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Effect of Pentoxifylline on Experimental-dextran Sulphate Sodium-induced Colitis in Rats

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Authors' contributions

This work was carried out in collaboration between all authors. Authors MAA and HZN responsible for: designed the study, data collection and analysis of the, practical part, writing the manuscript and statistical analysis. Authord FKM and NAA revised practical and writing process. All authors read and approved the final manuscript.

Original Research Article

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ABSTRACT

Aims: Ulcerative colitis (UC) is considered as an idiopathic, chronic inflammatory bowel disease (IBD) with multifactorial agents. Tumor necrosis factor-alpha (TNF-α) has been suggested to be one of them. The study was designed to evaluate whether pentoxifylline (PTX), as TNF-α suppressor, has a beneficial effect in rats with dextran sulfate sodium (DSS) induced-colitis.
Study Design: Original research papers.
Place and Duration of Study: In both Anatomy Department, Faculty of Medicine, Menoufia University and Animal medicine & Infectious Diseases Department, Faculty of Veterinary Medicine Sadat City Branch , Menoufia University, Egypt , between April 2009 and August 2010.
Methodology: Fifty adult male rats were divided randomly and equally into: control group, model control group, colitis model group, PTX treated group and recovery group. Induction of colitis was made in colitis model by adding DSS to the drinking water; for three weeks (5% for one week followed by 3% of for two weeks). Rats in both PTX treated and model control groups received pentoxifylline for two weeks after the induction of colitis; by intraperitoneal injection (100 mg/kg/day; 1ml /rat). Colon mucosal inflammation and damage were assessed through; clinical, macroscopic, microscopic,

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morphometric and molecular assessments.
Results: Rats treated with oral administration of DSS for three weeks developed clinical and macroscopic signs of colitis. Treatment with PTX for two weeks, in the treated group or cessation of DSS for two weeks, in the recovery group relieved the colitis symptoms including: diarrhea, reduction in body weight, shortening and ulceration of the colon and extensive colonic damage. All of these were associated with a significant increase in TNF-α m RNA expression. However, an improvement was more significant among treated animals than that in the recovery one.
Conclusion: Pentoxyfylline seems to be an effective in the treatment of ulcerative colitis for further investigation. Also, the unclear dual role of mast cell in both induction and treatment of the disease should be considered in a further investigation.

Keywords: Experimental colitis; dextran sulphate sodium; tumour necrosis factor.

1. INTRODUCTION

Inflammatory bowel disease (IBD) is one of chronic inflammatory conditions that has sudden remissions and exacerbations clinical course with rectal bleeding and diarrhea [1]. Ulcerative colitis is considered as one of inflammatory bowel disease (IBD) that affects the lining of colon and rectum. It has many symptoms as; abdominal pain, cramping, bloody diarrhea, pus stool, fever, rectal pain, weight loss, nausea, vomiting, arthritis, mouth sores and children growth retard [2]. Immune dysfunction, intestinal bacterial flora and genetic liability are accused in disease development [*3*]. Dextran sulfate sodium (DSS) colitis paradigm is the most appropriate model that resembles the human phenotype [4]. Imbalance between inflammatory and anti-inflammatory cytokines is a corn stone in the development of ulcerative colitis [5]. Tumor necrosis factor-alpha (TNF-α) is one of the most important proinflammatory cytokines produced by macrophages in the colon. It which plays a central role in host defense and in the acute inflammatory response related to tissue injury [6]. Recently, researchers discovered that TNF-α is involved in the regulation of apoptosis, lipid metabolism, cell proliferation and differentiation, and coagulation [7].Therapies that reduced epithelial cell apoptosis and tumor necrosis factor-alpha levels led to clinical and histopathological improvement in experimental models of colitis [8]. Also, cancer risk was decreased among patients treated with TNF-α inhibitor [9]. Pentoxifylline, a methylxanthine derivative has anti-inflammatory properties and an inhibitory effect tumor necrosis factor-alpha formation [10]. Also, it has a role in the treatment of various types of vasculitis [11]. The present work was conducted to study the effect of pentoxyphylline on dextran sulfate sodium induced colitis in albino rats.

2. MATERIALS AND METHODS

2.1 Material

2.1.1 Reagents

- Dextran sulfate sodium salt; a product of leuconostoc spp, Sigma-Alorich (St Louis, MO, USA), was available in the form of powder. (M_w >500 kDa) that contains 0.5-2% phosphate buffer. It was dissolved in drinking water [12].
- Pentoxifylline (trentoximal amp): a product of (Alex. Co. for Egypharma, Egypt) and was available as ampoule. Each 5 ml ampoule contains 100 mg pentoxifylline.

- Feces occult blood was determined using occult blood test device (ACON Laboratories, Cairo, Egypt).
- TRIzol Reagents (Invitrogen, Carlsbad, California).

2.1.2 Animals

Healthy adult male albino Wistar rats, weighing 180±30g, were housed in a temperature conditioned room (24±2ºC) in a separate cages for at least one week before and through the experimental work, being maintained on a standard diet (composed of 20% casein, 15% corn oil, 55% corn starch, 5% salt mixture and 5% vitamins) (National Research Center, Egypt). Water was available ad-libitum. The study protocol was in accordance with the guideline for animal research and approved by the Ethical Committee of faculty of Medicine, Menoufia University.

2.1.3 Experimental protocol

Fifty adult male rats were randomly divided into (Fig.1):

Group I (control group)**:** consisted of ten rats. This group was further subdivided equally into two subgroups:

Subgroup IA: left without treatment and served as the untreated control group.

Subgroup IB: received 1 ml of distilled water intraperitoneal (IP) for two weeks.

Group II (model control group**):** consisted of ten rats received 100 mg of pentoxifylline /kg/day (1ml /rat, IP) for two weeks.

Group II (colitis model group): consisted of ten rats. The colitis was induced by adding DSS to drinking water; for three weeks (5% for one week followed by 3% for two weeks) [13].

Group III (treated group**):** consisted of ten rats. They received pentoxifylline by IP injection (100 mg /kg/day; 1ml /rat) for two weeks after induction of chronic colitis [14].

Group IV (recovery group)**:** consisted of ten rats. They were Left without treatment for two weeks after induction of colitis and they were accessed freely to regular water intake.

