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Effect of Methotrexate on the Jejunum of Adult Albino Rat and the Possible Protective Role of Vitamin A: Histological and Immunohistochemical Study

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Abstract

Background: Methotrexate (MTX) is an antineoplastic drug that is widely used in the treatment of neoplasms. Patients undergoing MTX therapy have a variety of side effects affecting many important organs.

Aim of the work: The aim of the present study was to detect the histological and the immunohistochemical adverse alterations that might occur in the jejunum of the adult albino rats following methotrexate therapy and to determine the possible protective role of vitamin A.

Material and Methods: Seventy two adult male albino rats were divided into six equal groups. In all groups the animals were sacrificed 24 hours after the last injection. The jejunal specimens were examined using H& E and Masson's trichrome stains and immunohistochemical examination to study the alkaline phosphatase activity using light microscopy. Morphometric study measuring villous height, crypt depth, number of goblet cells, percentage area of fibrosis and the optical density of alkaline phosphatase activity was done in all groups.

Results: The methotrexate treated groups' revealed different changes in the jejunum of rats. Distortion and cystic dilatation of the crypts, cellular shedding and dilated blood vessels were found. Morphometric study showed decreased villus height and goblet cells, increased the crypts depth. Immunohistochemical examination of the jejunal sections of the methotrexate treated groups showed weak reaction of alkaline phosphatase enzyme when compared with that of the control group.

Conclusion: It can be concluded that the toxic effect of methotrexate on the jejunum of rats can be partially improved with the concomitant use of vitamin A.

Keywords: Methotrexate; Jejunum; Histology; Vitamin A; Antioxidant

Introduction

Methotrexate (MTX) is used for the therapy of a wide range of neoplastic disorders including acute lymphoblastic leukemia, non-Hodgkin's lymphoma, breast cancer and testicular tumors [1]. MTX treatment may reduce the number of attacks in patients with recurrent acute anterior uveitis [2]. Recent literature reported that high dose of MTX regimens may be used in induction of abortion as well as liver cholestatic disorders [3]. As methotrexate (MTX) is an anti-metabolite drug, it is widely used in the treatment of many diseases such as rheumatoid arthritis and psoriasis [4].

Concerning the effect of MTX on the small intestine, Miyazono et al. [5] mentioned that the oxidative stress plays an important role in the MTX-induced small intestinal damage. Ciralik et al. [6] mentioned that methotrexate caused damage to the mucosa of small intestine leading to nausea, vomiting, diarrhea, stomatitis, decreased absorption and gastrointestinal (GI) ulceration. The latter authors also reported that biopsies of the small intestinal epithelium of patients treated with MTX exhibited an increase in the oxidative stress and a decrease in antioxidant defenses. Sener et al. [7] postulated that methotrexate exerted its cytotoxic effects on the rapidly dividing cancer cells as well as on the normal tissues with high cell proliferation rate such as the gut mucosa and the hematopoietic cells. Methotrexate also induced mucositis and nitrosative stress in the small intestine of rats [8].

Regarding the concomitant use of vitamin A with methotrexate, Yasuharu et al. [9] reported that vitamin A increased the integrity of small intestine by increasing its protein and lipid content without affecting the absorption of methotrexate. In addition, Swartz -Basile et al. [10] found that vitamin A was essential for normal growth and differentiation of the epithelial tissues. Yuncu et al. [11] assumed that vitamin A might be involved in the regulation of DNA and RNA synthesis in the crypt cells (stem cells) helping their regeneration which further supports the protective effect of vitamin A. Moreover, Ciralik et al. [6] suggested that vitamin A is among the antioxidants which might protect the jejunum from MTX damage.

The aim of the present study is to detect the histological and the immunohistochemical adverse alterations that may occur in the jejunum of the adult albino rats following short and long term methotrexate therapy (two modes of regimen). Determination of the possible protective role of vitamin A against MTX-induced damage will also be done.

Material and Methods

Chemicals

Methotrexate was supplied as methotrexate tablets in a

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concentration of 2.5 mg/tablet (a product of EBEWE pharma Austria). Two tablets were dissolved in 10 ml of saline solution to obtain a concentration of 0.5 mg/ml. Methotrexate was then administrated in a dose of 2.5 and 1 mg/kg for three successive days/week for a period of four weeks to detect its short and long term effects [4].

Vitamin A was supplied as vitamin A capsules in a concentration of 50000 IU/capsule (a product of El Kahira pharmaceutical company). Each capsule was dissolved in 10 ml of olive oil to obtain a concentration of 5000 IU/ml. It was given in a dose of 5000 IU/kg for five days (two days before and three days concurrently with methotrexate injection) for one week in short term treated group and for four weeks in long term treated group. All medications were given by intraperitoneal injection.

Animals

The present study was carried on 72 adult male albinos Sprague Dawely rats weighing 180-220 grams each. The animals were housed in separate cages (five rats per cage) under standard laboratory and environmental conditions with free access to food and water. They were obtained from the animal house of the Faculty of Medicine, Cairo University. Only male rats were used in this study to exclude the possible sex differences. The rats were acclimatized in the laboratory for a period of two weeks before carrying out the drug administration. The rats were divided into six equal groups 12 rats each. In all groups the animals were sacrificed 24 hours after the last injection.

Group I (Normal control): The rats of this group received nothing and according to the time of scarification were subdivided into:

- Subgroup Ia: The rats were sacrificed after one week.
- Subgroup Ib: The rats were sacrificed after four weeks.

Group II (Sham control): The rats of this group were subdivided into 2 subgroups 6 rats each:

- Subgroup IIa: The rats received saline solution in a dose of 0.2 ml/Kg body weight for three days [9].
- Subgroup IIb: The rats received saline solution in a dose of 0.2 ml/ Kg bodyweight for three successive days/week for four weeks [9].

Group III (short term methotrexate treated group): The rats were given methotrexate in a dose of 2.5 mg/kg for three successive days [4].

Group IV (long term methotrexate treated group): The rats were given methotrexate in a dose of 1mg/kg for three successive days/week for four weeks to detect the cumulative effect of methotrexate [4].

Group V (short term methotrexate and Vitamin A treated group): The rats were given methotrexate in a dose of 2.5 mg/kg for three successive days and vitamin A in a dose of 5000 IU/kg dissolved in olive oil [4] for five days (two days before and three days concurrently with methotrexate injection).

Group VI (long term methotrexate and Vitamin A treated group): The rats were given methotrexate in a dose of 1 mg/kg for three successive days/week for four weeks and vitamin A in a dose of 5000 IU/kg dissolved in olive oil [4] for five days/week for four weeks (two days before and three days concurrently with methotrexate injection). All medications were given by intraperitoneal injection.

The rats were sacrificed by inhalation of high dose of ether. The jejunal specimens were excised and fixed in 70% alcohol, trimmed, washed, dehydrated in ascending grades of alcohol, cleared in xylol and processed for paraffin sections of 5 µm thickness. The specimens were subjected to:

1-Histological examination: using H & E and Masson's trichrome stains.

2-Immunohistochemical examination: using DAB (diaminobenzidine) for studying the alkaline phosphatase activity using light microscopy.

