Potential protective role of barley's grains on methotrexate induced jejunal mucosal damage in male albino rats

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Abstract: Introduction: Methotrexate (MTX) is widely used as chemotherapy drug in the treatment of rheumatoid disease. MTX is known for its common effect in causing intestinal mucosal injury. Barley (*Hordeum vulgare* L.), an ancient grain, has been domesticated since 8000 B.C. possesses significant antioxidant, antiradical potentials and antiulcerative activity. **Aim of the Work:** To investigate the Potential protective effects of barley's grains on methotrexate induced jejunal mucosal damage in male albino rats by light and scanning electron microscope and morphometric study**. Material and Methods:** thirty adult male rats weighting 150-200 gm were used and randomly divided into three equal groups: Control group; MTX group was given 6 mg/kg body weight of Methotrexate for 5 days intraperitoneal injection; the third group was given barley grains at a dose of 200g /kg/day beside the usual food for 30 days plus intraperitoneal injections of methotrexate on the day 25, at the dose 6mg/kg /day for 5 days then sacrificed by cervical dislocation. Jejunal samples were excised for light and scanning electron microscopic study and morphometric study. **Results:** intraperitoneal administration of methotrexate induced marked changes in jejunal mucosa detected by both light and scanning electron microscope with severe erosions, exfoliation and ulcer formation. The addition of barley significantly decrease jejunal damage and could protect intestinal mucosa against the injurious effects of methotrexate. **Conclusions:** These results concluded that methotrexate combined with barley (*Hordeum vulgare* L.) in comparison with methotrexate induced less intestinal mucosal damage and this protective effect might be due to the anti-inflammatory and antiulcer activity of barley.

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Keywords: Methotrexate, barley, jejunal mucosal, rats.

Introduction:

Chemotherapy has been shown to alter mucosal morphology and gut function. Mucositis is an inflammatory, painful and debilitating condition that significantly interferes with anticancer therapy. One of the most common side effects of chemotherapy is gastrointestinal toxicity **(Tran *et al.,* 2003)**.

Methotrexate (MTX), a folic acid antagonist is widely used as a cytotoxic chemotherapeutic agent for leukemia and other malignancies. MTX has also been used for treatment of various inflammatory diseases such as psoriatic and rheumatoid arthritis **(Kolli *et al.,* 2007 and El-Boghdady, 2011).**

One of the major toxic effects of MTX is intestinal injury and enterocolitis **(Somi *et al.,* 2011).** The small intestinal damage induced by MTX treatment results in malabsorption and diarrhea. The malabsorption results in weight loss and disturbs the cancer chemotherapy of the patients **(Doan and Massarotti, 2005).**

Barley (Hordeum vulgare L.), wonderful ancient grain, has been domesticated since 8000 B.C., as a food for humans and animals used for various medicinal purposes, barley exhibited antioxidant, antiradical potentials and antiulcerative effects **(Yichen Xia, 2012).** Barley grains are an important dietary source of water soluble and fat soluble as well as insoluble antioxidants. These antioxidants include vitamin E, Tocotrienols, selenium, phenolic acids and phytic acid. These antioxidants are available through the gastrointestinal tract over long period after being consumed **(*Selim*, 2005 and Yichen Xia, 2012).**

Material and Methods

Materials:

Methotrexate brought from CIPLA LTD Verna industrial estate pharmaceutical company. Barley was purchased from local market at Sohag, Egypt**.**

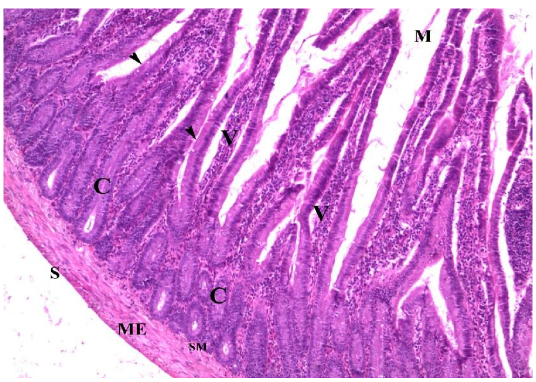
Animals:

Thirty adult male rats weighting 150-200 gm / each were used in the present study. All animals were housed under the same conditions and allowed food and water. Rats were randomly divided into three submucosa, and musculosa, the jejunal mucosa consisted of finger & leaf like villi, each villus had central core covering simple columnar epithelium (enterocytes) with goblet cells. The enterocytes appeared columnar in shape with oval basal nuclei and apical acidophilic cytoplasm. Goblet cells were unicellular glass shaped cells; its basal part is basophilic. Its apical part appears faint because of presence of mucinogen granules.

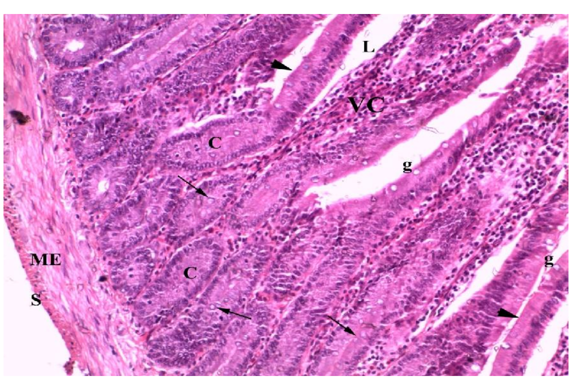
Between the villi there were invaginations called intestinal gland or crypts of Lieberkühn, which appeared simple tubular structures also lined by simple columnar epithelium.

The submucosa appeared as a loose connective tissue layer with comparatively large blood vessels.

The Muscularis externa consisted of two layers of smooth muscle; the inner one was circular and formed of spindle shaped smooth muscle fibers with fusiform nuclei and the outer one was longitudinal and appeared as circular sections (cells) with centrally located rounded nuclei.



