Original Article

The Possible Ameliorative Effect of Manuka Honey on Tartrazine Induced Injury of the Jejunal Mucosa with the Role of Oxidative Stress and TNF-alpha: Histological and Morphometric Study

Samah Kandeel and Heba E. M. Sharaf Eldin

Department of Histology and Cell Biology, Faculty of Medicine, Tanta University, Egypt

ABSTRACT

Introduction: Tartrazine is a water-soluble synthetic azo dye with a bright lemon yellow color used to enhance look, taste, smell, and quality of food. Its prolonged intakes can lead to serious health hazards through the induction of oxidative stress and tissue inflammation mediated by TNF- α. Manuka honey, is a dark honey derived from the manuka tree, possesses antioxidant, anti-inflammatory, antiulcer and antimicrobial effects.

Aim: This study aimed to investigate the injurious effect of tartrazine on the rat jejunal mucosa and the possible amelioration by Manuka honey in comparison to tartrazine withdrawal from the tissues through a histological, and morphometric study with the role of oxidative stress &TNF- α.

Materials and Methods: 40 adult male Wistar rats 180-200 grams used and divided into 4 groups (10 rats each): group 1 (control); group 2 (Tartrazine group (TZ)): 10 mg/kg/ day tartrazine given orally for 12 weeks; group 3 (Tartrazine+ Manuka honey group (TZ+MH)): 2.5 mg/kg/day of manuka honey 1 hour before tartrazine (10 mg/kg/ day) orally for 12 weeks; and group 4 (TZ recovery): 10 mg/kg/day tartrazine given orally for 12 weeks then left for another 12 weeks for recovery.

Results: Group 2 showed jejunal mucosal degenerative changes, significantly decreased villus height, mucosal thickness and crypt depth. Also, congested blood capillaries, mononuclear cellular infiltrations, significant decrease in the number goblet cells, and significantly increased collagen fibers. The significantly decreased proliferating cell nuclear antigen (PCNA) expression, and significantly increased expression of TNF-α were also noticed. Group 3 showed marked amelioration of the previous findings better than group 4.

Conclusion: Manuka honey ameliorates tartrazine-induced jejunal mucosal damage through its antioxidant and anti-inflammatory effects.

INTRODUCTION

Food-coloring additives play an essential role in the food supply industries[1]. Concerning food, the first noticeable feature is its color. Thus, food-coloring (dyes) is added to food products to restore its natural color that was wasted during the production process. Additionally, food dyes enhance the perceived zest and appeal to food, give them the desired look, and taste, besides the better smell, texture, as well as quality[2].

Tartrazine (TZ) (E 102; FD and C Yellow No. 5); is a water-soluble synthetic azo dye with a bright lemon yellow coloration derived from coal tar. Its usage in many developing countries is related to its stability as well as cheapness in comparison to other natural food dyes[3,4].

Tartrazine enters in the manufacture of many food products such as ice cream, yogurt, and soft drinks, besides cake mixes, custard, and candy. It is also widely used for the coloration of different cosmetics, human pharmaceuticals, and crayons in addition to the manufacture of textile dyeing, paper printing, color photography, as well as leather[4].

According to WHO, the accepted daily intake of TZ is 7.5 mg/kg/day[5]. However, in developing countries, many serious health hazards had been risen, resulting from the uncontrolled as well as unsupervised TZ intake. The prolonged use of TZ induces multiple health problems like indigestion, anemia, besides allergic reactions such as asthma and urticaria[6]. In addition to genotoxic, mutagenic effects as well as some behavioral changes such as irritability, restlessness and sleep disorders[7].

Tartrazine is a nitrous derivative that is reduced in the tissues into aromatic amine; the main metabolite is sulfanilic acid that has carcinogenic and mutagenic effects[8]. When TZ reaches the intestine it is reduced by the microflora, into sulfanilic acid and aminopyrazolone[9]. These metabolites are very toxic to the tissues as they are rapidly absorbed generating reactive oxygen species (ROS), resulting in

tissue oxidative stress damage[1]. Moreover, TZ possesses inflammatory actions on different tissues as proved by El-sakhawy *et al*., (2014)[4] during his study on the inflammatory effect of TZ on the stomach.