3-Morphometric study: sections stained with DAB and Masson's trichrome stain were examined by the image analyzer computer system to measure the optic density of alkaline phosphatase enzyme activity and the percentage area of fibrosis in each group. The data were obtained using Leica Qwin 500 image analyzer computer system (England). The image analyzer consisted of a colored video camera, colored monitor, hard disc of IBM personal computer connected to the microscope, and controlled by Leica Qwin 500 software. The image analyzer was first calibrated automatically to convert the measurement units (pixels) produced by the image analyzer program into actual micrometer units. Using the measuring field menu, the area, optic density and standard measuring frame of a standard area equal to 118476.6um^2 were chosen from the parameters. This step was repeated but the optic density was replaced by area percentage. In each chosen field the jejunal tissue was enclosed inside the standard measuring frame (Figures 1a and 1b) and then the optic density of alkaline phosphatase enzyme activity and the CT area were masked by a blue color to be measured (Figures 1a and 1b). Villus height, crypts depth and goblet cell counting also detected (Figures 1c and 1d).

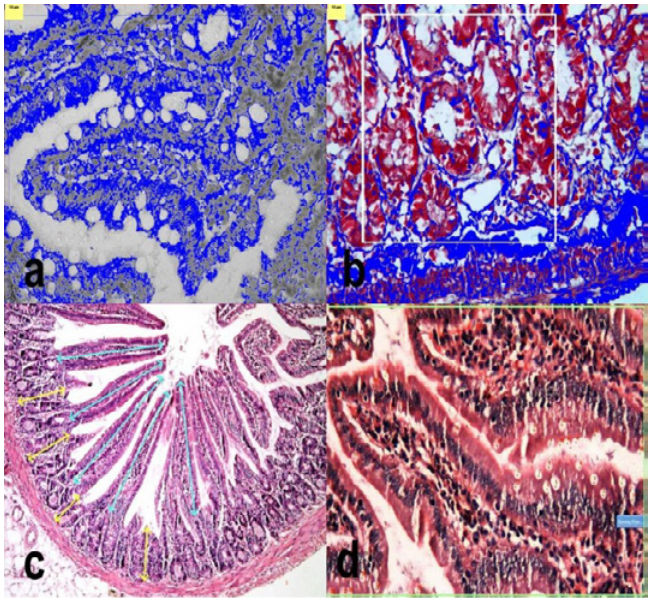

Figure 1: A copy of a display seen on the monitor's screen of the image analyzer of a cross section of rat jejunal specimen of methotrexate treated group showing the:
a) optic density of alkaline phosphatase enzyme activity masked by blue color inside the frame. (DAB x 400).
b) areas of connective tissue fibres masked by blue color inside the standard frame. (Masson's Trichrom x 400).
c) method of measuring the villus height (blue double head arrows) and crypts depth. (yellow double head arrows). (H & E x 100).
d) method of counting the goblet cells. (H & E x 400).

These measurements were done using an objective lens of magnification 40, i.e. of total magnification 400. The total area of fibrosis in each specimen was measured and the mean values for each group were obtained. The statistical package for the social science (SPSS version 7.5) was used on data analysis. Data was expressed as mean ± SD. One way analysis of variance (ANOVA) was used.

Results

Histological and Immunohistochemical results for control and sham control groups (groups I and II)

Light microscopic examination: The results of control and sham control are the same. The villi appeared as finger like projections thrown into the lumen (Figures 2a and 2b) and lined by a single layer of tall columnar cells with oval basal nuclei (Figure 2c). The lamina propria of villi covered by a single layer of tall columnar cells with oval basal nuclei (Figures 2b and 2d). The goblet cells were scattered

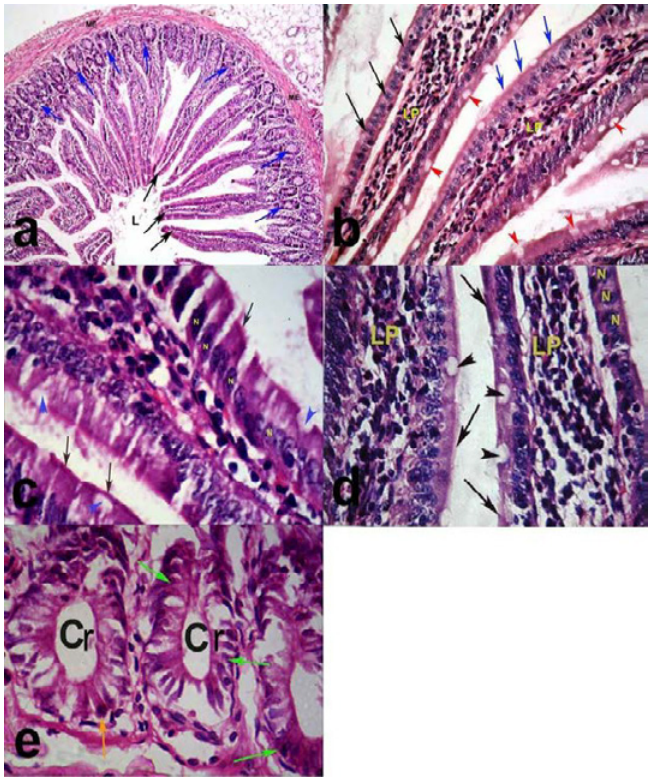

Figure 2: A photomicrograph of a cross section in the jejunum of adult male albino rat of group I (normal control groups) showing the:
a) villi (black arrows) that appear as finger like projections thrown into the lumen (L). The tubular crypts (blue arrows) arranged deep to the muscularis externa (ME). (H &E X 100)
b) lamina propria (LP) of the villi covered by a single layer of tall columnar cells with oval basal nuclei (black arrows). The goblet cells (arrow heads) are scattered in between the columnar cells. The brush border (blue arrows) is intact. (H &E X 200)
c) villi lined by tall columnar cells with oval basal nuclei (N) and the goblet cells (arrow heads) are scattered in between. The brush border (arrows) is intact. (H &E X 400).
d) lamina propria (LP) of the villi covered by tall columnar cells with oval basal nuclei (N) and the goblet cells (arrow heads) are scattered in between. The brush border (arrows) is intact. (H &E X 400)
e) crypts (Cr) lined by columnar epithelial cells with basal oval nuclei (green arrows)
and Panth cell (orange arrow). (H & E X 400).

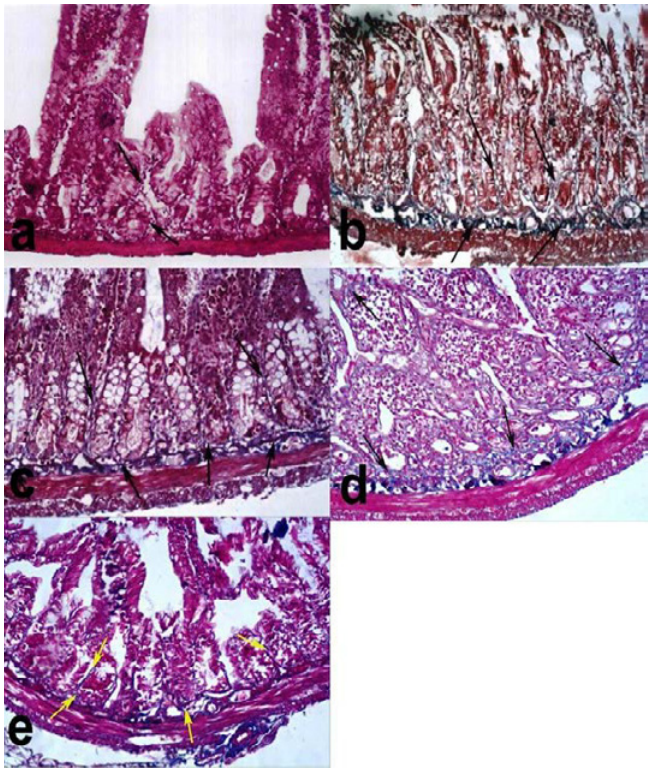

Figure 3: A photomicrograph of a cross section in the jejunum of adult male albino rat of:
a) group I showing minimal amount of collagen fibers (arrows) in the submucosa and in between the villi.
b) group III showing increased amount of collagen fibers in the submucosa and among the crypts (arrows).
c) group IV showing increased amount of collagen fibers in the submucosa and among the crypts (arrows).
d) group V showing mild amount of collagen fibers in the submucosa (arrows).
e) group VI showing mild amount of collagen fibers among the crypts and in the submucosa (arrows). (Masson's trichrom X 100).