(Fig. 1): A photomicrograph of a transverse section of a jejunum of control rat **(group1)** showing; mucosa **(M)** with finger and leaf like villi **(V)** covered by enterocytes with its striated border **(heads arrow)**, intestinal glands **(C),** submucosa **(SM)**, muscularis externa **(ME)** and serosa **(S). (H&EX100)**



(Fig. 2): A photomicrograph of a transverse section of a jejunum of control rat **(group1)** showing; villus core **(VC)**, striated border of enterocytes **(head arrows)**

equal groups, given the treatment orally and via intraperitoneal injection.

- Control group: was given the usual food.

-MTX group: given the usual food with intraperitoneal injections of methotrexate at the dose 6mg/kg /day for 5 days **(Kesik *et al.,* 2009).**

-The barley + MTX group: barley grains were given at a dose of 200g /kg/day beside the usual food for 30 days **(Rebolé *et al.,* 2010)** plus intraperitoneal injections of methotrexate on the day 25, at the dose 6mg/kg /day for 5 days **(Kesik *et al.,* 2009)** and at the end, the animals were sacrificed by cervical dislocation. Specimens from the jejunum were subjected to light and scanning electron microscopic studies **(Kolli *et al.,* 2007 & El-Boghdady, 2011).**

For light microscopic study:

Specimens were fixed in 10% formalin, processed and embedded in paraffin. Serial sections (5 microns) were prepared and subjected to hematoxylin and eosin stain **(Ross & Pawlina, 2011).**

For scanning electron microscopic study (SEM):

Pieces of the mucosal surface of the jejunum were washed with normal saline, rinsed with cocodylate buffer and placed in 2.5 % glutaraldehyde. Following fixation, the specimens were washed several times with cold cocodylate buffer and post-fixed in 1 % osmium tetroxide. They were dehydrated in a graded ethanol series, exposed to liquid CO_2 in a drying apparatus and coated with a thin layer of gold (10-15 um) deposited over the surface in vacuum evaporator **(Yamauchi *et al.,* 2006).** The specimens were examined with a Jeol-JSM-5400 LV scanning microscope in the Electron Microscopic Unit of Sohag University.

Morphometric methods and statistical studies:

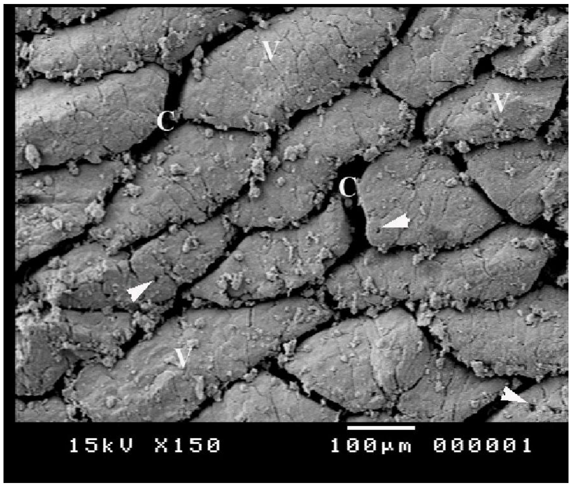
Measuring the diameter of mucosa thickness and goblet cells number was done by using Digimizer (image analyzer computer system). The mucosa thickness diameters and goblet cells number from the control and different experimental groups were measured from hematoxylin and eosin-stained sections, 5 sections were observed at X 20 objective from each animal of all groups. The mucosa thickness diameters were measured as the maximum diameter of each one. Results were expressed as mean value ± Standard deviation. The data were statistically analyzed using the independent T- test. A probability value of $P<0.05$ was considered significant and $P < 0.01$ was highly significant (**Acipayam *et al.,* 2013).**

Results:

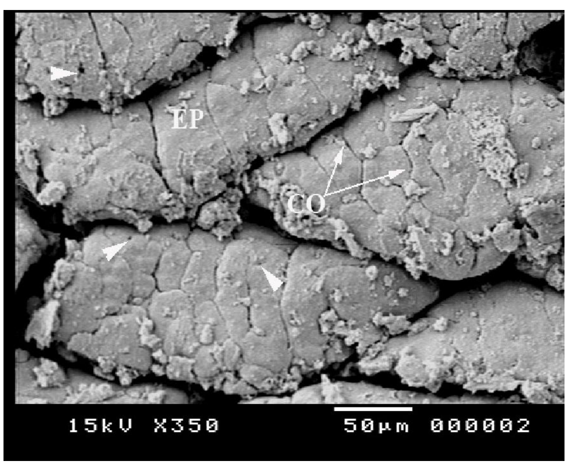
Control Group:

Histological study (figs: 1, 2, 3,4):

Specimens obtained from the control rats and stained with H & E showed the wall of the jejunum was formed of classic layers namely, mucosa, microvilli were seen giving the cell surface a granular texture.

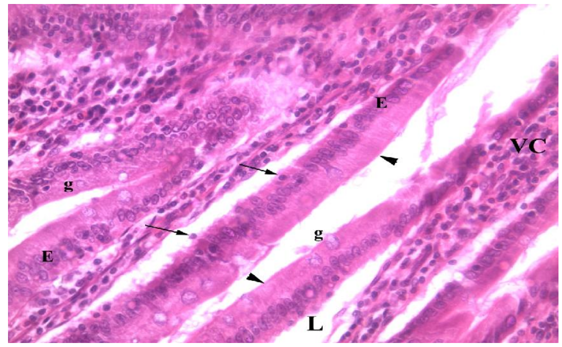


(Fig.5): A scanning electron micrograph of a jejunum of **control rat (group1)** showing intact epithelial surface of the villi **(V)** separated by crypts **(C),** goblet cell orifices **(arrows). (X 150)**

