Research Article

Histological Study of the Damaging Effect Induced by Cyclophosphamide on Intestinal Mucosa of Adult Male Albino Rat

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Abstract
Background and objectives: Cyclophosphamide is an effective anti-cancer drug widely used in the treatment of many types of cancers, e.g., acute and chronic leukemia, lymphomas, breast cancer, ovarian cancer, neuroblastoma, and sarcoma. It is also used in immunosuppression disorders. Several health hazards were reported after cyclophosphamide administration in animal and human models. Many adverse effects are related to cyclophosphamide and include chemotherapy-induced nausea and vomiting, bone marrow suppression, stomach ache, hemorrhagic cystitis, diarrhea, darkening of the skin and alopecia (hair loss). **Materials and methods:** Twenty- four adult male albino rats were divided into 2 groups (n=12 per group). **The control group (C-group)**, received only standard diet and water. **The cyclophosphamide 2 weeks group** (**CP-2W**), received intraperitoneal injection of a single sublethal dose of 300 mg /kg body weight and rats were sacrificed after 2 weeks. **Results:** There were marked structural changes in intestinal mucosa of CP-2D group in the form of Inflammation, degenerative changes and apoptosis. **Conclusion**: Single intra-peritoneal injection of cyclophosphamide causes massive distruction in the intestinal mucosa.
Keywords: Cyclophosphamide, anti-cancer drug, massive distruction

Introduction
Treatment of many tumors has been performed by chemotherapy and radiotherapy. As the incidence of cancer increases, chemotherapeutic drugs are becoming more widely used all over the world (Shi et al., 2017).

Cyclophosphamide is an effective anti-cancer drug used in the management of many types of cancers, e.g., acute and chronic leukemia, lymphomas, multiple myeloma, breast cancer, ovarian carcinoma, neuroblastoma, and sarcoma (Nabil et al., 2020). It is also used as an immune-suppressant for the treatment of auto-immune diseases (Madondo et al., 2016).

Several health hazards were reported after cyclophosphamide administration in animal and human models. Many adverse effects are related to cyclophosphamide and include nausea and vomiting, bone marrow suppression, hemorrhagic cystitis (Alhowail et al., 2019), diarrhea, alopecia (hair loss), or thinning of hair and pro-found gonadotoxicity (Khorwal et al., 2017).

The small intestine is a very important organ and plays many important functions in the human body. Compared with the duodenum and ileum, the jejunum is the one that has been most damaged by chemotherapeutic drugs (Xie et al., 2016). So, this study assesses the effect of cyclophosphamide on the histological structure of the jejunal mucosa.

Aim of the work
The aim of this study was to assess the structural changes which possibly occur in the intestinal mucosa of adult male albino rat due to the effect of cyclophosphamide injection.

Material and methods

This study was conducted in the Histology and Cell Biology Department, Faculty of Medicine, Minia University, Egypt. This work was carried on 24 adult male albino rats which were weighing approximately 150-250 gm, about 6-8 weeks and pathogenically free. Animals were obtained from the study animal house of Minia University laboratory animals growing center of the faculty of agriculture.

Rats were housed in clean plastic cages and fed a standard laboratory diet with free access to water and diet. Rats were maintained at a laboratory temperature ranged from 24-30ºC and exposed to 12 hours light and 12 hours dark cycle. Animals were acclimatized for 2 weeks before the experiment. All aspects of animal care and treatment were carried out according to the local guidelines of the ethical committee of the faculty of medicine of Minia University.

Reagents: -

1- **Cyclophosphamide** (**Endoxan®**) vial containing 1gm of yclophosphamide was purchased from Multipharma company, Egypt, was freshly prepared by diluting in 50 cm physiological saline and used with a single sublethal dose of 300 mg per kg body weight via intraperitoneal injection at the beginning of the experiment.

Experimental design: -

Animals were randomly divided into 2 groups (12 rats at each group) as following**:**

- **The Control Group (C-group):** Rats were received standard rat diet and water.
- **The Cyclophosphamide 2-weeks Group (CP -2W):** Rats were received intraperitoneal injection of a single sublethal dose of 300 mg of cyclophosphamide /kg body weight at the beginning of the experiment and rats were sacrificed after 2 weeks **(Owari et al., 2012a)**.
  - Rats were sacrificed by cervical decapitation under light halothane anesthesia.
  - Tissue samples of the Jejunum was immediately removed and carefully dissected for tissue preparation.