in between the columnar cells (Figure 2b -2d). The brush border was intact (Figure 2b-2d). The tubular crypts were arranged deep to the muscularis externa (Figure 2a) and lined by columnar epithelial cells with basal oval nuclei and Panth cell (Figure 2e). Minimal amount of collagen fibers were observed in the submucosa and in between the villi (Figure 3a).

Immunohistochemical examination: Immunohistochemical examination of the jeujenal sections of the control and sham control groups showed strong reaction of alkaline phosphatase enzyme along the brush border of the villi and among the crypts (Figure 4a).

Short term methotrexate treated group (Group III)

Light microscopic examination: Examination of this group revealed fusion of villi (Figures 5a, 5c and 5d), flattening of the villus surface epithelium (Figures 5c and 5d), loss of villus architecture (Figures 5c and 5d), blunting of the apices of the villi (Figures 5e, 6a and 6b), intravillous hemorrhage (Figures 5e and 6a) and shedding of the surface epithelium (Figures 5a, 5d, 6a and 6b). Distortion of the crypts (Figures 5a, 5c-5e), cystic dilatation of the crypts (Figure 5b), dilated blood vessels (Figure 5b), large goblet cells (Figures 6a-6c), apoptotic like lesion (Figures 6c and 6d), ghost nuclei (Figure 6c),

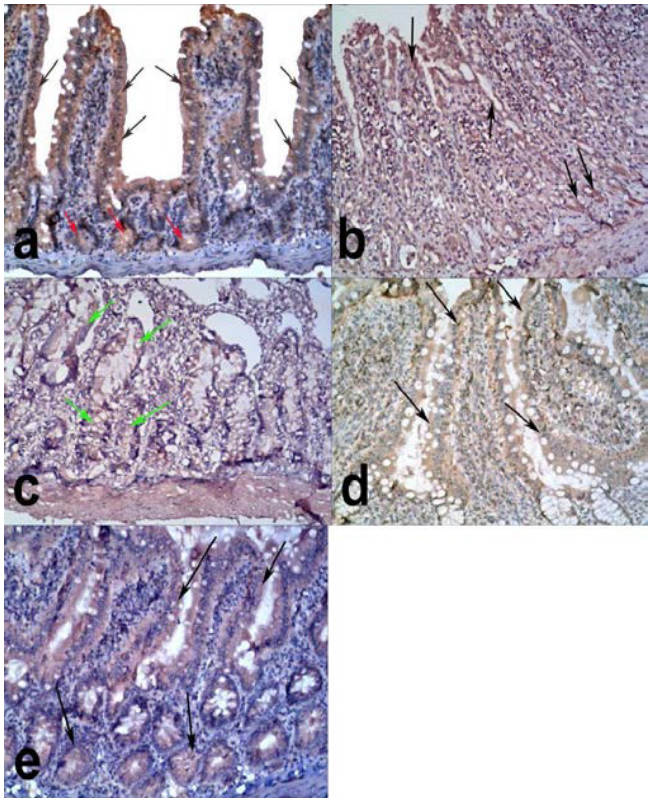

Figure 4: A photomicrograph of a cross section in the jejunum of adult male albino rat of:
a) group I showing strong reaction of alkaline phosphatase enzyme on the brush border of the villi (black arrows) and among the crypts (red arrows).
b) group III showing weak reaction of alkaline phosphatase enzyme (arrows).
c) group IV showing weak reaction of alkaline phosphatase enzyme on the brush border of the villi and among the crypts (arrows).
d) group V showing moderate reaction of alkaline phosphatase enzyme (arrows).
e) group IV showing moderate reaction of alkaline phosphatase enzyme on the brush border of the villi and among the crypts. (DAB X 100).

cellular vacuolations (Figure 6d) and few cells showing intravillous pyknosis (Figure 6b) were also seen in this group. Increased amount of collagen fibers in the submucosa and among the crypts were observed (Figure 3b).

Immunohistochemical examination: Immunohistochemical examination of the jeujenal sections of the short term methotrexate treated group showed weak reaction of alkaline phosphatase enzyme (Figure 4b).

Long term methotrexate treated group (Group IV)

Light microscopic examination: Examination of this group revealed shedding of most of cells covering the villi (Figures 7a and7b), loss of villi (Figures 7c-7e), fusion of villi (Figure 7f), flattening of surface epithelium (Figure 7f), ulceration (Figure 7g) and cellular infiltration (Figures 7c and 7g). Distortion of some crypts (Figures 7b-7d and 7f) cystic dilatation of the crypt (Figure 7e) and multiple cellular shedding inside the crypts (Figure 7e) were also seen in this group. Vacuolations, pyknosis, karryorehxis (Figure 7h), disappearance of intervillus space (Figures 7a and 7c), loss of crypts architecture (Figure 7h) and dilated blood vessel (Figures 7b and 7g) were also found in this group. Increased amount of collagen fibers was also seen in the submucosa and among the crypts (Figure 3c).

Immunohistochemical examination: Immunohistochemical examination of the jeujenal sections of the long term methotrexate treated group showed weak reaction of alkaline phosphatase enzyme on the brush border of the villi and among the crypts (Figure 4c).

Short term methotrexate and vitamin A treated group (Group V)

Light microscopic examination: Examination of this group revealed partial damage of villi (Figures 8a, 8b, 8d and 9b), increased mitotic activity (Figures 8d, 8f, 9a and 9b), focal intravillous hemorrhage (Figures 8c, 8e), numerous goblet cells (Figures 8e, 8f and 9a-9c), ulceration (Figures 8c and 8f) and nearly intact brush border, (Figures 8a, 9a, 9b). Distortion of the crypts with flattening of its epithelial lining (Figure 9c), few shedded cells inside the crypts (Figure 8b), apoptotic like lesion (Figures 9c and 9d), ghost nuclei (Figure 9d) and pleomorphism (Figures 9c and 9d) were also noticed. Mild amount of collagen fibers in the submucosa (Figure 3d) were observed.