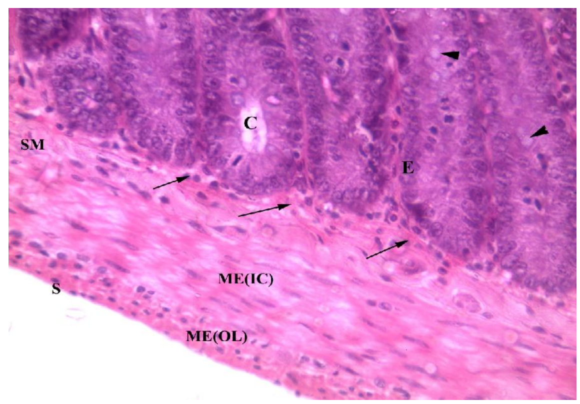


(Fig.6): A scanning electron micrograph of the magnification of the previous picture showing normal corrugations of the villi **(Co)**, enterocytes **(EP)** appear with hexagonal outlines, goblet cell orifices **(head arrows)**. (X **350)**

and goblet cells in the villi**(g)** and goblet cells**(arrows)** in the intestinal glands **(C),** muscularis externa **(ME)** and serosa **(S)**. **(H&EX200)**



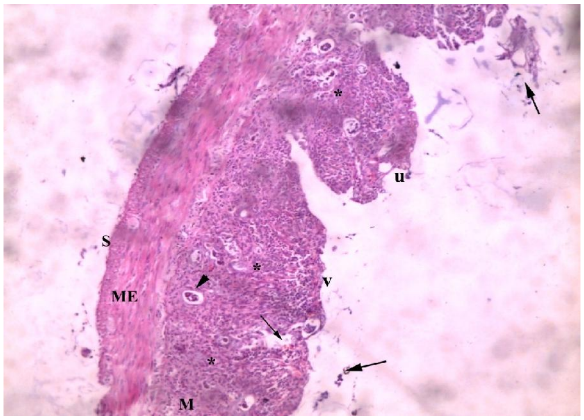
(**Fig.3):** A photomicrograph of a transverse section of a jejunum of **control rat (group1)** showing the shaft of a finger like villus covered by enterocytes (**E**) with its striated border **(head arrows)** and goblet cells on the surface of the villus **(g**), with connective tissue core **(VC)**, **lacteal (L)** and notice the intraepithelial lymphocytes **(arrows)**. (**H&E. X400)**



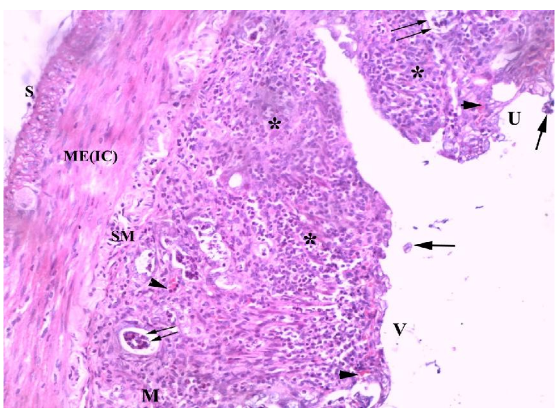
(Fig.4): A photomicrograph of transverse section of a jejunum of **control rat (group1)** at the base of the villus showing; intestinal glands **(C)** lined by enterocytes **(E)** and multiple goblet cells **(head arrows),** muscularis mucosae **(arrows),** submucosa **(SM)**, inner circular layer of muscularis externa **ME(IC)**, outer longitudinal layer of muscularis externa **ME(IC) and serosa (S)**. **(H&E. X400)**

SEM examination (figs: 5, 6, 7,8):

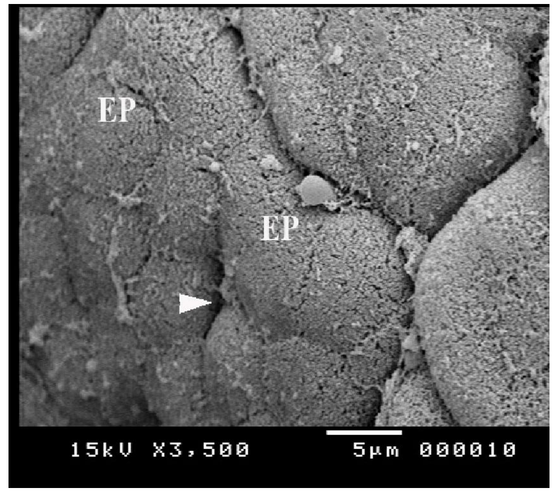
Examination of jejunal mucosa by scanning electron microscopy revealed that the jejunal mucosa appeared intact with Leaved, flattened and tongue shaped villi. Irregular clefts and corrugations were seen on the surface of the villus. Goblet cell orifices secreting mucus were seen. The enterocytes appeared flat topped or gently convex outwards and hexagonal in outlines, their packing giving rise to a honeycomb appearance on the villous surface with rod-shaped externa appeared thickened with multiple discontinuations in the inner circular layer.



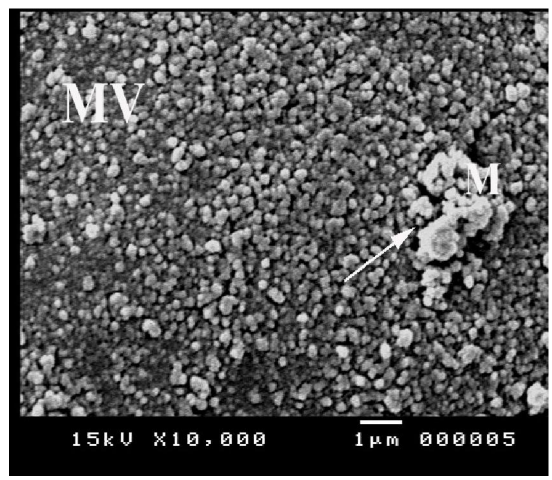
(Fig.9): A photomicrograph of a transverse section of the jejunum of **group2, treated with MTX** showing; fusion of the villi **(V)** with ulceration **(U)** and sloughing (**thick arrows)** of the mucosa **(M)** crypt necrosis (**head arrow**) and inflammatory infiltration of lamina propria (**star**) edema **(thin arrow),** muscularis externa **(ME)** and serosa **(S). (H&E. X100)**