Methods:

For light microscope examination: -

a) The Paraffin Technique (Suvarna et al., 2018):

The small intestine specimens were fixed in 10% neutral-buffered formalin at room temperature. After proper fixation, the samples were dehydrated in graded alcohol concentrations, cleared in xylene and embedded in paraffin wax then cut by a microtome. 3µm sections were flattened by floating in a hot water bath and then they were mounted on glass slides to be stained**.**

b) Staining with Hematoxylin and Eosin (H&E) (Suvarna et al., 2018):

Some sections, mounted on glass slides, were deparaffinized to be stained with H&E. They were put in Hematoxylin stain for 7 minutes, washed well in running tap water, then put in Eosin for 3 minutes and the excess stain was washed off in water. The sections were dehydrated by alcohol, cleared by xylene and then mounted on glass slides to be viewed by the light microscopy for the general histological analysis.

Result: The cytoplasm appeared red to pink while the nuclei stained blue.

Results

I) Histological Results:

- **Hematoxylin and Eosin results:**

1) The Control Group (C-group):

There was normal jejunal architecture which is consisted of four layers; mucosa, submucosa, musculosa and serosa. The mucosa showed leaf like villi covered by enterocytes with goblet cells. Enterocytes had eosinophilic cytoplasm and basal oval nuclei. Goblet cells were at intervals between these enterocytes. The villi showed a core of loose connective tissue extending from the lamina propria (Fig. 1).

2) The Cyclophosphamide 2 weeks Group (CP-2W):

There was noticeable histological changes if compared to the previous group.

The villi appeared Shorter and broader. Some villi showed distortion. In addition, shedding of the epithelium in the lumen was observed as well as formation of sub epithelial space. The lamina propria displayed inflammatory cell infiltration and congested capillaries (Fig.2).