Immunohistochemical examination: Immunohistochemical

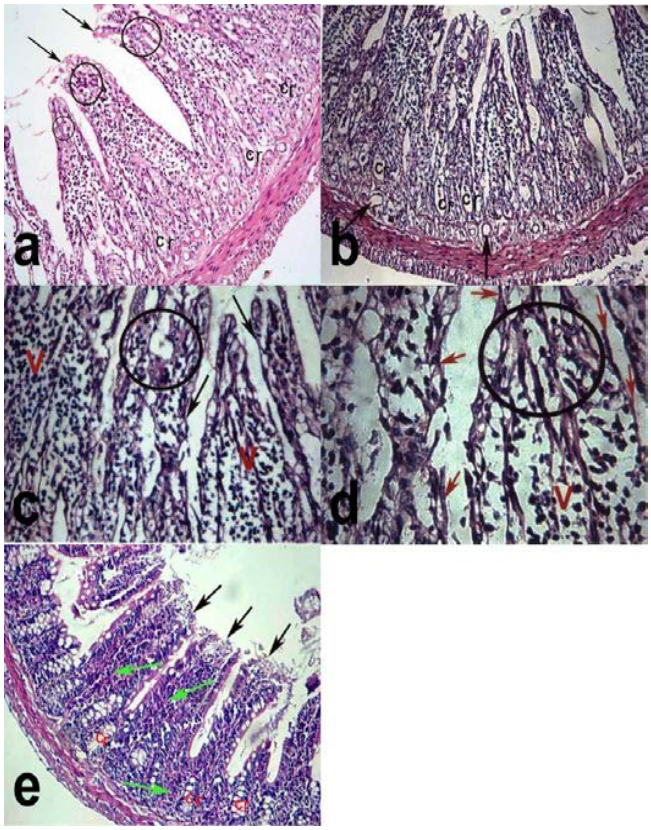

Figure 5: A photomicrograph of a cross section in the jejunum of adult male albino rat of group III (short term MTX treated group) showing:
a) fusion of villi (circle), shedding of the surface epithelium (arrows) and distortion of
the crypts (Cr). (H & E X100)
b) cystic dilatation of the crypts (Cr) at the bases of the villi and dilatation of the blood vessels (arrows). (H &E X100).
c) flattening of the villous surface epithelium (arrows) with loss of villus architecture (V) and fusion of villi (circle) especially near the apices. (H &E X200.)
d) flattened and shedded cells of the villous surface epithelium (arrows) with the loss of the villus architecture (V) and fusion of the villi (circle). (H &E X400)
e) showing distortion of the crypts (Cr) with blunting of the apices of the villi (black arrows) and intravillous hemorrhage (green arrows). (H & E X 100).

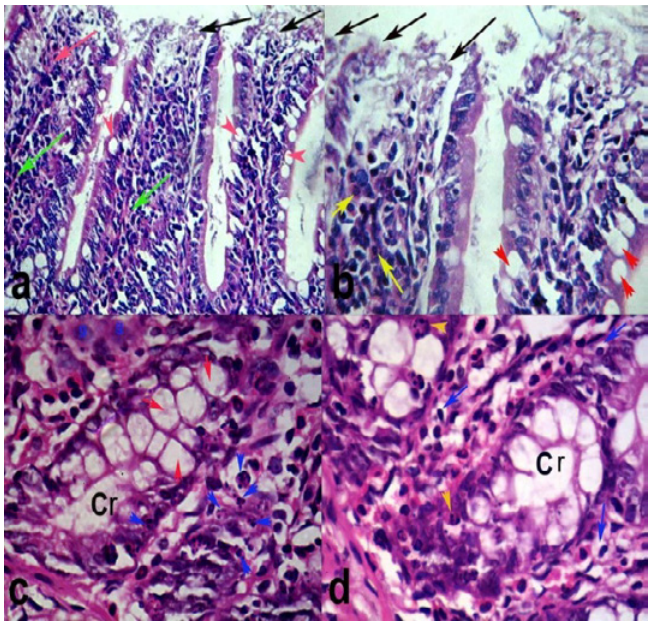

Figure 6: A photomicrograph of a cross section in the jejunum of adult male albino rat of group III showing:
a) blunting and shedding of the apices of villi (red and black arrows) with large goblet cells (arrow heads) and the intravillous hemorrhage (green arrows). (H & E X 200)
b) shedding and blunting of the apices of the villi (black arrows) with large goblet cells
(arrow heads). Few cells showed intravillous pyknosis (yellow arrows).
(H & E X 400).
c) distortion of the crypt (Cr), large goblet cells (short red arrows), apoptotic like lesion (arrow heads) and ghost nuclei (g). (H & E X 400)
d) apoptotic like lesion (arrow heads), distortion of the crypt (Cr) and cellular vacuolations (blue arrows). (H & E X 400).

examination of the jeujenal sections of the short term methotrexate and vitamin A treated group showed moderate reaction of alkaline phosphatase enzyme (Figure 4d).

Long term methotrexate and vitamin A treated group (Group VI)

Light microscopic examination: Examination of this group revealed blunt tipped villi (Figure 10a) and numerous large goblet cells (Figures 10a and 10b), few shedded cells at the tip of the villi (Figure 10b), almost intact the brush border (Figure 10c), shedding of the apices of the villi (Figure 10c), almost normal crypts lined by columnar epithelium (Figure 10d). Mild amount of collagen fibers among the crypts and in the submucosa (Figure 3e) were observed.

Immunohistochemical examination: Immunohistochemical examination of the jeujenal sections of the long term methotrexate and vitamin A treated group showed moderate reaction of alkaline phosphatase enzyme on the brush border of the villi and among the crypts (Figure 4e).

Morphometric measurements

All the measurements of the jejunum regarding the different experimental groups were summarized in Table 1.

The Villus height: The villus height of short term methotrexate treated group (group III) decreased significantly in comparison with the corresponding control group. With treatment with vitamin A (group V), there was a significant increase in villus height. With long duration of exposure to methotrexate (group IV), there was a significant decrease in the villus height when compared with the corresponding control group and insignificant increase in the villus height when compared with short term methotrexate treated group (group III). Much improvement was observed in the villus height in long term methotrexate and vitamin A treated group (group VI) when compared with both short term methotrexate treated group (group III) and long term methotrexate treated group (group IV).