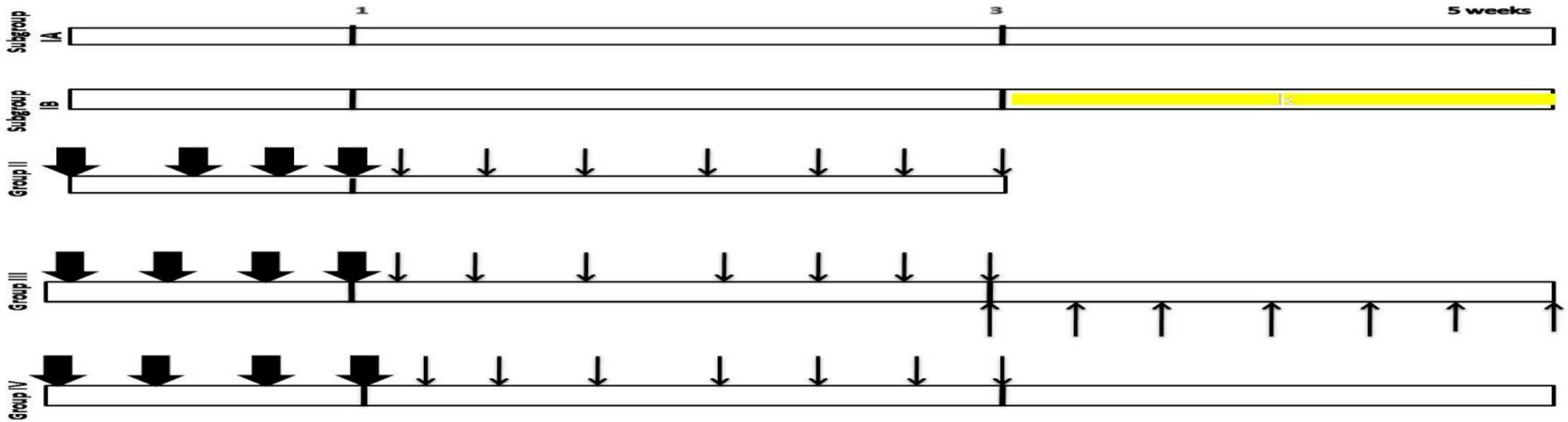

Fig. 1. schematic representation of experimental protocol. The filled box indicates distilled water injection. The downward arrows indicate oral DSS; thick arrows3% of DSS &thin arrows 3% of DSS. The upward arrows indicate PTX injection.

2.2 Methods

2.2.1 Morphological (clinical) assessment

Weight change percentage assessment & activity index assessment [15] (Table 1) were evaluated and scored every week during the experimental period.

Table 1. Scoring of the disease activity index (DAI)

Score	Weight changes (%)	Stool consistency	Occult/gross rectal bleeding
0	1	Normal[1]	Normal
1	1~5	Normal	Occult blood +
2	5~10	Loose stool[2]	Occult blood ++
3	10~15	Loose stool	Occult blood +++
4	>15	Diarrhea[3]	Gross bleeding

[1]Normal stool: well formed pellets; [2]Loose stools: pasty and semi-formed stools which do not stick to the anus; [3]Diarrhea: liquid stools that stick to the anus. The total clinical score was calculated by summation of the previous parameters. It was classified as: mild activity: ranged from 1-4, moderate activity: 5-8, & maximal activity: 9-12.

2.2.2 Macroscopic assessment

At the end of each detected period, the animals were sacrificed by cervical dislocation. The abdominal cavities were rapidly opened and the entire colons were removed and lengthen. Specimens were taken for real time PCR and microscopic analysis and then the remainder part of the colon was opened examined, scored and photographed for visible mucosal damage [16] (Table.2).

Table 2. Mucosal damage score

Score	Gross morphology
0	No damage
1	Localized hyperemia, but no ulcers or erosions
2	ulcers or erosions with no significant inflammation
3	ulcers or erosions with inflammation at one site
4	Two or more major sites of ulceration and /or inflammation
5	Two or more major sites of inflammation and ulceration extending >1cm along the length of the colon

1-8(mild activity), 9-18(moderate activity), & 19-27 (maximal activity)

2.2.3 Microscopic assessment

The colonic samples were fixed, dehydrated, embedded in paraffin, deparaffinized, cut and stained by Hematoxyllin and eosin (H&E), periodic acid schiff alcian blue (PAS-Ab) & Masson trichrome stains [17]. Hematoxyllin and eosin slides were examined and scored for histopathological evaluation. The slides were coded to prevent observer bias during evaluation. All tissue sections were examined in a blinded fashion by experienced histopathologist [15] (Table 3).

Table 3. Histopathological score of colitis with little modification

Score	Destruction of epithelium and/ or glandular crypts	Dilation of glandular crypts	Depletion and loss of goblet cells	Inflammatory cell infiltration	Edema	Hemorrhagic mucosa	Crypt abscess	Apoptosis	Dysplasia
0	morphologically normal	normal aspect	normal aspect	absence of infiltration	absent	absent	absent	absent	absent
1	focal destruction	focal dilation	slightly depleted goblet cells	infiltrate at the subepithelial and lamina propria level or crypt bases	focal	focal	focal	focal	focal
2	zonal destruction	zonal dilation	zonal or moderately depleted goblet cells	infiltration reaching muscularis mucosa	zonal and/or moderately diffuse	zonal	zonal	zonal	zonal
3	diffuse and/or mucosal ulceration involving submucosa and/or diffuse crypt loss	diffusely dilated crypts	diffusely or complete depletion of goblet cells	severe and extensive infiltration reaching submucosa and/or involving muscularis propria	extensive and severe	diffuse	diffuse	diffuse	diffuse

1-2 (mild activity),3-4 (moderate activity) & 5 (maximal activity).