(Fig.10) a magnification of the previous picture showing; fusion of the villi **(V)** with ulceration **(U)** of the mucosa, sloughing of epithelium **(arrows)**, crypt necrosis **(double arrows)**, inflammatory infiltration (**stars**) and hemorrhage **(head arrows)** of lamina propria, inflammatory infiltration of submucosa (SM), muscularis externa shows discontinuations in its inner circular layer (ME(IC)) and intact serosa(S). (H&E. X200)



(Fig.7): A scanning electron micrograph of the magnification of the previous picture showing the hexagonal outlines of enterocytes **(EP)** and arrangement giving the honey comb appearance goblet cell orifice **(head arrow)** appears intact. **(X3500)**

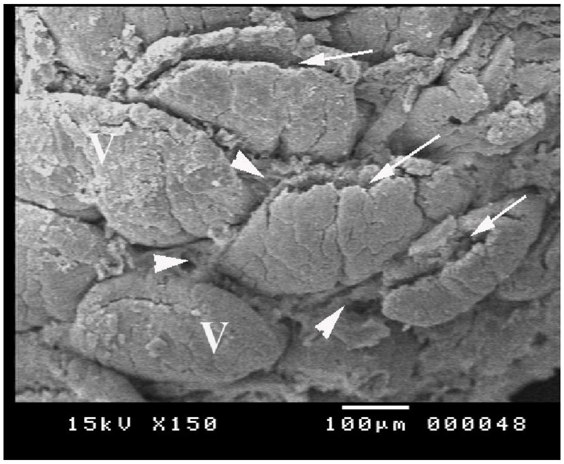


(Fig. 8): A scanning electron micrograph of a magnification of the previous picture showing closely backed microvilli **(MV)** giving granular appearance, goblet cell orifice **(arrow)** discharging mucus **(M). (X10, 000)**

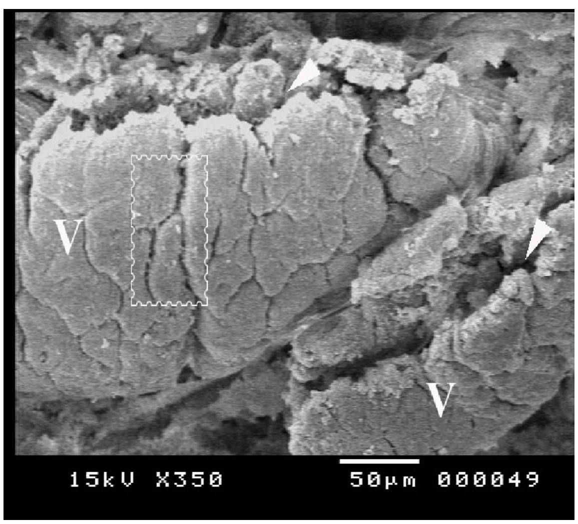
MTX Group:

Histological study (figs: 9, 10,11,12):

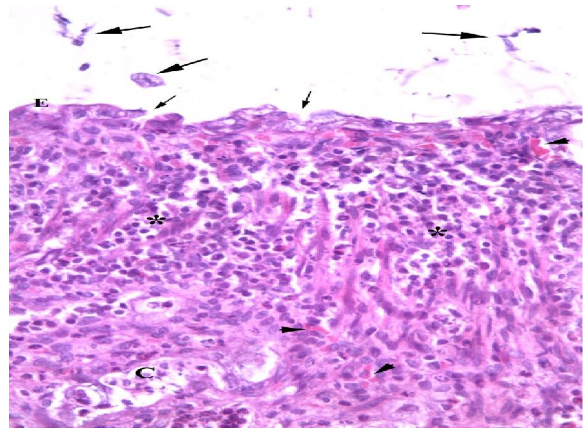
Intraperitoneal injection of methotrexate induced congestion and multiple hemorrhagic erosions and ulcerations in the rat jejunum. The mucosal surface revealed the villi fused together forming thick& irregular mucosal folds with edema, heamorrahge and massive inflammatory cell infiltrations. Areas of epithelial loss were seen. Goblet cells decreased in number with intestinal crypts of Lieberkühn showed reduction in number with hypoplasia. The muscularis with disarranged microvilli and absent goblet cell orifices.



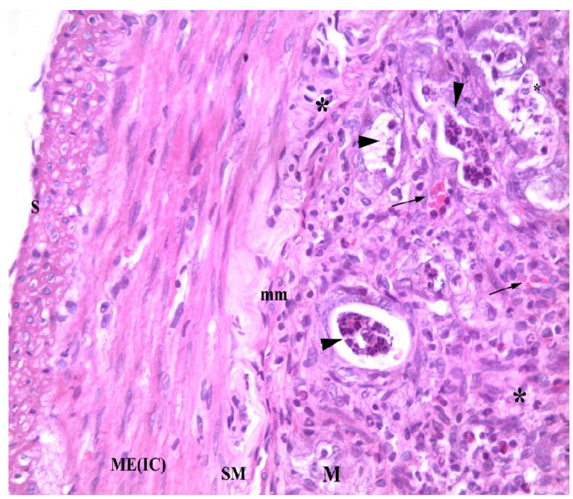
(Fig.13): A scanning electron micrograph of the jejunum of **group2, treated with MTX** showing villi **(V)** with destruction of their tips **(arrows)** with disturbance of the crypts in between villi **(head arrows)**. **(X150)**