Manuka honey (MH) is a unifloral dark honey derived from the manuka tree (Leptospermum scoparium) of the family Myrtaceae present in New Zealand as well as Australia, Eastern region. It possesses antioxidant, anti-inflammatory, antiulcer and antimicrobial effect[10]. MH comprises the highest amount of phenolic as well as flavonoid compounds in comparison to the other honey types concerned for ROS scavenging activity. Moreover, it can reduce tissue inflammation besides reversal of tissue damage[11].

Based on the previously mentioned data, this study was presented to investigate the injurious effect of TZ on the rat jejunal mucosa and the possible amelioration of such injury by MH in comparison to TZ withdrawal from the tissues through a histological, immunohistochemical and morphometric study.

MATERIALS AND METHODS

Chemicals and Reagents

Tartrazine (Sigma, USA), Manuka honey (Product Code: FLO-63024, Flora, New Zealand), sodium pentobarbitone (Abbott Lab., Chicago, IL, USA), phosphate-buffered saline (El Gomhuria Co., Tanta, Egypt), anti- proliferating cell nuclear antigen (PCNA) antibody (Santa Cruz Biotechnology Inc., USA), anti-TNF-α (Abcam, Cambridge, USA), biotinylated goat anti-polyvalent antisera (Dako, Inc., USA), secondary antibody (Vector Laboratories, Burlingame, CA, USA).

The Experimental Animals

Forty adult male Wistar rats weighting (180-200 grams) obtained from the animal house, Tanta University, Egypt. Then, kept at 22 ± 1°C, and a relative humidity of 60 ± 5%. Additionally, animals exposed to a twelve-hour light/dark cycle and kept in clean properly ventilated cages under the same environmental circumstances with free access to a balanced diet and water ad libitum. Rats also were left to become accustomed for a week before the start of the experiment.

The experiment was carried out following the guidelines for the care and use of experimental animals in Tanta University, Faculty of Medicine, Egypt and approved through the Local Ethics Committee of the Faculty (Approval code: 34365/1/21).

The Experimental Groups

Rats were divided into the following experimental groups:

- Group1 (Control group): rats were divided into two further subgroups (5 rats each); subgroup 1a: rats left without any treatments and subgroup 1b in which rats were given 1 ml distilled water daily through an intragastric tube for a period equal to their corresponding experimental groups.

- Group 2 (Tartrazine group (TZ)): 10 rats were given tartrazine in a dose of 10 mg/kg/ day orally by intragastric tube for 12 weeks according to Himri *et al*., (2011), Elbanna *et al*., (2017)[9,12].

- Group 3 (Tartrazine+ Manuka honey group (TZ+MH)): 10 rats were given an oral dose of 2.5 mg/kg/day of manuka honey 1 hour before the oral dose of tartrazine (10 mg/kg/ day) by an intragastric tube for 12 weeks. Manuka honey dose was adjusted according to Almasaudi *et al*., (2016), Almasaudi *et al*., (2017)[10,13].

- Group 4 (Recovery group (TZ recovery)): 10 rats were given 10 mg/kg/day tartrazine orally for 12 weeks then left for another 12 weeks for recovery.

By the last day of the experiment, animals were anesthetized by i.p injection of pentobarbital (40 mg/kg)[14]. Then, the jejuna rapidly were dissected out, washed with normal physiological saline and were processed for histological and immunohistochemical examination.

Processing for histological examination[15]

Jejunal specimens were fixed in 10% formalin buffered saline for 3 days. Then, dehydrated in ascending grades of alcohol, cleared in two changes of xylene and finally embedded in hard paraffin. Later, by a rotatory microtome (Leica, US); 5 µm sections obtained.