2.2. 4 TNF-α mRNA expression assessment

From each rat, 10 mg of distal colonic tissue (2 mm^3) was taken and placed into a micro centrifuge tube that was stored at -80°c.The tubes containing the tissues were removed from the -80°C freezer placed on dry ice to prevent thawing. Total RNA of 100 mg colonic tissue was purified by TRIzol Reagents according to the manufacturer's instructions. Serial dilution was used for the determination of TNF-α mRNA expression level. Relative real-time PCR was carried out with 12µl of SYBR Green PCR mastermix containing AmpliTaq Gold (Bio-Rad, Hercules, CA). Then 45 cycles of PCR were performed in a thermal cycler, using the following conditions: initial denaturation at 95 for 10 min, followed by 35 amplification cycles at 95 for 30 s, at 60 for 30 s, at 72 for 30s min, extension at 72 for 10 min. At the end of the 45 cycles, further extension was continued for 7 minutes at 68º. The primers were TNF-α sense, 5´- CTGCCCCAATCCCTTTATT-3´; TNF-α antisense, 5´-CCCAATTCTCTTTTTGAGCC-3´ (Sigma-Aldrich, Egypt). The amount of TNF-αmRNA, normalized to the relative GAPDH control, was determined using the QEC21313 real-time PCR detection system, analyzed by Rotor-Gene Q Series Software 1.7. The sequences of the GAPDH (M32599) primers were: 5'-TCTCCCTCACAATTTCCATCCCAG-3' (forward primer) and 5'-GGGTGCAGCGAACTTTATTGATGG-3' (reverse primer) (Sigma-Aldrich, Egypt). The standard curve was used to detect the Ct values that were used for expressing the relative level of mRNA expression. Data analysis was performed with the comparative threshold (Ct) method [18].

2.2.5 Morphometric studies

1. The mean number of goblet cells in five (5) fields from five (5) sections (stained by Alcian blue-PAS) from each animal, of all groups, were counted using the 40X objective.
2. Mean area % of collagen fiber content of colonic mucosa in 5 fields from 5 sections, stained by Masson's trichrome from each animal of all groups, were measured using the 40X objective.

2.2.6 Statistical analysis

All statistical analyses were performed with SPSS14.0 statistical package for Microsoft Windows. Measurement data were expressed as mean ±SD. One-Way ANOVA test analysis followed by Post Hoc test were used to compare the variables among groups. P <0.05 was considered statistically significant**.**

3. RESULTS

3.1 Clinical Examination

Clinically in compare with the normal control group at zero day, the three experimental groups; colitis, recovery and PTX treated groups showed steady decrease in mean body weight % started from the first week and it became significant at the third week; *P<0.0001*. However, in both recovery and PTX groups, the body weight began to increase again at the 4th week. At the end of the study (5th week) and in compare to colitis group, both treated and recovery groups showed significant (*P<0.0001)* increase in % of body weight. The increase of body weight in the treated group was significance than that in the recovery group *(P<0.0001)*. Although, both were still significant decrease from the control at the day 0 *(P<0.0001)* (Fig. 2).

N.B. All control groups (subgroup Ia, subgroup Ib and group II) showed a nearly similar clinical, macroscopic, microscopic and molecular data with no significant difference (*p>0.05).* Both subgroup Ia & group II were neglected for simplification.

Also, compared the control groups with the other experimental groups, the clinical manifestation of colitis appeared on the rats in the following order: diarrhea and loose stools at the first week; hemoccult positive stools at the second week and gross bleeding at the third week. The clinical activity score was time dependent and they were mild in the first week, moderate in the second week and severe in the third week. Also, the score was significantly increased from first week up to third week after DSS administration (*P* < 0.0001). On the other hand, in compare to the colitis group, the clinical activity score was significantly decreased *(P* < 0.0001) in both treated and recovery groups. The treated group showed a significance decease in the activity score than the recovery one (P <0.0001) (Table. 4). During all experimental period, the mortality rate between rats was 0%.

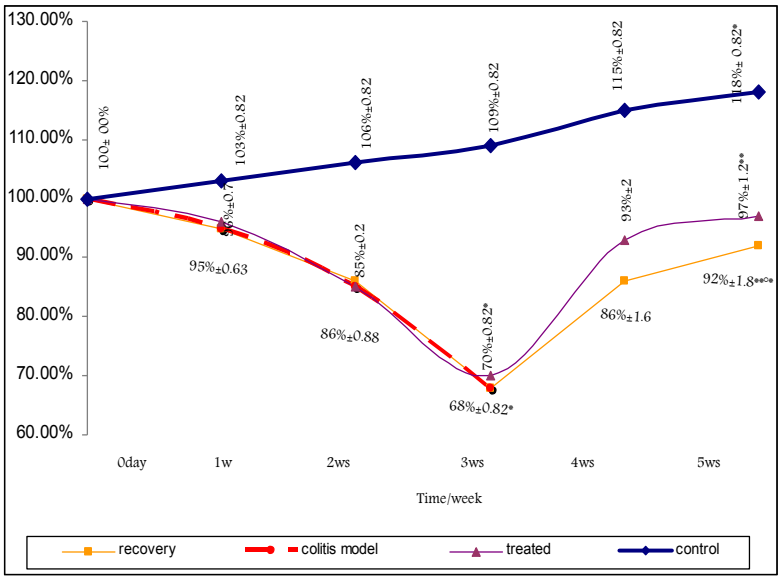

Fig. 2. Body weight gain (%; Mean ±SD) started from day 0.

** a significant difference from control. (A; P<0.0001), ** a significant difference from colitis group (P<0.0001).*

° a significant difference from treated group. The data of colitis model were omitted for simplification.

3.2 Macroscopic Assessment

Macroscopically, the colon length assessment of colitis groups showed a significant shortening comparing to the control group; *P* < 0.0001. The colon appeared pale and empty. However, at the end of the fifth week the colon length of both PTX treated and recovery groups showed a significant increase from colitis group; *P< 0.0001* (Fig.3 &table 5). However, the treated group was still significance from the recovery group; *P* <0.0001.

Also, in compare to the control group, mucosal damaged assessment in the colitis group showed significant loss; P <0.0001 of mucosal hustration with areas of hyperemia, hemorrhage, edema, ulceration and erosion scattered along the entire colon. The mucosal

lesions were more pronounced in the distal colon than in the proximal and middle colon (Fig. 4.). However, comparing with colitis group, both PTX treated and recovery groups showed significant reduction in the gross pathological changes; P < 0.0008 (Table 5.). The treated group was still significance from the recovery group; *P* <0.0001.