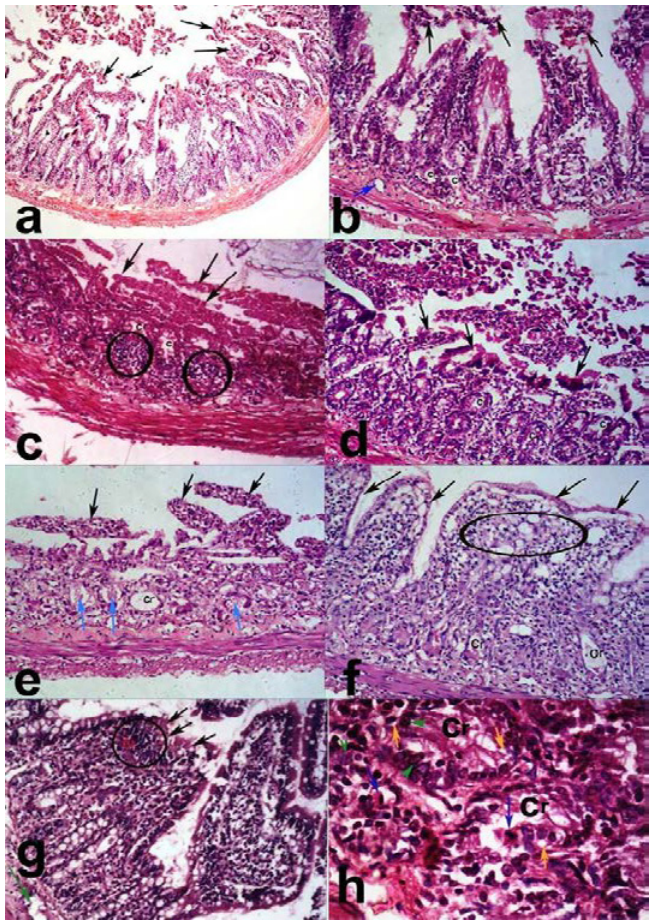

Figure 7: A photomicrograph of a cross section in the jejunum of adult male albino rat of group IV (long term MTX treated group) showing:
a) shedding of most of cells covering the villi (arrows) with disappearance of the intervillous space. (H & E X 100)
b) shedding of most of cells covering the villi (black arrows), distortion of the crypts (Cr) and dilated blood vessel (blue arrow). (H & E X 100)
c) loss of the villi (black arrows), disappearance of the intervillous space and distortion
of the crypts (Cr) with cellular infiltration (circle). (H & E X 200).
d) loss of villi (arrows) with distortion of some crypts (Cr). (H & E X 200).
e) loss of villi with cystic dilatation of the crypt (Cr) and multiple cellular shedding inside the crypts (blue arrows). (H & E X 200)
f) fusion of villi (circle) with flattening of surface epithelium (arrows) and distortion of the crypts (Cr). (H & E X 200).
g) ulceration (black arrows), dilated blood vessel (green arrow) and extensive cellular infiltration (CI). (H & E X 200).
h) showing loss of crypts architecture (Cr) and cellular vaculations (orange arrows). Few cells showed pyknosis (blue arrows) and karryorehxis (arrow heads) (H & E X400).

	Villous height			Crypts depth		Goblet cells		Area % of fibrosis		Optic density of alkaline phosphatise enzyme activity	
Group	Mean ± SD	P value		Mean ± SD	P value	Mean ± SD	P value	Mean ± SD	P value	Mean ± SD	P value
I	428.1 ± 43			159.6 ± 13		35.2 ± 1.9		6.8 ± 2.7		56.6± 6	
II	426.4 ± 42			157.6 ± 12		34.5 ± 1.6		7.4 ± 2.5		55.2±5.4	
III	144.1 ± 48	I	.000**	181.7 ± 26	.000**	23.4 ± 1.4	.049*	31.6 ± 3.9	.000**	28.2±4.18	.000**
		IV	1.000		.033*		.064		.592		.124
		V	.000**		.000**		.000**		.000**		.000**
		VI	.000**		.000**		.000**		.000**		.165
IV	155.8 ± 32	I	.000**	197.9 ± 18	0.000**	17.8 ± 1.5	.000**	36.8 ± 7.9	.000**	26.8±3.3	.000**
		V	.000**		0.000**		.000**		.000**		.003**
		VI	0.027*		0.001**		.000**		.000**		.063
V	401.7 ± 29	I	.068	159.4 ± 4	0.189	74.3 ± 1.7	.000**	11.6 ± 2	.764	42.7±2.6	.000**
		VI	.000**		0.154		.000**		1.000		.048*
VI	207.0 ± 3	I	.000**	150.6 ± 20	0.177	40.5 ± 1.4	.063	13.6 ± 3.7	.879	32.1±2.1	.039*

* = p value is significant
** = p value is highly significant

Table 1: the mean ± SD of the villous height, crypts depth, goblet cells, area % of fibrosis and 0ptic density of alkaline phosphatise enzyme activity of the jejunum among the different experimental groups.

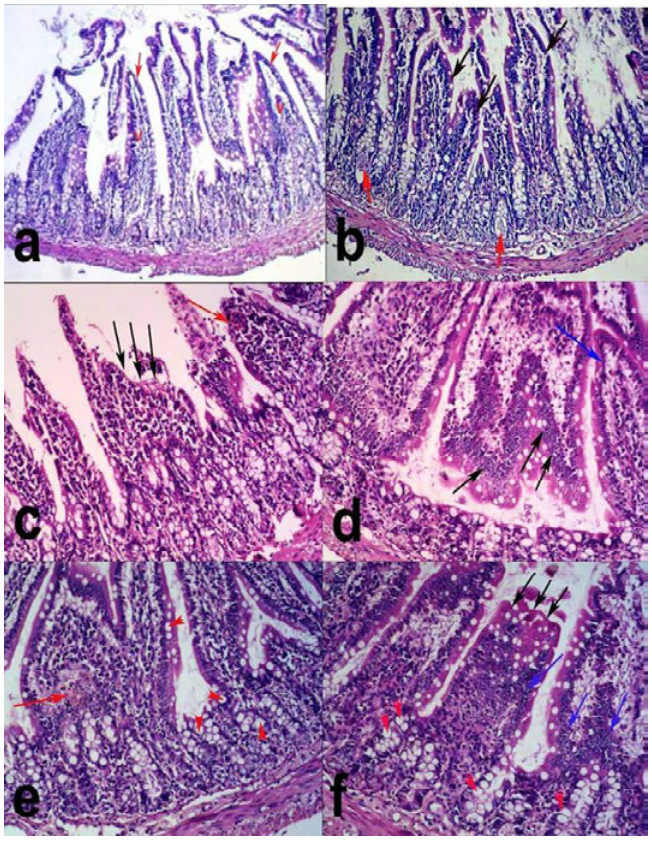

Figure 8: A photomicrograph of a cross section in the jejunum of adult male albino rat of group V (short term MTX and vitamin A treated group) showing:
a) partial damage of villi (V) and nearly intact brush border (arrows). (H &E X100)
b) partial damage of the villi (black arrows) with few cells shedded inside the crypts (red arrows). (H & E X 100)
c) ulceration (black arrows) and focal intravillous hemorrhage (red arrow). (H & E X 200).
d) partrial damage of villi (blue arrow) and increased mitotic activity (black arrows). (H & E X 200).
e) numerous goblet cells (arrow heads) and focal intravillous hemorrhage (arrow). (H & E X 200).
f) numerous goblet cells (arrow heads), ulceration (black arrows) and increased mitotic activity (blue arrows) (H & E X 200).

The Crypts depth: The crypts depth of short term methotrexate treated group (group III) increased significantly in comparison with the corresponding control group. Concomitant treatment with vitamin A (group V), there was a significant decrease in crypts depth. With long duration of exposure to methotrexate (group IV), there was a significant increase in the crypts depth when compared with both the corresponding control group and short term methotrexate treated group (group III). Much improvement was observed in the crypts depth in long term methotrexate and vitamin A treated group (group VI) when compared with both short term methotrexate

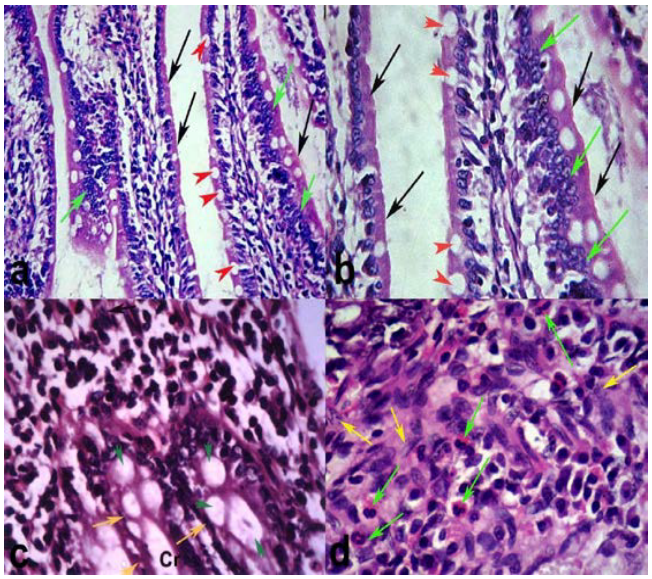

Figure 9: A photomicrograph of a cross section in the jejunum of adult male albino rat of group V showing:
a) nearly intact brush border (black arrows), increased mitotic activity (green arrows) and numerous goblet cells (arrow heads). (H & E X 200)
b) partial damage of villi with nearly intact brush border (black arrows), increased mitotic activity (green arrows) and numerous goblet cells (arrow heads). (H & E X 400).
c) distortion of the crypts (Cr) with flattening of its epithelial lining (yellow arrows), numerous goblet cells (arrow heads) and apoptotic like lesion (black arrow) with pleomorphism. (H & E X 400).
d) pleomorphic cellular changes with ghost nuclei (yellow arrows) and apoptotic like lesion (green arrows). (H & E X 400).