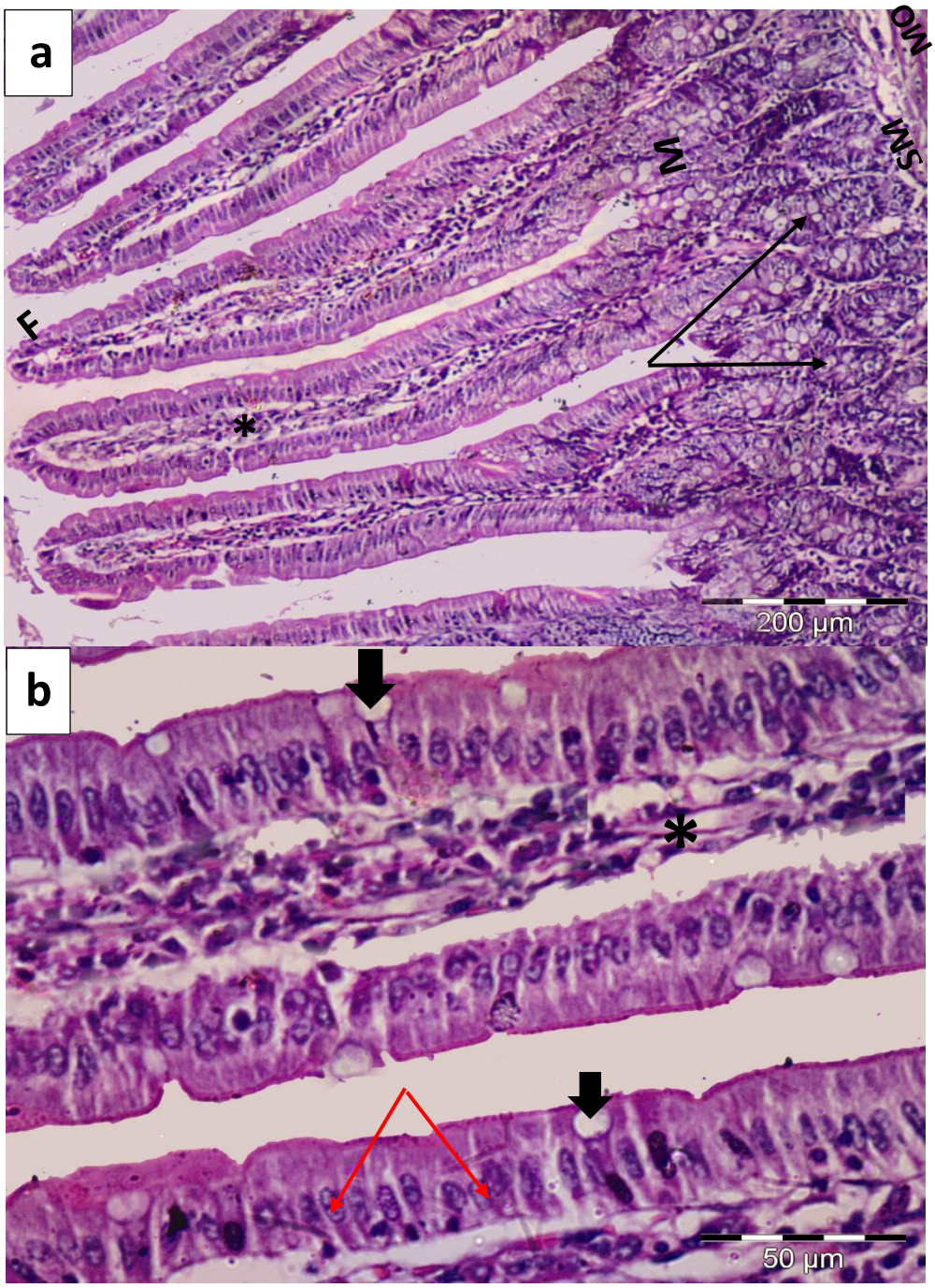

Fig. 1: photomicrographs of the jejunum of control group showing: a) showing intestinal mucosa (M), submucosa (SM), musculosa (MO), and showing leaf like (F) villi covered with simple columnar epithelium with goblet cells and basal jejunal crypts (arrows). b) The villi are covered with enterocytes with oval basal nuclei (red arrows) and goblet cells (thick arrow). The luminal surface of enterocytes has a regular striated border. The villi have a core of connective tissue (*) extending from the lamina propria. H and E a x10, b x 40.