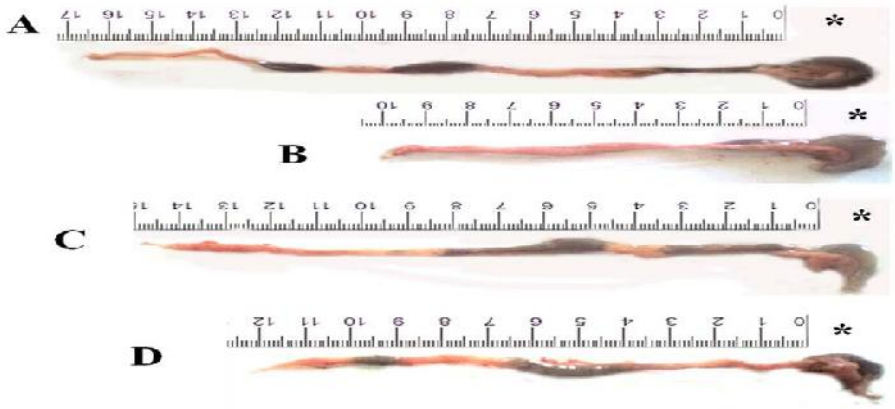

Fig. 3. a macroscopic view of the whole colon and cecum (stars) showing the changes in colonic length in different groups of rat as follows:

(A) The control group: the colon appears long, red and fill by stool. (B) The colitis group: The colon appears marked short, pale and devoid of stool when compared with control group. (C) The PTX group: The colon appears nearly as control group (A). (D) The recovery group: The colon appears red fill by stool and its shortness is less severe when compared with the colitis group (B). *(Sony digital camera x3)*

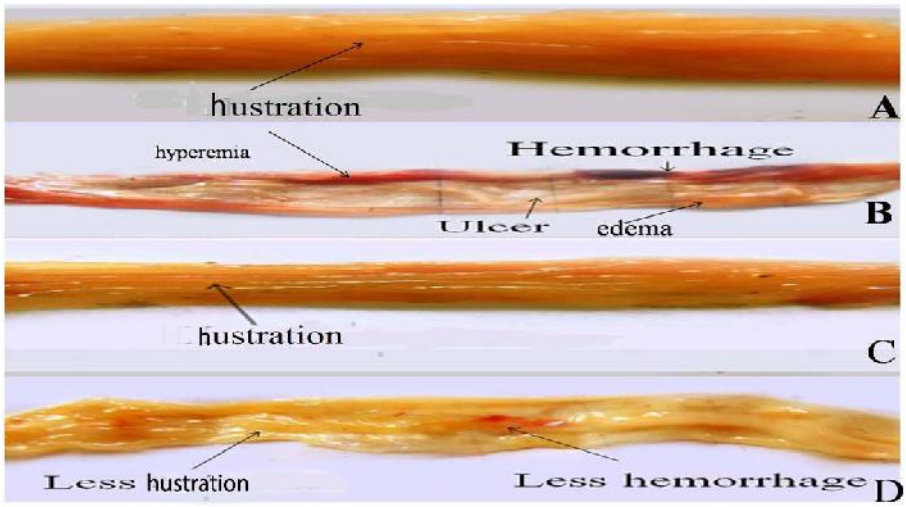

Fig. 4. A macroscopic view of the colon mucosa showing the changes in colonic mucosa in different groups of rat as follows:

(A) The control group shows typical hustration all over the colon mucosa. (B) The colitis group shows areas of hyperemia, hemorrhage, edema and ulceration. (C) The PTX treated group, the colon mucosa is more or less similar to control one (A). (D) The recovery group, the colon mucosa shows faint hustration and slit hemorrhage. (Sony digital camera x3)

Table 4. Clinical activity score (Mean ±SD) in different groups starting from zero day up to five weeks

/group activity	control 0day	colitis 0day	treated 0day	recovery 0day	control 1w	colitis 1w	treated 1w	recovery 1w	control 1W	Colitis 2w	treated 2w	recovery 2w	control 3w	colitis 3w	treated 3w	recovery 3w	control 4w	treated 4w	recovery 4w	control 5w	treated 5w	recovery 5w
bleeding	00±0	00±0	00±0	00±0	00±0	0.8± 0.04	0.9± 0.06	1.1± 0.06	00± 0	2.4± 0.7	2.4± 0.7	2.43± 35	00±0	3.4± 0.5	3.1± 0.7	3.6± 0.9	00±0	2.1± 0.06	2± 0.8	00±0	0.3± 0.07	.0.9± 0.09
Diarrhea	0.3± 0.07	0.3± 0.07	0.1± 0.03	0.1± 0.03	0.3± 0.07	1.1± 0.06	1± 0.04	1.1± 0.06	o.2± 0.4	2.1± 0.7	2.1± 0.7	2.23± 0.01	0.2± 0.06	3.3± 0.7	3.6± 0.5	2.9± 0.42	o.18± 0.4	1.6± 0.07	2.5± 1.1	o.2± 0.4	0.3± 0.05	1.7± 0.3
weight	0.2± 0.04	0.1± 0.03	O.2± 0.01	0.1± 0.03	0.1± 0.07	1± 0.00	1± 0.08	1± 0.02	o±0	3± .01	3± 0.5	3± 0.11	o± 0	4± 0.1	4± 0.55	4± 0.14	o± 0	2.1± 0.08	2.8± 0.6	o± 0	0.9± 0.07	2± .0.12
sum	0.5± 0.11	0.4 0.1	0.3± 0.04	0.2± 0.06	0.4± 0.14	2.9*± 0.1	2.9*± 0.18	3.2*± 0.15	0.2± 0.4	7.5*± 1.41	7.43*± 0.19	7.46*± 0.47	0.2± 0.06	10.7*± 1.3	10.7*±. 0.67	9*± 1.46	o.18± 0.4	5.8**± 0.21	7.5**°± 1.1	o.2± 0.4	1.5**± 0.19	4.6**°± 1.32

a significant difference from control at zero day. (P<0.0001), a significant difference from colitis(P<0.0001),° a significant difference from treated group (P<0.0001). W: week*

3.3 Microscopic Assessment

3.3.1 Slide examination

Histological examination of the distal colon in normal control rat showed normal colon architecture mucosa (Fig. 5,6,7a.). The goblet cell showed apparent normal number and activity (+PAS- Ab) (Fig. 8a) and the submucosa showed slight collagen fibers deposition (Fig.9a.).