(Fig.14): A scanning electron micrograph of the magnification of the previous picture showing destruction of the epithelium of villi **(V)** with ulcerations and exposure of their underlying connective tissue core **(head arrows). (X350)**



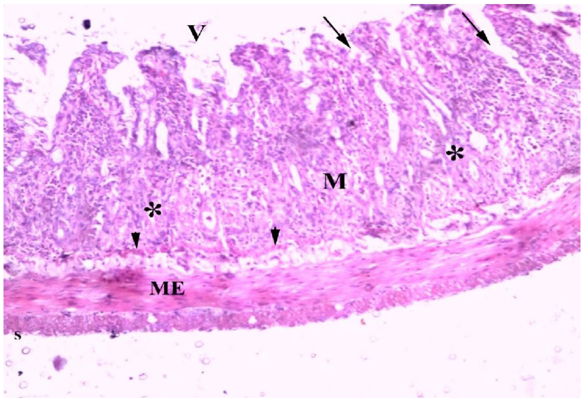
(Fig. 11): A photomicrograph of a transverse section of the jejunum of **group2, treated with MTX** showing the tip of the fused villi with sloughing of epithelium **(large arrows)** and ulcerations **(small arrows),** flattened enterocytes with pyknotic nuclei **(E),** hemorrhage **(head arrows),** inflammatory infiltrations and dispersion in between the cells **(stars)** of the lamina propria and crypt necrosis **(C)**. **(H&E. X400)**



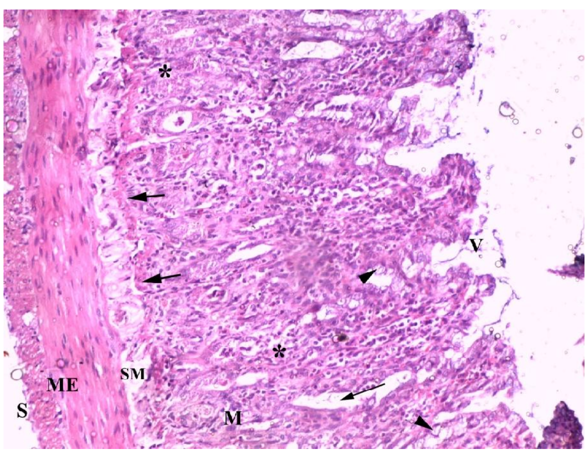
(Fig. 12): A photomicrograph of a transverse section of the jejunum of **group2, treated with MTX** showing; the basal part of mucosa, with inflammatory cell infiltrations **(stars)** of base of the villi and submucosa **(SM),** hemorrhage in lamina propria **(arrows),** crypt necrosis **(head arrows),** muscularis mucosa **(mm)**, discontinuations in the inner circular layer of muscularis externa **(ME(IC))** and serosa**(S)**. **(H&E. X400)**

SEM examination (figs: 13, 14, 15, 16):

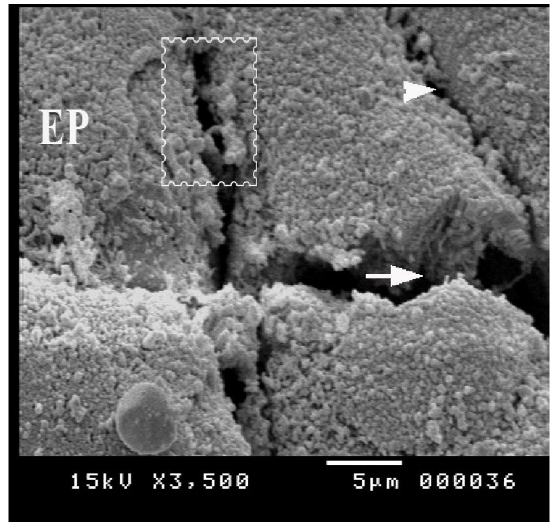
Examination of jejunal mucosa of this group by scanning electron microscopy revealed areas of erosion and ulceration at the tips of the villi with exposure of the underlying connective tissue, the surface appeared with deeply cutting corrugations



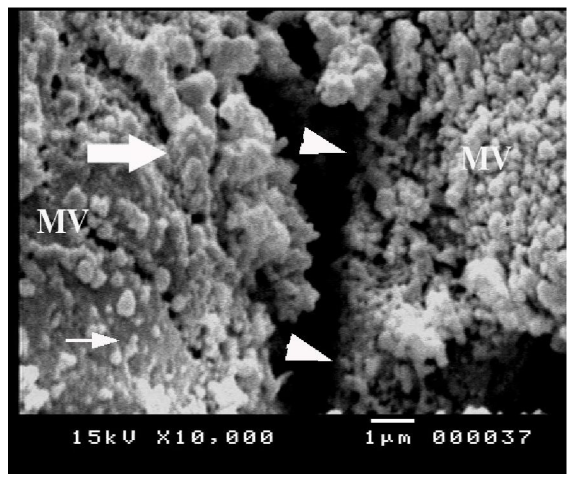
(Fig. 17): A photomicrograph of a transverse section of the jejunum of **group3, treated with MTX and barley** showing; mucosa **(M)** with thick finger like villi **(V)**, crypts appear in between villi **(arrows)**, inflammatory infiltrations of lamina propria **(stars)**, muscularis mucosae **(head arrows)**, muscularis externa **(ME)** appears intact with its 2 layer and serosa **(S). (H&E X100)**



(Fig. 18): A magnification of the previous picture showing; mucosa **(M)** with thick finger like villi **(V)**, crypts appear in between villi **(thin arrow),** goblet cells **(head arrows)** inflammatory cell infiltrations of lamina propria **(stars)**, muscularis mucosae **(thick arrows),** submucosa **(SM)**, muscularis externa appears intact with its 2 layer **(ME)** and serosa**(s). (H&E X 200)**