Hematoxylin and Eosin (H&E) stain processing

Slides contained jejunal sections were deparaffinized by xylene, and hydrated in descending grades of alcohol. After that, sections were stained with hematoxylin for 10 min followed by counterstaining in 1% Eosin solution. Lastly, sections were dehydrated, cleared and mounted in Canada balsam. Hematoxylin stained nuclei blue, and eosin-stained the cytoplasm as well as connective tissue fibers with pink coloration.

Periodic Acid Schiff's (PAS) stain processing

Slides were deparaffinized, rehydrated, and then oxidized by 0.5% periodic acid for 5 min. followed by staining with Schiff's reagent for 20 min. Then, washing with sulfurous water for 2 min and tap water for 10 min. Finally, sections were counterstained with hematoxylin for 20 seconds, then dehydrated, cleared and mounted. Neutral mucopolysaccharides appeared with magenta coloration.

Mallory's trichrome stain processing

Specimens were deparaffinized, rehydrated then washed in distilled water. At that time, re-fixation in Bouin's solution established followed by staining with Wiegert's iron hematoxylin then with Biebrich scarlet-acid fuchsine solution. Now, sections were differentiated in the phosphotungstic acid solution until the collagen is not red and then put directly in aniline blue stain and differentiate in 1% acetic acid solution. Lastly, sections dehydrated then cleared in xylene. Collagen fibers stained blue, nuclei were red and the cytoplasm appeared orange.

Immunohistochemical processing[16]

Jejunal sections of 5 μm thickness were put on positive slides then deparaffinized, and rehydrated. Then, placed in 0.3% hydrogen peroxide/methanol for 20 min to block endogenous hydrogen peroxidase activity followed by washing with PBS. After that, 10% normal goat serum was added to the sections. Followed by incubation overnight at 4°C in a humid chamber with mouse monoclonal anti- PCNA (1:75) (Santa Cruz Biotechnology)[17] and rabbit polyclonal anti-TNF-α (1:500) (Abcam, USA)[18]. After that, sections were incubated with the biotinylated goat anti-rabbit secondary antibody (1:200, Vector Labs, Peterborough, UK) 30 minutes at room temperature followed by the addition of 3, 3′-diaminobenzidine (DAB) chromogen. Eventually, sections were counterstained with Mayer's hematoxylin and examined by a light microscope (Olympus, Japan). Nuclear staining for positive PCNA immune reaction seen while in TNF-α; brown cytoplasmic reaction noticed. For negative control; sections were routinely processed but the primary antibodies were replaced by PBS. On the other hand, the positive control for PCNA was the tonsils (GeneTex.com) while that of the TNF-α was the brain (Abcam).

Morphometric study and analysis

The following measurements were evaluated by using H&E stained sections, and a light microscope (Olympus, Tokyo, Japan) at x 200 magnification. Ten randomly selected fields from 10 different slides from each experimental group were chosen and the following measurements were performed:

A. The mean heights of the jejunal villi starting from the tip of each villus to the junction between villi and crypts (expressed in μm) (15 villi examined/section).

B. Total mucosal thickness (μm) which was measured from the top of each villus to the muscularis mucosa.

C. Mean depths of the intestinal crypts (μm) were measured starting from the junction between villi and crypts until the bases of the intestinal crypts.

The number of goblet cells was evaluated; through which 15 villi per section were examined at a magnification of × 200 using an Olympus, Japan light microscope.

The area percentage (%) of collagen fibers (Estimation of the bluish coloration at the mucosal areas) was evaluated by using 10 Mallory's trichrome stained images at a magnification of x 400 through the Image J (Java 8. 1.6.0. 2017. NIH, USA) software.

The number of cells with PCNA positive nuclei were counted at a magnification of x 200 magnification in a total of 100 cells in the intestinal crypts. Finally, a percentage (%) was determined for the positive cells.

The number and intensity of the TNF-α immunohistochemical positive epithelial and macrophage cells were evaluated in ten microscopic images (× 400 magnification), then the Image J program (Java 8. 1.6.0. 2017. National Institutes of Health, Bethesda, MD, USA) was used to measure the number of cells per 1 mm.