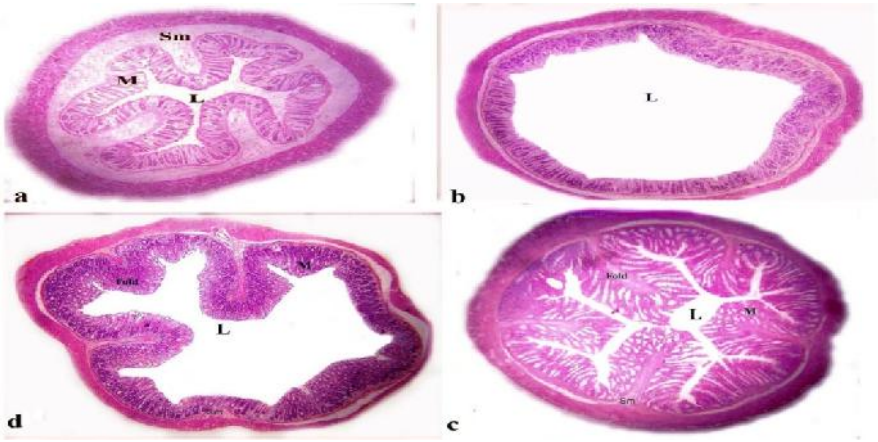

Fig. 5. (a) Transverse sections (T.S.) of distal colon of control rat. (b)T.S. section of distal colon in a colitis rat shows absence of the mucosal folds with wide lumen. (c)The folds reappear completely in the treated rat. (d) The folds reappear partially in the recovery rat. (H & E x40)

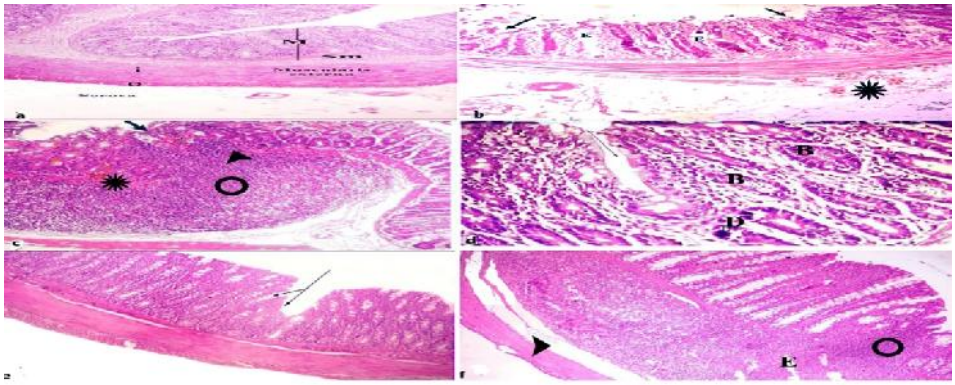

Fig. 6. (a) T.S of distal colon of control rat shows the typical four layers. (b) T.S of distal colon of colitis rat shows fissuring mucosa (arrows), edematous areas (E), serosal hemorrhage (star) and distorted crypts. (c) T.S of distal colon of colitis rat shows hemorrhage (star), ulceration (arrow) with diffuse cellular infiltration (circle) and splitting of muscularis mucosa (arrow head). (d) T.S of distal colon of colitis rat shows crypt abscess (B), wide & branched crypt (branched arrow) and dysplasia (D). (e) T.S of distal colon of PTX treated rat shows rat shows: almost normal structure but some crypts are still wide and branched (branched arrow). (f) T.S of distal colon of recovery rat shows edematous submucosal areas (E), inflammatory cellular infiltration (circle) and separation of submucosa from musculosa (arrow head).
(H &E x100)

Comparing with the control group, histopathological assessment of colitis group showed absence of mucosal folding and widening of the colon lumen (Fig. 5b.). The mucosa showed ulceration, edema and crypts distortion (Fig. 6b.). Mucosal hemorrhage, muscularis mucosa splitting (Fig. 6c), crypt abscess and dysplasia (Fig. 6d) could also be detected. Both mucosa and submucosa showed a diffuse lymphocytic cellular infiltration (6 c) that was accompanied with other polymorphic types of cells such as: macrophage, eosinophils, plasma cell, mast cell, neutrophils, apoptotic cells (7b) and fibroblast. Serosal hemorrhage (Fig. 6b.) and splitting of musculosa were also noticed (Fig. 6c). Also, there was an apparent marked decrease in the goblet cell number and activity (Fig. 8b) that was accompanied with increased in submucosal collagen fibers (9b).

Comparing with the colitis group, histopathological assessment of PTX group showed complete reappearance of mucosal folding (Fig.5c), marked disappearance of pathological picture of ulcerative colitis (Fig. 6e) and presence of multiple mitotic Figures and new blood vessels formation as a sign of regeneration(Fig. 7c&d). In addition, there was an apparent increase in number and activities of the goblet cells (Fig.8c) and a moderate decrease in the submucosal collagen fibers (Fig. 9c). Lastly, a fairly notable improvement of histopathological picture could be detected in the recovery group (5d) with still presence of diffuse cellular infiltration, cystic crypts and slight hemorrhage in the lamina propria and increase in the submucosal vascularity (Fig. 6f). The goblet cells showed apparent increase in number and activity (Fig.8d) with apparent slight decrease in submucosal collagen fibers (Fig. 9d).