(Fig.15): A scanning electron micrograph of the magnification of the previous picture showing a group of epithelial cells **(EP)** with deeply cutting corrugations **(head arrow),** with exposure of their underlying connective tissue **(arrows). (X3500)**

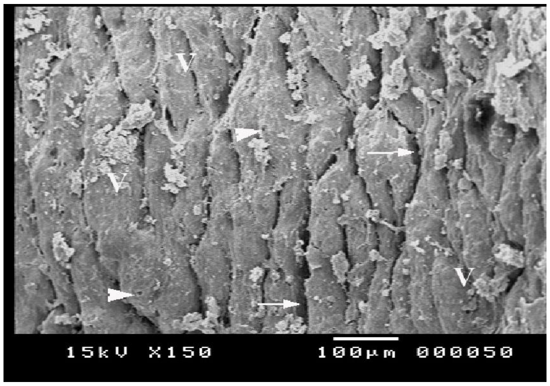


(Fig.16): A scanning electron micrograph of the magnification of the previous picture showing absence **(thin arrow**), disarrangement and fusion **(thick arrow)** of the microvilli and discontinuation of epithelial cell surface (**head arrows) X10,000.**

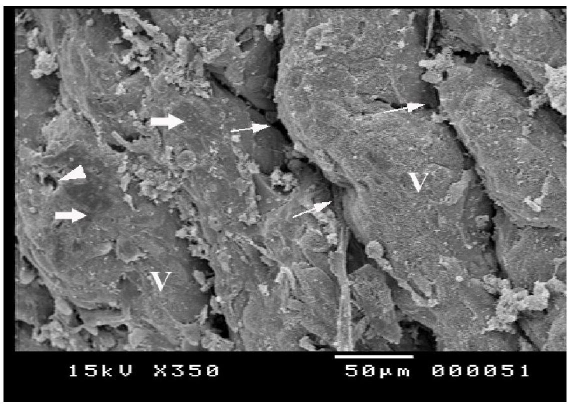
Combined barley +MTX Group:

Histological study (figs: 17, 18, 19, 20):

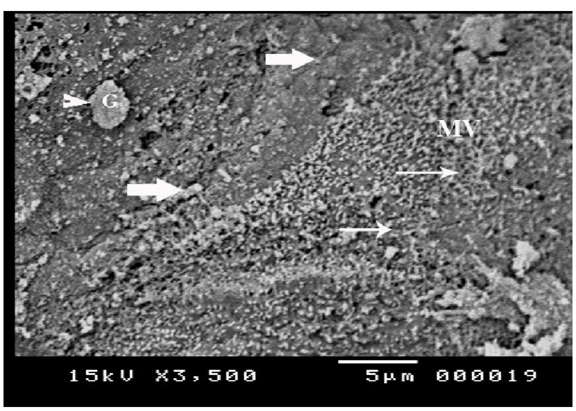
The mucosa of this group showed thick finger shaped villi with cuboidal enterocytes with goblet cells presence. Crypts were seen in between the villi. Lamina propria & submucosa showed moderate cellular infiltrations and hemorrhage. The muscularis externa showed normal architecture.



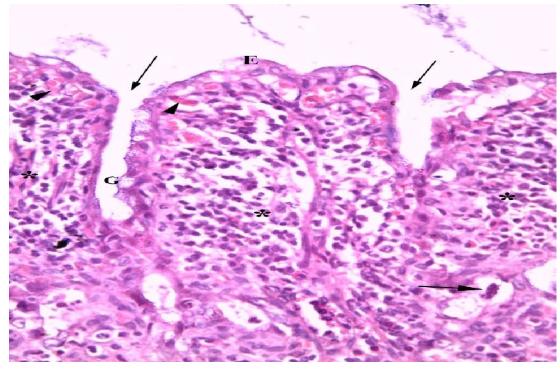
(Fig.21): A scanning micrograph of the jejunum of **treated rat (group3)** showing intact villi **(V)**, crypts seen in between **(arrows)** and goblet cell openings**(head arrows). (X 150)**



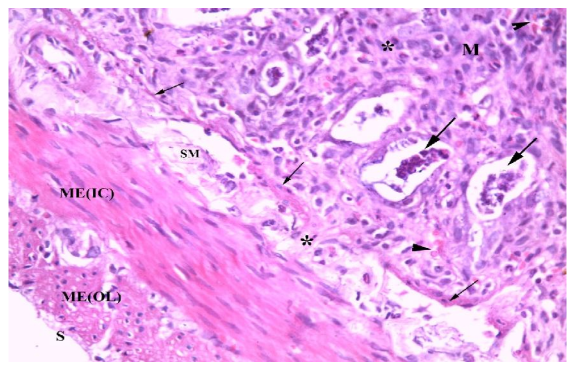
(Fig.22): A scanning micrographs of the magnification of the previous picture showing intact villi **(V)**, with crypts in between **(thin arrows),** orifice of the goblet cell **(head arrow)** and areas of absent microvilli **(thick arrows)**. **(X 350)**



(Fig.23): A scanning micrographs of a jejunum of **treated rat (group3)** showing granular surface of microvilli **(thin arrows)**, areas of absent microvilli **(thick arrows)** and goblet cell discharging mucus **(G). (X 3500)**



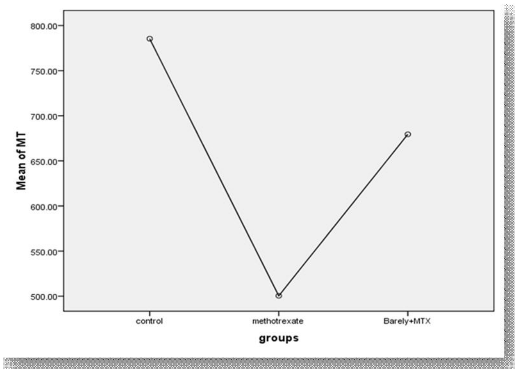
(Fig. 19): A photomicrograph of a transverse section of the jejunum of **group3, treated with MTX and barley** showing; the tip of the finger like villus covered with cuboidal enterocytes **(E)** and goblet cells **(G),** crypts appear in between villi **(thin arrows),** inflammatory infiltrations **(stars)** and hemorrhage **(head arrows),** crypts necrosis **(thick arrow) still present**. **(H&E X400)**