Statistical analysis

The statistical values were presented as means ± SD. Where the two-sample Student's t-test or the Mann-Whitney's U-test after evaluation by F-test were used to compare the different experimental groups. Finally, the differences between the experimental groups were considered significant if $P < 0.05$.

RESULTS

H & E results

Group 1 (control group) showed jejunal mucosa with epithelium, lamina propria, and muscularis mucosa. The mucosa consisted of long finger-like villi with striated border. Each villus covered by epithelium consisted of tall columnar absorptive cells with eosinophilic cytoplasm and basally situated oval nuclei. Goblet cells were seen scattered between enterocytes; showing cup-shaped apex and basally situated nuclei. Also, there were intraepithelial lymphocytes. The villi also contained a small lacteal vessel lined with simple squamous epithelium. Additionally, intestinal glands or crypts of Lieberkühn were seen extending from the bases of villi into the lamina propria and some cells showed mitotic figures. Its lining epithelium verified pyramidal shaped paneth cells with basally situated nuclei and apical eosinophilic granules (Figure 1).

In group 2 (TZ group); some of the villi appeared shorter with broad apical surfaces while others revealed atrophy in association with areas of lost epithelium. Its lining enterocytes appeared cuboidal besides degeneration, and atypical aggregation at the tips of some villi towards the luminal surface. There were also congested blood capillaries and inflammatory cellular infiltrations in the lamina propria. Furthermore, the intestinal crypts presented degenerative changes and wide spaces between them (Figure 2).

Regarding group 3 (TZ+ MH), amelioration of TZ histopathological previous findings appeared in comparison to the short blunt villi with degenerated and atypical epithelial appearance in group 4 (TZ recovery) (Figure 2).

The villus height of group 2 (TZ group) showed a significant decrease when compared to group 1 (control group). While in group 3 (TZ+MH); a significant increase was noticed when compared to group 2 (TZ group) & 4 (TZ recovery). On the other hand, group 4 (TZ recovery) revealed a non-significant difference compared to group 2 (TZ group) (Figure 3).

The total mucosal thickness of group 2 showed a significant decrease in comparison to group 1. Whereas, group 3 showed a significant increase when compared to group 2 & 4. Conversely, group 4 showed a non-significant difference when compared to group 2 (Figure 4).

The depths of the intestinal crypts of group 2 showed a significant decrease in comparison to the control. However, in group 3; a significant increase was observed when compared to group 2 and 4. On the contrary, the group 4 displayed a non-significant difference when compared to group 2 (Figure 5).

PAS results

The control PAS-stained sections revealed strong PAS-positive reaction of the brush border of enterocytes in addition to the magenta red colorations of goblet cells. Despite that, group 2 displayed interruption of the PAS-stained brush border with a decreased number of goblet cells. On the other hand, group 3 showed a picture similar to the control group. While group 4 showed a moderate PAS-positive reaction of the brush border of enterocytes in addition to moderate number of goblet cells (Figure 6).

The number of goblet cells of group 2 showed a significant decrease when compared to the control group. But, in group 3; a significant increase was detected when compared to both group 2 and 4. Inversely, a non-significant difference for the group 4 was demonstrated as compared to group 2 (Figure 7).

Mallory's trichrome results

The control group showed few collagen fibers in the lamina propria of the intestinal villi as well as around the intestinal crypts. In contrast, group 2 revealed an increase in its amount especially in the villi, and around the degenerated glands. While, group 3 showed normal collagen content in both villi and around the crypts to be similar to the control group. While in group 4, moderate amount of collagen fibers still visible in both villi and crypts (Figure 8).

The area percentage of the mucosal collagen fibers of group 2 showed a significant increase in comparison to the control group. However group 3 showed a significant decrease when compared to group 2 and 4. On the other hand, group 4 showed a non-significant difference as compared to group 2 (Figure 9).