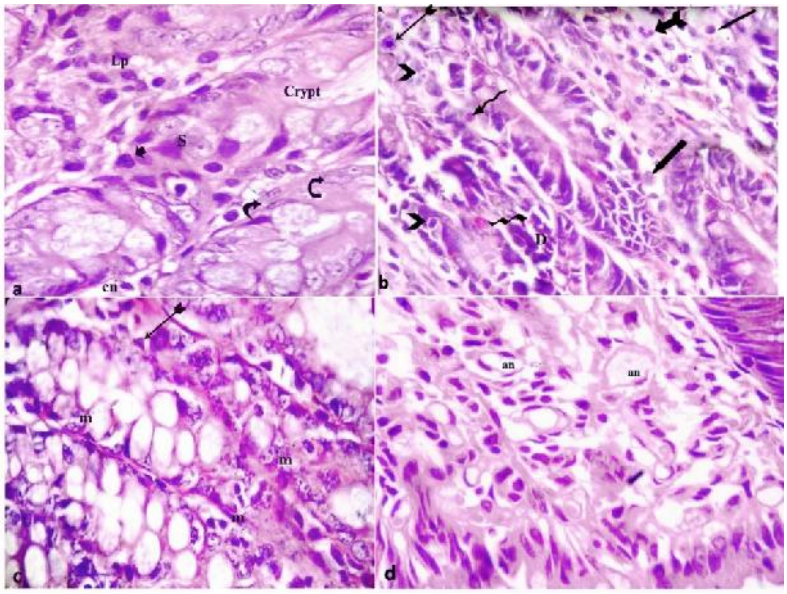

Fig. 7. (a) T.S of distal colon of control rat showing normal crypts and lamina propria (LP) with connective tissue cells nuclei (cn). Also, there is absorptive cells (curved arrow), goblet cells (annular arrow), enteroendocrine cells (tailed arrow head) and stem cells (S) inside the crypts. (b) T.S in distal colon of colitis rat shows dysplasia in the crypts (D) and different types of inflammatory cells in the lamina propria as: macrophage (thick pen); eosinophils (corrugated line); plasma cell (corrugated arrow); mast cell (thin crossed arrow); neutrophils (thick crossed arrow) and lymphocytes (thin pen). Notice the presence of apoptotic cells (hollow arrows head). (c) T.S in distal colon of treated rat shows reactive cells mitotic Figures (m) and mast cells (thin crossed arrow). (d) T.S in distal colon of treated rat shows angiogenesis (an) as a sign of regeneration. (H &E x1000)

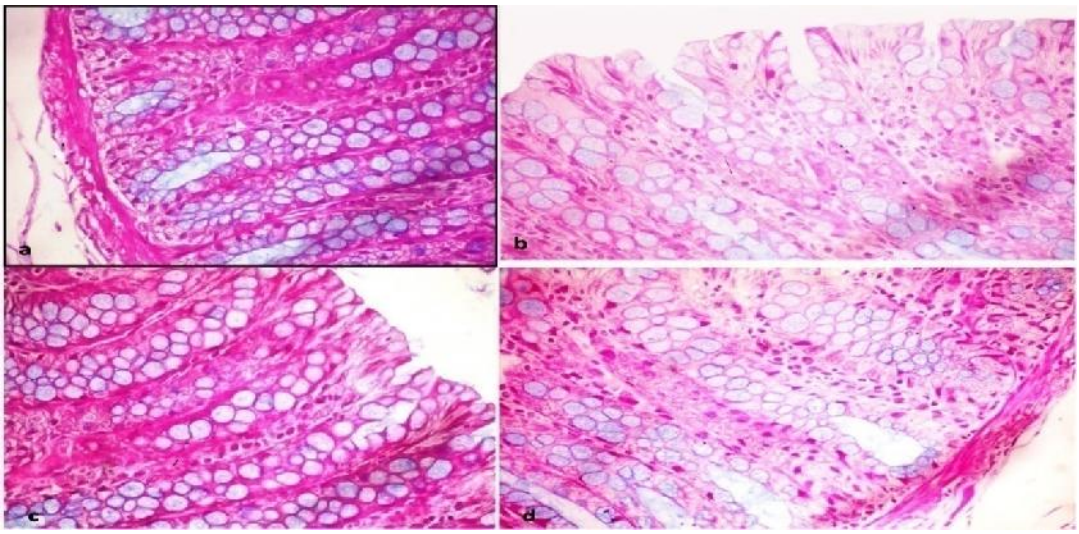

Fig. 8. (a) T.S of distal colon of control rat. (b) T.S of distal colon of colitis rat shows marked decrease in goblet cell number and mucin. (c) T.S of distal colon of treated rat shows restored goblet cells number and mucin content. (d) T.S of distal colon of recovered rat shows fairly decrease in goblet cells. (PAS- Ab X 400)

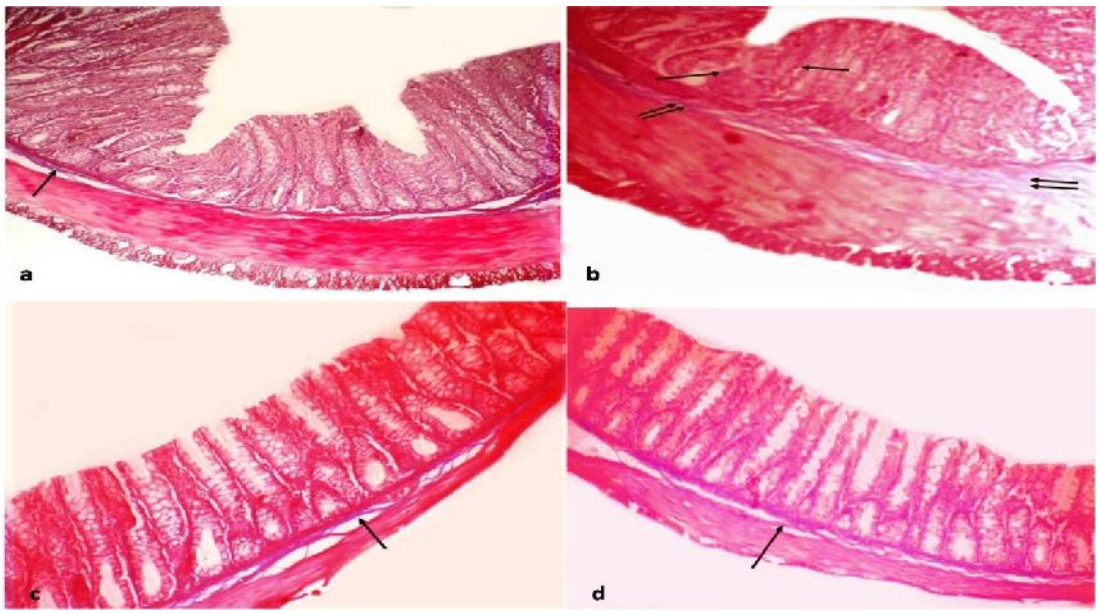

Fig. 9. (a) T.S in rat distal colon of control rat shows collagen fibers (arrows). (b) Collagen fibers increased in colitis rat (arrows). (c). Collagen fibers decrease in treated rat. (d) T.S of distal colon of recovered rats shows decreased collagen fibers. (MTx100)

3.3.2 Mean number of goblet cells

As compared to the control group, the colitis group showed a significant decrease in the mean number of goblet cells *(P<0.0001).* However as compare to colitis group, a significant increase in mean number of goblet cells was detected in both treated and recovery groups *(P<0.0001*). However, the treated group still showed a significance increase in mean number of goblet cells than the recovery group *(P<0.0001)* (Table 5).