(Fig.20): A photomicrograph of a transverse section of the jejunum of **group3, treated with MTX and barley** showing; the basal part of mucosa **(M)**, inflammatory cell infiltrations **(stars)**, hemorrhage **(head arrows)**, crypt necrosis **(thick arrows),** muscularis mucosa **(thin arrows)**, submucosa **(SM),** Intact inner layer of muscularis externa **(ME(IC))**, outer layer of muscularis externa **(ME(OL))** and serosa **(S)**. **(H&E X400)**

SEM examination (figs: 21, 22, 23, 24):

Scanning electron microscopy of this group showed plate -shaped villi with intact epithelial. Goblet cells openings were seen discharging mucus, microvilli on the enterocytes surface were absent in areas, disarranged and loosely backed in other areas.



Histogram (1): showing the mean diameter of mucosal thickness in control and experimental groups.

Goblet cell number in the villi (Table.2, Histogram.2):

The mean numbers of the goblet cells of the villi were, in control (group1): 36.1379 ± 6.07527; in MTX-group (group2): 2.43839 ±.26261, in barley + MTX (group3): 15.6000 ± 2.19089.

1-Mean number of goblet cell in villi in group2 show a very highly significant (P=0.000) decrease when compared with group1.

2-Mean number of goblet cell in villi in group3 show a very highly significant (P=0.000) decrease when compared with group1.

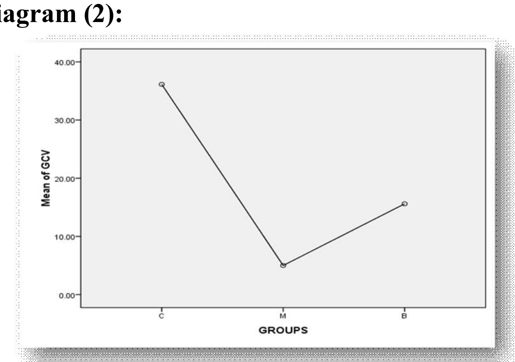
3-Mean number of goblet cell in villi in group3 show a highly significant (P=0.003) increase when compared with group2.

Table 2: showing the mean the mean goblet cell number in the villi in control and experimental groups.

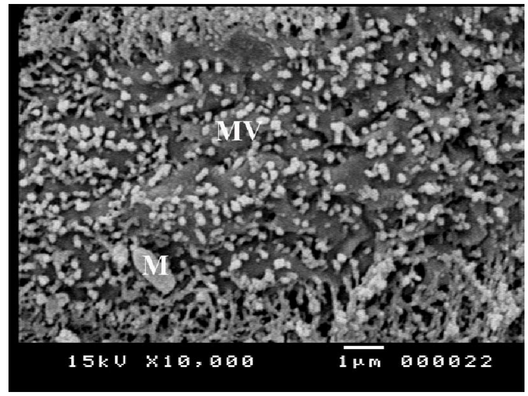
Parameters	Goblet cell (In villi)
Control	36.1379 ± 6.07527
MTX	2.43839 ±.26261***
Barley +MTX	15.6000 ± 2.19089***

Values are mean ± SD; P>0.05 --->non-significant, P>0.05 (*) ---->significant difference, P>0.01 (**) ----> high significant difference, P>0.001 (***) ----> very high significant difference

Diagram (2):



Histogram (2): showing the mean number of goblet cells in control and experimental groups.



(Fig.24): A scanning micrographs of the magnification of the previous picture showing scanty rod shaped microvilli **(MV)** and goblet cell discharging mucus **(M). (X 10000)**

Morphometric Study:

Mucosal thickness (Table 1, Histogram 1):

The mean diameters of the mucosa thickness (MT) were, in control (group1): 785.4200±262.620 pixels; in MTX-group (group2): 500.417±190.837pixels; in barley + MTX (group3): 679.486±85.339 Pixels.

1- Mean mucosal thickness in group2 show a highly significant.

(P=0.000) decrease when compared with group1.

2- Mean mucosal thickness in group3 show non-significant (P=0.199) decrease when compared with group1.

3- Mean mucosal thickness in group3 show a highly significant.

(P=0.000) increase when compared with group2.

Table 1: showing the mean diameter of mucosal thickness in control and experimental groups.

Parameters	Mucosa thickness (MT)
Control	785.4200±262.620
MTX	500.417±190.837***
Barley +MTX	679.486±85.339***

Values are mean ± SD; P>0.05 --->non-significant, P>0.05 (*) ---->significant difference,
P0.01 (**) ----> high significant difference, P>0.001 (***) ----> very high significant difference

Diagram (2):

dihydrofolate reductase and this in turn reduces the cellular supply of pyrimidine deoxythymidine triphosphate **(Xian *et al.,* 1999, Boukhettala *et al.,*2009 and Change *et al.,*2013)**. This impairs DNA and RNA synthesis this leads to decreased protein content in the cells as a result; reduced crypt cells replication and this resulted in shortening of the villi and a decrease in the mucosal surface area available for absorption **(Iqbal *et al.,* 2001 and Vardia *et al.,* 2008).**

Goblet cells in rats treated with MTX were present but less than normal, some were swollen and some were ruptured. This is in consistence with the results of **Rebolé *et al.* (2010), Acipayam *et al.* (2013) and Kesik *et al.* (2009)** who reported that goblet cells in the small intestine of methotrexate treated rats showed decrease or depletion after methotrexate administration.