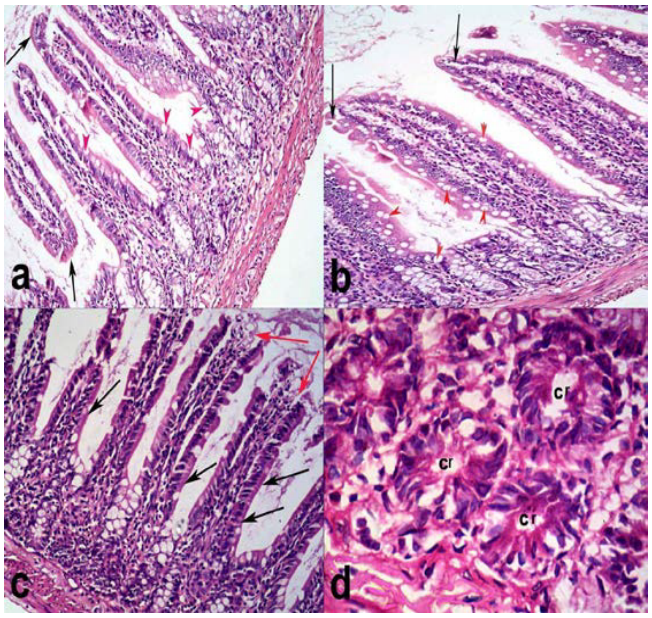

Figure 10: A photomicrograph of a cross section in the jejunum of adult male albino rat of group VI (long term MTX and vitamin A treated group) showing:
a) blunt tipped villi (arrows) and numerous large goblet cells (arrow heads). (H & E X100).
b) few shedded cells at the tip of the villi (arrows) and numerous large goblet cells (arrow heads). (H & E X 200).
c) shedding of the apices of the villi (red arrows) and almost intact the brush border (black arrows). (H & E X 200).
d) almost normal crypts (Cr) lined by columnar epithelium. (H & E X 400).

treated group (group III) and long term methotrexate treated group (group IV).

The number of goblet cells: The number of goblet cells of short term methotrexate treated group (group III) decreased significantly in comparison with the corresponding control group. With concomitant treatment with vitamin A (group V), there was a significant increase in the number of goblet cells. With long duration of exposure to methotrexate (group IV), there was a significant decrease in the number of goblet cells when compared with the corresponding control group and insignificant decrease in the number of goblet cells when compared with short term methotrexate treated group (group III). There was a significant increase observed in the number of goblet cells in long term methotrexate and vitamin A treated group (group VI) when compared with both short term methotrexate treated group (group III) and long term methotrexate treated group (group IV).

The mean area percentage of fibrosis: The mean area percentage of fibrosis of short term methotrexate treated group (group III) increased significantly in comparison with the corresponding control group. With treatment with vitamin A (group V), there was a significant decrease in the mean area percentage of fibrosis. With long duration of exposure to methotrexate (group IV), there was a significant increase in the mean area percentage of fibrosis when compared with the corresponding control group and insignificant increase in the mean area percentage of fibrosis when compared with short term methotrexate treated group (group III). Much improvement was observed in the mean area percentage of fibrosis in long term methotrexate and vitamin A treated group (group VI) when compared with both short term methotrexate treated group (group III) and long term methotrexate treated group (group IV).

The optical density of the alkaline phosphatase enzyme activity: The optical density of the alkaline phosphatase enzyme activity of short term methotrexate treated group (group III) decreased significantly in comparison with the corresponding control group. With concomitant treatment with vitamin A (group V), there was a significant increase in the optical density of the alkaline phosphatase enzyme activity. With long duration of exposure to methotrexate (group IV), there was a significant decrease in the optical density of the alkaline phosphatase enzyme activity when compared with the corresponding control group and insignificant decrease in the optical density of the alkaline phosphatase enzyme activity when compared with short term methotrexate treated group (group III). The optical density of the alkaline phosphatase enzyme activity in long term methotrexate and vitamin A treated group (group VI) was improved when compared with short term methotrexate treated group (group III) and long term methotrexate treated group (group IV) but the improvement was insignificant.

Discussion

Methotrexate (MTX) is one of the most effective antineoplastic drugs inspite of its undesirable side effects on the different organs. Methotrexate was a structurally related to folic acid and acted as an antagonist to that vitamin [12]. Lower doses of MTX had been shown to be very effective for the management of rheumatoid arthritis and psoriasis due to the inhibition of enzymes involved in purine metabolism, leading to accumulation of adenosine or the inhibition of T cell activation and suppression of intercellular adhesion molecule expressed by T cells [13].

In the present study, the short and long term methotrexate treated groups showed the presence of fusion of villi and loss of intervillus spaces. This was in agreement with several authors [4,14] who observed fusion of the villi in rats treated with MTX. With long duration of exposure to the drug the fusion of the villi was seen more obvious with loss of intervillus space with subsequent decrease in the surface area of absorption from the jejunum. This finding might be attributed to the direct irritant, cytotoxic and oxidative effect of the drug as previously reported [5]. One of the most characteristic finding of short term methotrexate treated group (group III) in the present work was the loss of villus architecture. Similar finding was noticed by many reseaerches [15, 16] who suggested that the folic acid analogue, MTX, primarily inhibited DNA synthesis by binding to the enzyme dihydrofolate reductase which might lead to an inhibition of proliferation in the crypts of the small intestine.

Garipardic et al. [17] believed that the oxidative stress played an important role in MTX induced mucosal damage and also, fibrosis might cause loss of villus architecture.

In the current work, most of the villi were covered with flattened or cuboidal cells in long term methotrexate treated group (group IV) as previously reported [18]. This might be related to the antimitotic effect of MTX with wide spread apoptosis of the stem cells in the villus tip [16]. Blunting of the apices of the villi was noticed in the current study in short term methotrexate treated group (group III) and progressed to loss of the villi in long term methotrexate treated group (group IV). This was in agreement with previous reports [16,19] suggesting that the injury to the villous tip was due to exposure of the mucosa to the erosive effect of pancreatic and biliary secretions causing aut-odigestion of the intestinal epithelium. Sukhotnik et al. [20] also observed strong inhibitory effects of MTX on enterocyte proliferation using brumodeoxy-uridine (BrdU), a marker for cell proliferation,

which was considered as a major mechanism responsible for decreased intestinal cell mass and mucosal hypoplasia with loss of villi.