3.3.3 Mean area % of collagen fibers of colonic mucosa

As compared to the control group, the colitis group showed a significant increase in the mean area % of collagen fibers *(P<0.0001).* However as compared to colitis group, a significant decrease in the mean area % of collagen fibers was detected in both treated and recovery groups*(P<0.0001).* However, treated group still showed a significant decrease than the recovery group *(P<0.0001)* (Table 5).

Table 5. Colon length (cm), mucosal damaged score, number of goblet cell & percentage (%) of collagen fibers

Experimental group	Control	Colitis model	Treated	Recovery
Mean colon length± SD	16.55±1.06	7.6±1.41*	0.48** **±**15.5	13±0.57**°
Mucosal damage score± SD	00±00	3.4±0.5*	0.40±0.32**	0.90±0.51**°
Mean number of Goblet cell ±SD	153.2±3.36	92.56±1.92*	147.45±1.51**	124±2.89**°
Mean area % of collagen fibers± SD	5.20%±0.7	19.60±0.2*	6.40±0.02**	9.10±0.05**°

a significant difference from control group (P<0.0001), **- a significant difference from colitis group (P<0.0001) & ° a significant difference from treated group(P<0.0001*).** SD, standard deviation

3.3.4 Tissue damage scoring assessment

Compared with control, the colitis group showed: a significant increase in the tissue damage; *P <0.0001.* On the other hand, in compare to colitis group, at the end of the fifth week, both PTX treated and recovery groups showed a significant reduction in the tissue damage; *P* < 0.0001. However, the PTX treated group showed a significance reduction of tissue damage than that of the recovery group; *P <0.0001* (Table 6).

Table 6. Histopathological score in different groups expressed in mean ±SD

Scores / Groups	Control	Colitis model	Treated	Recovery
Apoptosis	0.1±0	2.7±0.68	0.1±0.32	0.1±0.32
Crypt abscess	0±0	1.5±0.85	0.3±0.48	0.9±0.47
Dysplasia	0±0	1.6±0.97	0.1±0.32	0.5±0.7
Hemorrhage	0±0	0.4±0.07	0.4±0.7	0.6±0.7
Edema	0±0	2.5±0.7	0.2±0.63	0.3±0.48
Inflammatory cell infiltration	0±0	2.9±0.32	0.3±0.48	1.6±0.7
Depletion of goblet cells	0±0	2.8±0.42	0.2±0.42	1.1±0.32
Dilatation of crypts	0.1±0	1.8±0.63	0.2±0.42	1.6±0.7
Destruction of epithelium	0±0	2±0.82	0.3±0.48	1.1±0.73
Sum	0.2±0	18.2±5.46	2.1±4.25**	7.8±5.12**°

**a significant difference from control. (P<0.0001), **a significant difference from colitis(P<0.0001)& ° a significant difference from treated (P<0.0001).*

3.4 TNF -α expression assessment

Compared with internal control, colonic TNF-α mRNA increased one fold in all experimental control with no significant difference; *P>0.05*. However, TNF-α mRNA of the colitis group was increased up to 6 folds and it was significant; *P*<0.0001. Moreover, both PTX treated and recovery groups showed increase of TNF-α mRNA ; 1.8 folds and 3 folds respectively, but they showed a significant decrease in TNF-α mRNA expression from colitis group; *P*<0.0001. However, the treated group still showed a significance decrease in TNF-α mRNA expression than that of the recovery group; *P<0.0001* (Table 7).

Table 7. Mean expression of TNF–α m RNA regarding internal control

Groups	Internal control	Control	Colitis model	Treated	Recovery
TNF-α mRNA Expression	21±0.11	25±0.11	126±00*	37.8±0.13**	65±0.17**°

** significant difference from control. (A ; P<0.0001), ** significant difference from colitis (B ;P<0.0001)& ° significant difference from treated (P<0.0001).*

4. DISCUSSION

The exact pathogenesis of the ulcerative colitis disease is still unknown. However, it is always accompanied with TNF-α elevation. Pentoxyfylline has an inhibitory effect on tumor necrosis factor-alpha production. Pentoxyfylline is cheap and low toxic in chronic uses [19].

In this work, the colitis was proved in colitis model group; clinically by a significant decrease in the body weight and a significant increase in the clinical score activity [20]. In addition, in this research, the colitis model might be chronic as it was time dependent and there were severe pathological changes that accompanied with dysplasia (13). In this study, we considered the colitis picture might be a severe one, as the clinical finding correlated with colon shortage, weight loss and pathological changes [21]. In addition, a significant increase in morphological mucosal damage score might confirm the severity and the chronicity of the colitis [22&23]. Clinically, many researchers [24] recorded the same picture in biopsies taken from ulcerative colitis patients.