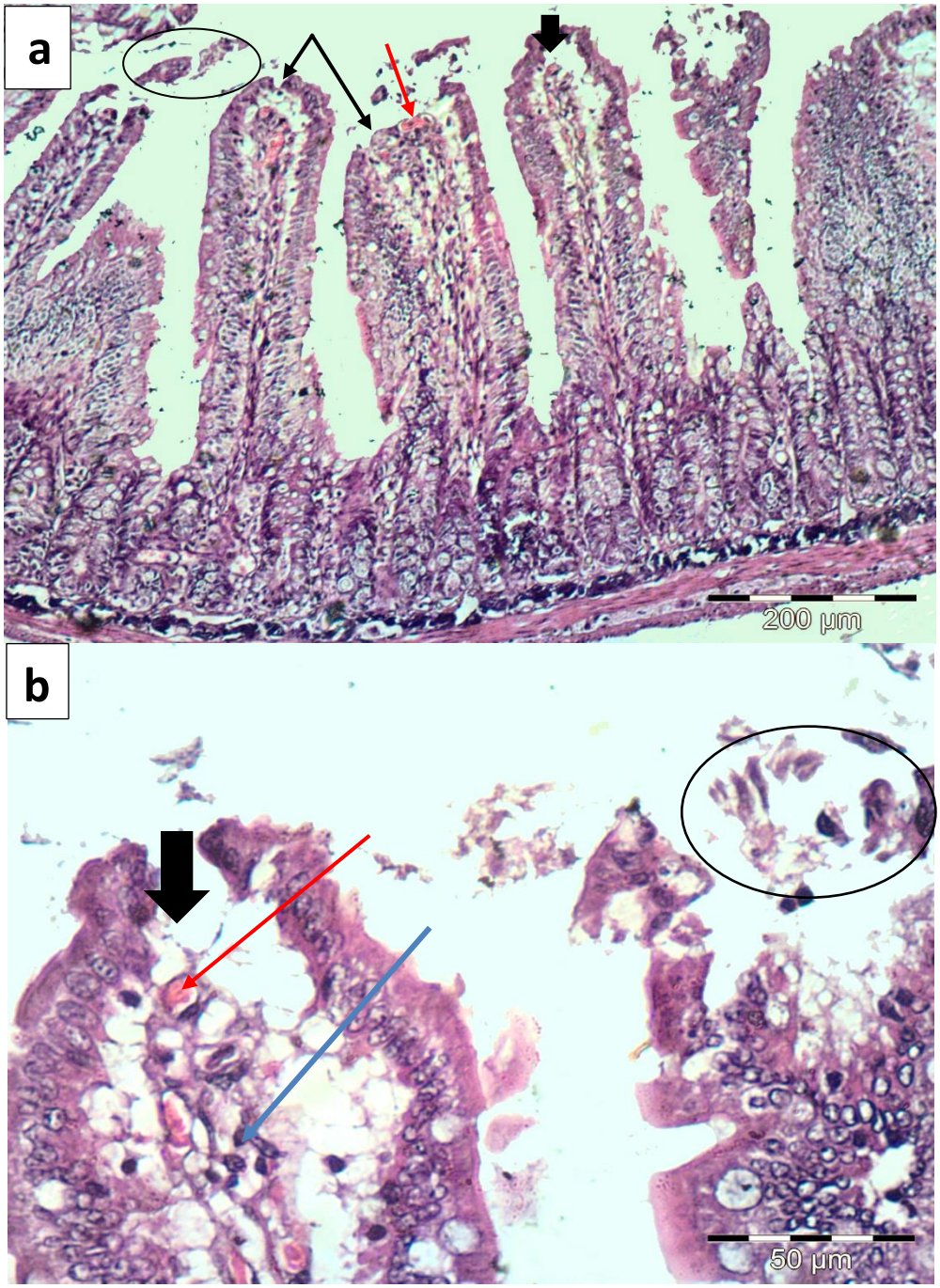

Fig. 2: photomicrographs of jejunal section of CP-2W group showing: a) shortening and broadening of the villi (arrows), shedding of the epithelium of the villi in the lumen (circle) and formation of sub epithelial spaces (thick arrows). b) lamina propria showed mononuclear cellular infiltration (blue arrow) and congested capillaries (red arrow). H and E a x 10, b x 40.

Discussion

Cyclophosphamide is an effective anti-cancer drug used in the management of many types of cancers, e.g., acute and chronic leukemia, lymphomas, multiple myeloma, breast cancer, ovarian carcinoma, neuroblastoma, and sarcoma (Bhattacharjee et al., 2017). It is also used as an immunosuppressant for the treatment of autoimmune diseases (Madondo et al., 2016).

Several health hazards were reported after cyclophosphamide administration in

animal and human models. The clinical use of this drug is restricted due to its side effects which include nephrotoxicity, heaptotoxicity, neurotoxicity, cardiotoxicity, immunotoxicity, urotoxicity, vomiting, nausea, alopecia, and bone marrow suppression (Ahlmann and Hempel, 2016).

High doses of anticancer drugs can damage the intestinal mucosa leading to clinical problems as bacterial translocation, diarrhea and dyskinesia (Owari et al., 2012b) as they cause severe tissue oxidative stress and massive cellular damage, thereby increasing apoptosis, and death of cancer and healthy cells (Abdel-Hafez et al., 2017).

In this study, twenty- four adult male albino rats were divided into 2 groups (n=12 per group). The control group (C-group), received only standard diet and water. Cyclophosphamide 2 weeks group (CP-2W), received intraperitoneal injection of a single sublethal dose of 300 mg/kg body weight and rats were sacrificed after 2 weeks (Owari et al., 2012a).

By using hematoxylin and eosin, there was preserved histological architecture of the jejunum in control group (C-group).

In CP-2W group, there was Shortening, broadening and distortion of the villi. In addition, sub epithelial spaces were formed and the epithelial cells were desquamated and shedded in the lumen. These results were in accordance with another study (Abdel-Hafez et al., 2017) which stated that CP mediated a clear inflammatory response, resulted in sub-epithelial edema, hemorrhage and massive tissue destruction.

The lamina propria showed prominent congested blood vessels and inflammatory cellular infiltration. These results were in agreement with other researcher (Hamsa and Kuttan, 2010) who stated that CP induced oxidative stress in rats. Oxidative stress activated multiple intracellular signaling pathways, thus causing up regulation of proinflammatory cytokine production. This was due to the accumulation of inflammatory neutrophils, macrophages and lymphocytes through the release of free radicals.

CP plays a major role in the development of injury to different tissues. Intestinal epithelial cells are especially susceptible to CP, which produced intestinal oxidative stress and immunosuppression, leading to intestinal mucosal barrier dysfunction (Wang et al., 2019).

Conclusion

Consequently, the previously mentioned data indicated that CP has toxic effect on the jejunum. Inflammation, degenerative changes and apoptosis could be the mechanisms through which CP induces its toxic effect on the jejunum.

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