mucus. This was confirmed by the electron microscopic study that revealed intact microvilli and goblet cells filled with mucous globules. The goblet cell number over the villi was apparently increased compared to FU3 group, indicating complete cell renewal and migration of goblet cells to the villi.

## CONCLUSION

Administration of 5-FU results in marked histological and ultrastructural degenerative changes in rat small intestinal mucosa which are mostly recovered 9 days after 5-FU injection with some residual ultrastructural changes.

## REFERENCES

- Cabana, E.M., 1999. Cellular degeneration and infiltration. Pathology notes Dr. EM Cabana home on the www. <http://www2.mozcom.com/~emcdvm/>.
- Cameron, S., A. Schwartz, S. Sultan, I.M. Schaefer and R. Hermann *et al.*, 2012. Radiation-induced damage in different segments of the rat intestine after external beam irradiation of the liver. *Exp. Mol. Pathol.*, 92: 243-258.
- Carneiro-Filho, B.A., I.P.F. Lima, D.H. Araujo, M.C. Cavalcante and G.H.P. Carvalho *et al.*, 2004. Intestinal barrier function and secretion in methotrexate-induced rat intestinal mucositis. *Digestive Dis. Sci.*, 49: 65-72.
- Duncan, M. and G. Grant, 2003. Oral and intestinal mucositis-causes and possible treatments. *Aliment. Pharmacol. Ther.*, 18: 853-874.
- Fata, F., I.G. Ron, N. Kemeny, E. O'Reilly, D. Klimstra and D.P. Kelsen, 1999. 5-Fluorouracil-induced small bowel toxicity in patients with colorectal carcinoma. *Cancer*, 86: 1129-1134.
- Gamble, M. and L. Wilson, 2002. The Hematoxylin and Eosin. In: *Theory and Practice of Histological Techniques*, Bancroft, J.D. and M. Gamble (Eds.). 5th Edn., Churchill Livingstone, London, New York, pp: 130.
- Gwavava, N.J., C.R. Pinkerton, J.F. Glasgow, J.M. Sloan and J.M. Bridges, 1981. Small bowel enterocyte abnormalities caused by methotrexate treatment in acute lymphoblastic leukaemia of childhood. *J. Clin. Pathol.*, 34: 790-795.
- Hapani, S., D. Chu and S. Wu, 2010. Eosinophilic pneumonia associated with bleomycin in a patient with mediastinal seminoma: A case report. *J. Med. Case Rep.*, Vol. 4. 10.1186/1752-1947-4-126
- Kolli, V.K., P. Abraham and S. Rabi, 2008. Methotrexate-induced nitrosative stress may play a critical role in small intestinal damage in the rat. *Archiv. Toxicol.*, 82: 763-770.
- Labejof, L., I. Mororo, P. Galle, G. Barbosa, M.I. Severo and A.H. de Oliveira, 2010. Effect of dose rate of irradiation on ultrastructure of duodenal mucosa. *World Acad. Sci. Eng. Technol.*, 68: 664-671.