In the colitis group of the present study, a significant decrease in the goblet cells number and acid mucin activity added a pathogenic cause in IBD. As, many researchers described mucus [25] as a mucosal surface gel layer with a protective function [26]. One striking point of this investigation was the distortion and loss of the crypts. The loss of crypts might increase the permeability to the inflammatory cells [27]. In the present study, the detected polymorphic cellular infiltration in the colon mucosa, especially polymorphic neutrophil (PMN), might be a cause of loss of the epithelium, loss of goblet cells and crypt damage. As over activation of PMN might play a critical role in microcirculation and increase in the amounts of TNF-α and IL-1b secretions [28]. In this work, the crypt abscess confirmed the activity of colitis disease as it is a hallmark of colitis activity in human UC [15]. Rupture of crypt abscess might be a cause of the mucosal ulcer [29]. The cellular apoptosis recorded in the present work was in accordance with others who described that apoptosis of enterocytes and lymphocytes [30] as well as epithelial cell [31] were the main reasons for UC and inflammation. But this contra directory with others [32] who said that apoptosis of lymphocytes is delayed in UC. It may be explained by caspase-3 activity [33] or p53 and Fas R/Fas L pathways [34]. In the present study, the severe cytological abnormalities (hyperchromasia, increased nuclear/cytoplasmic ratio, irregular nuclear outline) that

associated with epithelio-glandular hyperplasia may be a high grade dysplasia as they were not associated with increased number of typical mitosis as classified by American Association for Cancer Research [35]. The chronicity of the colitis in this research might be a cause of dysplasia. As, there were a significantly increased risk of colorectal cancer in patients with ulcerative colitis after ten years, if their involvement were beyond the splenic flexures [36]. So, the DSS model could be used as a colitis-associated dysplasia model.

In the present work, a significant increase in mean area % in collagen fibers area might be due to increase in fibroblast activity that presented also in this work [37]. The fibrous tissue replacement by collagen fibers, degenerated muscle layer [38] and serosal fat and hemorrhage (detected in the present work) might be a cause colonic stricture observed in colitis patients. The previous clinical investigations recorded a collagen deposition in the intestinal biopsy samples from patients with IBD and it was associated with cellular inflammation and some gene expressions [39], one of them must be TNF-α expression; as the present study proved [40].

In the present study, a significant increase in the TNF-α mRNA expression level in the colitis rats was on going with other researchers. They considered it as an implicating element in ulcerative colitis [41&42]. As the significant increase in TNF-α mRNA expression was accompanied with a significant elevation in quantitative indices of inflammation (clinical activity, macroscopic and microscopic damage scores), we can say TNF-α might be one of the key inflammatory mediators in the pathogenesis of UC. This study can consider PTX as an effective therapy in ulcerative colitis. As, the treated group showed: significant clinical, macroscopic and microscopic improvements that accompanied with a significant reduction in colonic TNF-α mRNA expression level. Also, a sign of regeneration such as: appearance of wide and branched crypt, increase in newly formed blood vessels (angiogenesis), increase in the mitotic activity and mast cellular infiltration associated the previous data improvement. Other previous experimental studies [43&44] came in accordance with the research PTX finding. These studies proved the efficiency of PTX treatment in decease the severity of the disease, especially if it associated TNF-α mRNA with monoclonal antibodies [45].

The significant improvement of PTX treated group parameters might be mediated via the following actions of PTX: its natural killer cell activity (apparent reduction in the inflammatory cells) and a significant suppression of TNF-α production [38] as the present work recorded. Many researchers [46] reported that in patients with inflammatory bowel disease, PTX concentration of 25 mg/ml led to inhibition of TNF-α secretion up to 50%. Many researchers believed that the wide and branched crypts might play an important role in crypt repair. As, each branch progressed upward to create an independent crypt [47]. Also, the presence of angiogenesis, in this work, might accelerated the healing process through replacement of congested blood vessels that might produce ischemia and prevent sweeping of the massive cellular infiltration and accumulated toxins. Another important sign was the high rate of mitotic activity, reported inside the crypts that might be responsible for restore of goblet cell number and in turn acid mucin protective activity. Basal stem increase in goblet cell number might [48] invite us to say that PTX either stimulates and/or recovers the stem cell proliferation activity.

Although mast cells has a role in allergy and anaphylaxis, it also has a protective role in wound healing and defense against pathogens [49].This opinion is ongoing with this study that reported accumulation of proliferated mast cells in the colitis group as well as in the treated group. So, the mast cells reported in the treated group could assure the PTX treatment activity in UC as many researchers reported them either with colonic tissue repair,

in the late phase of the disease [50], or in the recovery state [22]. So, dual effects of mast cells in UC must astonish us. A surprising effect discovered in this study was the ability of PTX to prevent UC complication as: there was a significant decrease in collagen fibers deposition and in turn fibrosis that responsible for colonic shortage and stricture. Also, there was apparent histological disappearance of dysplasia in PTX treated group. So PTX may have a role in prevention of cancer colon risk rise. Other researchers supported our data [51&52], as they emphasized the efficacy of PTX in patients with collagenous colitis and its experimental antifibrotic effect.

Lastly, the exact mechanism of colonic recovery, in the recovery group, might be due to recovery of the immune system, fostering microcirculation and epithelial regeneration [53]. Also, it may be due to reactivation body's natural healing and self-repair ability by removing toxins accumulated in tissues that disrupt biochemistry, obstruct the channels of circulation and elimination and prevents proper nutrition from reaching the tissues. Also, our data go in a line with the previous clinical observation [24]. They noticed that patients with proctitis or left-sided colitis usually had a more benign course, as up to 20% can have sustained remission in the absence of any therapy. In the present study, the withdrawal of the DSS in the recovery group showed a significant less improvement in all colitis parameters (clinical activity, macroscopic and microscopic damage scores) than treatment with PTX in treated group. It might be due to the less significant improvement in the TNF-α expression, collagen fibers area %, and in goblet cell number, in addition to apparent absence of angiogenesis and mitotic activity. Finally, the false recovery improvement is still considered as just a remission stage that might be followed by uncontrollable exacerbations. So, to exam this hypothesis the further studies might elongate the time of recovery and PTX treatment groups. One of the limitations of our study is the relatively short follow-up period, which cannot provide to assess the long-term effect of PTX on colonic inflammation. Also, this will clarify a difference between significant improvements in both treated and recovery group. Another limitation is that we did not examine the serum level of TNF-α serum level.

5. CONCLUSION & RECOMMENDATION

Pentoxyfylline is effective in colitis treatment as a TNF-α inhibitor for further investigation. Also, the unclear dual role of mast cell, in induction and in treatment of UC, should be considered in further investigation.

CONSENT

Not Applicable.

ETHICAL APPROVAL

All authors hereby declare that "Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985) were followed, as well as specific national laws where applicable. All experiments have been examined and approved by the Faculty of Medicine, Menoufia University Ethical Committee.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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