Complete shedding of the surface epithelium was a characteristic finding in the present study in both short and long term methotrexate treated groups. This was in agreement with Jahovic et al. [21] who found desquamation of surface epithelium in rats treated with MTX. Some authors [5] suggested that this finding was due to the cytotoxic effect of MTX on the intestinal cells while others [18] related it to the inhibition of the cell proliferation in the small intestinal mucosa. The mucosal damage in the present study progressed to ulcer formation in long term methotrexate treated group (group IV). This was also detected by Gao and Horie [22] who noticed neutrophil aggregation in the intestinal mucosa after MTX administration to rats and suggested that neutrophil accumulation in the intestinal mucosa played a key role in the potentiating action of MTX- induced ulceration. Ulceration of the jejunal mucosa noticed in this work might also be due to the decreased number of goblet cells in MTX treated rats causing deficiency in the secretion of mucus by these cells affecting their protective role. In this study, it was found that the alkaline phosphatase enzyme activity of short and long term methotrexate treated groups decreased and the decrease was statistically significant in comparison with the corresponding control group. This was in agreement with many studies [4,23,24] that attributed this finding to the oxidative stress that caused inactivation of the enzyme and the affection of the brush border of the villi. The significant decrease in the activity of alkaline phosphatase enzyme encountered in the present work might be due to the inhibitory effect of MTX on DNA synthesis [15] with subsequent decrease in protein formation resulting in decreased enzyme synthesis. Intravillus hemorrhage was observed in the current study in short term methotrexate treated group (group III). This was also observed by Kremer [25] who attributed that to the increased transendothelial and transepithelial permeability causing inflammation, on the other hand Garipardic et al. [17] discussed that these changes may be due to the antiplatelet action of nitrous oxide or the folic acid deficiency that might cause severe anemia and bleeding.

Distortion of the crypts was also noticed in the present study in short and long term methotrexate treated groups. This was also observed in one study [18] which explained this by inhibition of proliferation of the cells in the crypts of the small intestine.

Cystic dilatation of the crypts in the present work, with flattening of its epithelial lining was observed in short and long term methotrexate treated groups. Similar finding was detected and explained by the antimitotic effect of MTX with wide spread apoptosis of the stem cells in the crypts [11,14].

In the current study, the crypts depth of short and long term methotrexate treated groups increased significantly in comparison with that of the corresponding control group while the villus height decreased significantly in both groups. Similar findings were reported by several authors [16,26] in rats treated with MTX. However other studies [27,28] related this finding to a reduction of intestinal mucosal protein and DNA contents together with starting recovery by proliferation of crypt epithelium. In disagreement with these findings, some studies [4, 29] reported significant decrease in the crypts depth due to cytotoxicity of the drug and the resulting inflammation.

In the present study, the short and long term methotrexate treated groups showed dilated blood vessels. Similar findings were found by many previous studies [16,17]. This might be due to inflammation associated with MTX treatment that increased the transendothelial and transepithelial permeability [25]. Marked cellular infiltrate in the lamina propria was observed in the long term methotrexate treated group (group IV) in the present work. This was in agreement with many previous studies [6,11,16] who observed cellular infiltration in the lamina propria after MTX administration. Miyazono et al. [5] explained that this infiltration to the reactive oxygen species (ROS) production. In this study, it was found that the number of goblet cells of short and long term methotrexate treated groups decreased and the decrease was statistically significant in comparison with that of the corresponding control group. This was in agreement with many authors [4,14,16] who found depletion of goblet cells and attributed that to the antimitotic effect of MTX. In contrast, Xian et al. [30] reported that there were repopulation and accumulation of the goblet cells four days after MTX treatment and interpreted that to the commencement of epithelial proliferation.

One of the most characteristic finding of short term methotrexate treated group (group III) in the present work was the presence apoptotic like lesion. Similar finding was reported by different studies [31-33].

Cellular vacuolations were also noticed in the current study in the short and long term methotrexate treated groups. This was in agreement with Leitão et al. [34] who noticed the same finding under transmission electron microscope and referred these vacuolations to the occurrence of large residual bodies (secondary lysosomes) containing partially degraded fragments of damaged epithelial cells. Cells showing cytoplasmic and nuclear degeneration with disappearance of their cellular boundaries (ghosts' nuclei) in addition to cellular pyknosis were noticed in this study in the short term methotrexate treated group (group III) and karryorehxis was seen in long term methotrexate treated group (group IV). In agreement with Leitão et al. [34] and Miyazono et al. [5] such finding might be attributed to the oxidative stress.

In the present study, increased amount of collagen fibers in the submucosa and among the villi was observed in the jejunum of rats of short term methotrexate treated group (group III) and this fibrosis was more obvious in the jejunum of rats of the long term methotrexate treated group (group IV). This was in agreement with Dadhania et al. [4] who found that there was a significant increase in the collagen fibers in the short and long term methotrexate treated group when compared with that of the corresponding control group. The possible cause of this fibrosis was either due to the direct toxic effect of the drug or a result of the inflammation [33]. The present work showed that the use of vitamin A in conjunction with methotrexate (groups V and VI) improved partially the histological finding that occurred in the cells of the jejunum as compared to those of the methotrexate treated groups. The improvement was in the form of few shedded cells at the tip of the villi, numerous goblet cells, partial restoration of villus architecture, and significant increase of the villus height, increased mitotic activity and nearly intact brush border. Almost normal crypts lined by columnar epithelium with basal oval nuclei, increased number of crypts, significant decrease of the crypts depth and few cells shedded inside the crypts were also noticed. Similar findings were reported by different authors [11,14,16]. This improvement was explained by Yuncu et al. [11] who suggested that vitamin A might be involved in the regulation of DNA and RNA synthesis in the crypt cells. A significant increase in the number of goblet cells was observed in groups V and VI when compared to that seen in both short and long term methotrexate treated groups. This matched with the finding of Antar et al. [16] who believed in the protective role of vitamin A.

In the present study, much improvement was observed in the mean area percentage of fibrosis seen in groups V and VI when compared

with that noticed in the methotrexate treated groups. This finding was confirmed statistically by the significant decrease in the area percentage of fibrosis with the use of vitamin A. Similar finding was reported by previous studies [4,6] which confirmed the beneficial antioxidant effects of vitamin A. In the current study, much improvement was noticed in the alkaline phosphatase enzyme activity in short term methotrexate and vitamin A treated group when compared to that observed in the short term methotrexate group (group III). This finding was confirmed statistically by the significantly increased optical density of the alkaline phosphatase enzyme activity following the use of vitamin A. Yasuharu et al. [9] confirmed the protective role of vitamin A and reported that the lipid and protein contents of the small intestinal cells were significantly higher in mice treated with MTX plus vitamin A than in mice treated with MTX alone without affecting the intestinal absorption of MTX.

On the other hand, in long term methotrexate and vitamin A treated group (group VI) the increase in the optical density of the alkaline phosphatase enzyme activity was insignificant when compared with that seen in the long term methotrexate treated group (group IV). Similar finding was reported by Dadhania et al. [4] who attributed this differential protection to the different antioxidant reserve as well as to the adaptive response of the cells at the target site to the threshold of the chemical insult. Further, the lack significant protection in long term study could be attributed to persistence of low level of oxidative stress over long period of time and the change in the pH of intestine. It can be concluded that vitamin A has a protective role against the adverse alterations and improves partially the toxic changes that may occur in the jejunum of the adult albino rats following short and long term methotrexate therapy and it is advised to be given prior and throughout the methotrexate therapy.