In contrast with this finding, **Verburg *et al.* (2000)** detected that there is selective sparing of goblet cells in the intestine of methotrexate-treated rats and this serve a very well protective function in epithelial defense via their secretion of mucins and trefoil factor.

In rats treated with MTX muscularis externa appeared thicker than control and interrupted in the inner circular layer indicating edema and friability of the tissue and intact in other.

These results are in agreement with **Soares *et al.* (2011)** who reported that methotrexate-induced intestinal mucositis affects the intestinal muscular wall leading to delayed gastric emptying and gastrointestinal transit. These observations clearly suggest that inflammation of the gastrointestinal tract causes significant affection in gut wall and motility **(Soares *et al.*, 2011) and (Hierholzer *et al.,* 2004).**

In our study, the light microscopic results revealed that rats treated with MTX and barley showed intact jejunal mucosa with thick finger shaped villi with cuboidal enterocytes with goblet cells appeared. Crypts appeared in between the villi and moderate inflammatory cell infiltration with intact muscular layer.

These results are in agreement with **Kanauchi *et al.* (1997,1998)** reported that barley possesses preventive effects against the intestinal mucosal damage and diarrhea in a methotrexate induced enteritis model in rats where jejunal villi revealed structure similar to that of the villi of control group and this is by increasing mucosal protein, DNA and RNA contents.

Bamdad *et al.* (2011), Morel and Cottam, (2007), Madhujith and Shahidi, (2007) stated that barley exhibited antioxidant, antiradical potentials and antiulcerative effects as it causes suppression effects on the ulcerogenesis in gastric stress ulcer when

Discussion

Mucositis is a debilitating side effect of cytotoxic chemotherapy and radiotherapy. It involves inflammation and mucosal ulceration of the alimentary tract, resulting in symptoms including pain, abdominal bloating, nausea, vomiting and diarrhea, and may significantly impair treatment compliance in cancer patients and in patients with rheumatoid arthritis **(Cwikiel *et al.,* 1996, Tran *et al.,* 2003, Duncan and Grant, 2003 and Sonis *et al.,* 2004).**

Methotrexate was chosen in this experiment as it is widely used drug in cancer therapy and in arthritis and is known to have several side effects. Enterocolitis due to intestinal damage is one of the most frequent and severe side effects of MTX **(somi *et al.,* 2011, Acipayam *et al.,* 2013, Al-Motabagani, 2006 and kolli *et al.,* 2007).**

Barley is an important dietary source of water soluble and fat soluble as well as insoluble antioxidants. These antioxidants include vitamin E, Tocotrienols, selenium, phenolic acids and phytic acid. These antioxidants are available through the gastrointestinal tract over long period after being consumed **(Selim, 2005 and Yichen Xia, 2012).**

In the present study the potential protective effects of pre-treatment of barley grains on methotrexate induced jejunal damage in rat models were investigated**,** General observations were found that animals received methotrexate showed malaise from the day following injection, diminished activity and ruffling of the fur.

These results are in agreement **with Loehry and Creamer,(1969) and Taminiaut *et al.* (1980)** who reported that rats injected with methotrexate showed obvious malaise from the day following injection, Anorexia, weight loss, diminished activity, ruffling of the fur.

Light microscopic results revealed that rats treated with MTX showed fused villi with flattened enterocytes, also areas of erosions and ulcerations with reduced hypoplastic crypts of Lieberkühn, lamina propria & submucosa showed massive inflammatory cell infiltrations and areas of Hemorrhage.

These results are in agreement with **Keefea *et al.* (2000), El-Boghdady, (2011), Chang *et al.* (2013), Yüncü *et al.* (2004), de Koning *et al.* (2007) and Vardia *et al.* (2008)** who reported that MTX-treated rats showed villus shortening and fusion with variable degrees of ulceration decrease in the number of crypt cells, crypt loss, inflammatory cell infiltrations and hemorrhages in the lamina propria.

Methotrexate induced intestinal damage could be related to alterations of metabolism and not to the normal desquamation or apoptotic processes, MTX acts on crypt cells by inhibiting the enzyme contained in the diet as this may be due to the fraction containing phenolic compounds and water soluble organic compound.

Also **Deguchi *et al.* (2000) and Abd El-Aziem *et al.* (2002)** showed that barley has a protective role against genotoxic, biochemical and histopathological alterations and these, were possibly due to its higher contents of antioxidant substances.

In our study the scanning electron microscopic results revealed that rats treated with MTX showed altered jejunal mucosal surface with erosions and ulcerations at the villi tips with exposure of the underlying connective tissue, microvilli were absent in areas and fused disarranged in other areas on the edges of ulcerations.

These results are in agreement with **Jeynes and Altmann, (1978), Jahovic *et al.* (2004) and de Koning *et al.* (2007)** who reported that scanning electron microscopy in the MTX group revealed villus damage desquamation of surface epithelium, glandular degeneration and the cellular pleo-morphism and loss of microvilli.

In our study the scanning electron microscopic results revealed that rats treated with MTX and barley showed jejunal mucosa with plate - shaped villi with intact epithelial surface with goblet cells discharging mucus. Microvilli were absent in areas and scanty disarranged in other areas.

These results are in agreement with **Selim, (2005)** who reported that in animals treated with Monosodium Glutamate (MSG) Scanning electron microscopic examination showed deformed villi in some areas while' other villi exhibited sloughing of their tips. On the contrary, in animals treated with MSG simultaneously with barley, the villi revealed structure almost similar to that of the villi of control group.

Also these results are in agreement with **Vardi *et al.* (2008)** who reported that in animals treated with methotrexate (MTX) Scanning electron microscopic examination showed rats with loss of the microvillar structures. The antioxidant and protective effects of barley have been documented also by **Kalra and Jood (2001), Dongowski *et al.* (2002).**

Conclusion to the previous results; barley has potential protective role against methotrexate induced intestinal damage when used with chemotherapy course

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