- Lalla, R.V. and D.M.K. Keefe, 2011. Mucositis (Oral and Gastrointestinal). In: The MASCC Textbook of Cancer Supportive Care and Survivorship, Oliver, I.N. (Ed.). Springer, New York, pp: 241-248.
- Leitao, R.F., R.A. Ribeiro, A.M. Lira, L.R. Silva and E.A. Bellaguarda *et al.*, 2008. Glutamine and alanyl-glutamine accelerate the recovery from 5-fluorouracil-induced experimental oral mucositis in hamster. *Cancer Chemother. Pharmacol.*, 61: 215-222.
- Levine, S. and A. Saltzman, 2004. Hydropic degeneration of the anterior pituitary gland (adenohypophysis) in uremic rats. *Toxicol. Lett.*, 147: 121-126.
- Logan, R.M., A.M. Stringer, J.M. Bowen, R.J. Gibson, S.T. Sonis and D.M.K. Keefe, 2009. Is the pathobiology of chemotherapy-induced alimentary tract mucositis influenced by the type of mucotoxic drug administered? *Cancer Chemother. Pharm.*, 63: 239-251.
- Longley, D.B., D.P. Harkin and P.G. Johnston, 2003. 5-fluorouracil: Mechanisms of action and clinical strategies. *Nat. Rev. Cancer*, 3: 330-338.
- Magne, N., P.Y. Marcy, E. Chamorey, E. Guardiola and X. Pivot *et al.*, 2001. Concomitant twice-a-day radiotherapy and chemotherapy in unresectable head and neck cancer patients: A long-term quality of life analysis. *Head Neck*, 23: 678-682.
- McCarthy, G.M., J.D. Awde, H. Ghandi, M. Vincent and W.I. Kocha, 1998. Risk factors associated with mucositis in cancer patients receiving 5-fluorouracil. *Oral Oncol.*, 34: 484-490.
- Pritchard, D.M., C.S. Potten and J.A. Hickman, 1998. The relationships between p53-dependent apoptosis, inhibition of proliferation and 5-fluorouracil-induced histopathology in murine intestinal epithelia. *Cancer Res.*, 58: 5453-5465.
- Soares, P.M.G., J.M.S.C. Mota, A.S. Gomes, R.B. Oliveira and A.M.S. Assreuy *et al.*, 2008. Gastrointestinal dysmotility in 5-fluorouracil-induced intestinal mucositis outlasts inflammatory process resolution. *Cancer Chemother. Pharmacol.*, 63: 91-98.
- Soares, P.M.G., R.C.P. Lima-Junior, J.M.S.C. Mota, P.F.C. Justino and G.A.C. Brito *et al.*, 2011. Role of platelet-activating factor in the pathogenesis of 5-fluorouracil-induced intestinal mucositis in mice. *Cancer Chemother. Pharmacol.*, 68: 713-720.
- Somosy, Z., 2000. Radiation response of cell organelles. *Micron*, 31: 165-181.
- Somosy, Z., G. Horvath, A. Telbisz, G. Rez and Z. Palfia, 2002. Morphological aspects of ionizing radiation response of small intestine. *Micron*, 33: 167-178.
- Sonis, S.T., 2010. Regimen-related gastrointestinal toxicities in cancer patients. *Curr. Opin. Support. Palliat. Care*, 4: 26-30.
- Stringer, A.M., R.J. Gibson, R.M. Logan, J.M. Bowen, A.S. Yeoh, J. Hamilton and D.M. Keefe, 2009. Gastrointestinal microflora and mucins may play a critical role in the development of 5-fluorouracil-induced gastrointestinal mucositis. *Exp. Biol. Med.*, 234: 430-441.
- Torres, D.M., K.L. Tooley, R.N. Butler, C.L. Smith, M.S. Geier and G.S. Howarth, 2008. Lyprinol™ only partially improves indicators of small intestinal integrity in a rat model of 5-fluorouracil-induced mucositis. *Cancer Biol. Ther.*, 7: 295-302.
- Totty, B.A., 2002. Mucins. In: *Theory and Practice of Histological Techniques*, Bancroft, J.D. and M. Gamble (Eds.). 5th Edn. Churchill Livingstone, London, New York, pp: 175.
- Woods, A.E. and J.W. Stirling, 2008. Electron Microscopy. In: *Theory and Practice of Histological Techniques*, Bancroft, J.D. and M. Gamble (Eds.). 6th Edn., Churchill Livingstone, London, New York, pp: 601-636.
- Yanez, J.A., X.W. Teng, K.A. Roupe, M.W. Fariss and N.M. Davies, 2003. Chemotherapy induced gastrointestinal toxicity in rats: Involvement of mitochondrial DNA, gastrointestinal permeability and cyclooxygenase-2. *J. Pharm. Pharm. Sci.*, 6: 308-314.