References

1. Jensen SB, Mouridsen HT, Reibel J, Brünner N, Nauntofte B (2008) Adjuvant chemotherapy in breast cancer patients induces temporary salivary gland hypofunction. Oral Oncol 44: 162-173.
2. Muñoz-Fernández S, García-Aparicio AM, Hidalgo MV, Platero M, Schlincker A, et al. (2009) Methotrexate: an option for preventing the recurrence of acute anterior uveitis. Eye (Lond) 23: 1130-1133.
3. Novak K, Swain MG (2008) Role of methotrexate in the treatment of chronic cholestatic disorders. Clin Liver Dis 12: 81-96, viii.
4. Dadhania VP, Tripathi DN, Vikram A, Ramarao P, Jena GB (2010) Intervention of alpha-lipoic acid ameliorates methotrexate-induced oxidative stress and genotoxicity: A study in rat intestine. Chem Biol Interact 183: 85-97.
5. Miyazono Y, Gao F, Horie T (2004) Oxidative stress contributes to methotrexate-induced small intestinal toxicity in rats. Scand J Gastroenterol 39: 1119-1127.
6. Ciralik H, Bulbuloglu E, Cetinkaya A, Kurutas EB, Celik M, et al. (2006) Effects of N-acetylcysteine on methotrexate-induced small intestinal damage in rats. Mt Sinai J Med 73: 1086-1092.
7. Sener G, Eksiglu-Demiralp E, Cetiner M, Ercan F, Yegen BC (2006) Beta-glucan ameliorates methotrexate-induced oxidative organ injury via its antioxidant and immunomodulatory effects. Eur J Pharmacol 542: 170-178.
8. Kolli VK, Abraham P, Rabi S (2008) Methotrexate-induced nitrosative stress may play a critical role in small intestinal damage in the rat. Arch Toxicol 82: 763-770.
9. Nagai Y, Horie T, Awazu S (1993) Vitamin A, a useful biochemical modulator capable of preventing intestinal damage during methotrexate treatment. Pharmacol Toxicol 73: 69-74.
10. Swartz-Basile DA, Rubin DC, Levin MS (2000) Vitamin A status modulates intestinal adaptation after partial small bowel resection. JPEN J Parenter Enteral Nutr 24: 81-88.
11. Yuncu M, Eralp A, Koruk M, Sari I, Bagci C, et al. (2004) Effect of vitamin A against methotrexate-induced damage to the small intestine in rats. Med Princ Pract 13: 346-352.
12. Howland RD, Mycek MJ, Harvey RA, Champe PC (2004) Anticancer drug in: Pharmacology. (3rd edn), Lippincott Williams & Wilkins. Philadelphia, USA.
13. Johnston A, Gudjonsson JE, Sigmundsdottir H, Ludviksson BR, Valdimarsson H (2005) The anti-inflammatory action of methotrexate is not mediated by lymphocyte apoptosis, but by the suppression of activation and adhesion molecules. Clin Immunol 114: 154-163.
14. Vardi N, Parlakpinar H, Ozturk F, Ates B, Gul M, et al. (2008) Potent protective effect of apricot and beta-carotene on methotrexate-induced intestinal oxidative damage in rats. Food Chem Toxicol 46: 3015-3022.
15. Genestier L, Paillot R, Quemeneur L, Izeradjene K, Revillard JP (2000) Mechanisms of action of methotrexate. Immunopharmacology 47: 247-257.
16. Antar L, Elfadaly A, Eldeeb D (2005) Vitamin A protects jejunal mucosal cells from methotrexate-induced damage in rats. Egypt J Anat 28: 347-368.
17. Garipardic M, Bakan V, DavutoÄŸlu M, Sayar H, KurutaÅŸ EB (2010) Oxidative stress and protective effect of erythropoietin on methotrexate-induced esophageal damage. J Pediatr Hematol Oncol 32: 108-112.
18. Yamamoto A, Itoh T, Nasu R, Kajiwara E, Nishida R (2013) Sodium alginate inhibits methotrexate-induced gastrointestinal mucositis in rats. Biol Pharm Bull 36: 1528-1534.
19. Bajin-Katic K, Stankov K, Kovaeevic Z (2004) Changes of biochemical parameters in rat intestinal mucosa induced by methotrexate and effects of enteral administration of glutamine. Arch Oncol; 12: 35-38.
20. Sukhotnik I, Shteinberg D, Lulu S Bashenko Y, Mogilner JG et al. (2008) Transforming growth factor-alpha stimulates enterocyte proliferation and accelerates intestinal recovery following methotrexate-induced intestinal mucositis in a rat and a cell culture model. Pediatr Surg Int; 24: 1303–1311.
21. Jahovic N, Sener G, Cevik H, Ersoy Y, Arbak S et al. (2004) Amelioration of methotrexate-induced enteritis by melatonin in rats. Cell Biochem Funct 22: 169- 178.
22. Gao F, Horie T (2002) A synthetic analog of prostaglandin E1 prevents the production of reactive oxygen species in the intestinal mucosa of methotrexate-treated rats. Life Sci 71: 1091–1099.
23. Naruhashi K, Nadai M, Nakao M, Suzuki N, Nabeshima T, et al. (2000) Changes in absorptive function of rat intestine injured by methotrexate. Clin Exp Pharmacol Physiol 27: 980-986.
24. Sogabe N, Mizoi L, Asahi K, Ezawa I, Goseki-Sone M (2004) Enhancement by lactose of intestinal alkaline phosphatase expression in rats. Bone 35: 249-255.
25. Kremer JM (2004) Toward a better understanding of methotrexate. Arthritis Rheum 50: 1370-1382.
26. Carneiro-Filho BA, Lima IP, Araujo DH, Cavalcante MC, Carvalho GH, et al. (2004) Intestinal barrier function and secretion in methotrexate-induced rat intestinal mucositis. Dig Dis Sci 49: 65-72.
27. Verburg M, Renes IB, Meijer HP, Taminiau JA, Büller HA, et al. (2000) Selective sparing of goblet cells and paneth cells in the intestine of methotrexate-treated rats. Am J Physiol Gastrointest Liver Physiol 279: G1037-1047.
28. Alan S and James L (2005) The cell in: Human histology. (3rd edn). Elsevier. Mosby, USA.
29. Koppelmann T, Pollak Y, Mogilner J, Bejar J, Coran AG, et al. (2012) Dietary L-arginine supplementation reduces Methotrexate-induced intestinal mucosal injury in rat. BMC Gastroenterol 12: 41.
30. Xian CJ, Couper R, Howarth GS, Read LC, Kallincos NC (2000) Increased expression of HGF and c-met in rat small intestine during recovery from methotrexate-induced mucositis. Br J Cancer 82: 945-952.
31. Gibson RJ, Keefe DM, Thompson FM, Clarke JM, Goland GJ, et al. (2002) Effect of interleukin-11 on ameliorating intestinal damage after methotrexate treatment of breast cancer in rats. Dig Dis Sci 47: 2751-2757.
32. Gibson RJ, Bowen JM, Cummins AG, Keefe DM (2005) Relationship between dose of methotrexate, apoptosis, p53/p21 expression and intestinal crypt proliferation in the rat. Clin Exp Med 4: 188-195.
33. Kumar KM, Abba AK, Fausto N (2005) Robbins and Cotran pathological basis of disease. 7th ed. Library of congress, Philadelphia, Pennsylvania, USA.
34. Leitão RF, Brito GA, Oriá RB, Braga-Neto MB, Bellaguarda EA, et al. (2011) Role of inducible nitric oxide synthase pathway on methotrexate-induced intestinal mucositis in rodents. BMC Gastroenterol 11: 90.