PCNA immunohistochemical results

Positive control of the tonsil showed positive PCNA immunohistochemical reaction (Figure 10 A2) (Ref. Dignostic Biosystems, USA). The control group examination revealed many PCNA positive nuclei of the cells lining the intestinal glands. In group 2, significantly decreased number of PCNA positive nuclei were observed when compared to the control group. Meanwhile, the intestinal gland cells with PCNA positive nuclei were significantly increased in group 3 when compared to the group 2 and 4. While group 4 expressed a non-significant difference in the number of PCNA positive nuclei when compared to group 2 (Figures 10,11)

TNF-α immunohistochemical results

Positive control of brain tissue showed positive TNF-alpha immunohistochemical reaction (Figure 12 A2) (Ref. IHCWorld, USA). The control group revealed few epithelial as well as macrophage cells with low TNF-α expression. However, in group 2; the number and the intensity of TNF-α-expression of both epithelial and macrophage cells increased significantly in comparison to the control group. Conversely, group 3 showed a significant reduction of the number as well as intensity of TNF-α-expression of reactive epithelial and macrophage cells when compared to groups 2 and 4. Moreover, group 4 revealed a non-significant difference when compared to group 2 (Figures 12& 13).
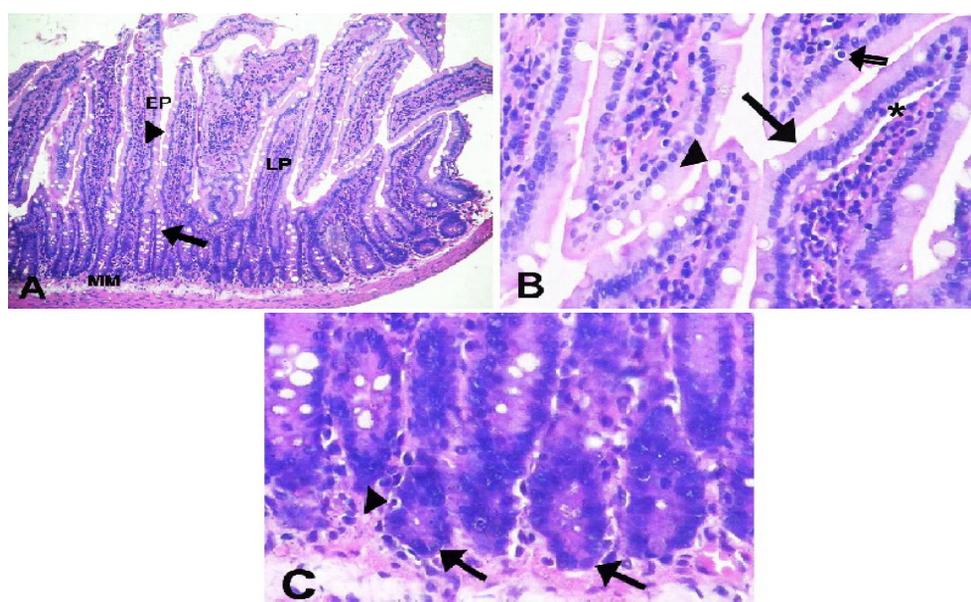

Fig. 1: Effect of manuka honey on tartrazine-induced injury of the jejunal mucosa of rats stained by H & E. A) Control (group 1): epithelium (EP), lamina propria (LP) and muscularis mucosa (MM), long finger-like villi with striated border (►) and intestinal glands (→) (H&E X 200). B) Higher magnification of image A: villi covered by tall columnar absorptive cells with eosinophilic cytoplasm and basal oval nuclei (→), goblet cells with cup-shaped apex and basal nucleus (►), intraepithelial lymphocytes (double arrow) and a small lacteal vessel lined with simple squamous epithelium (*) (H&E X 1000). C) Higher magnification of image A: paneth cells with basal nuclei and apical eosinophilic granules (→), and mitotic figures (►) (H&E X 1000).
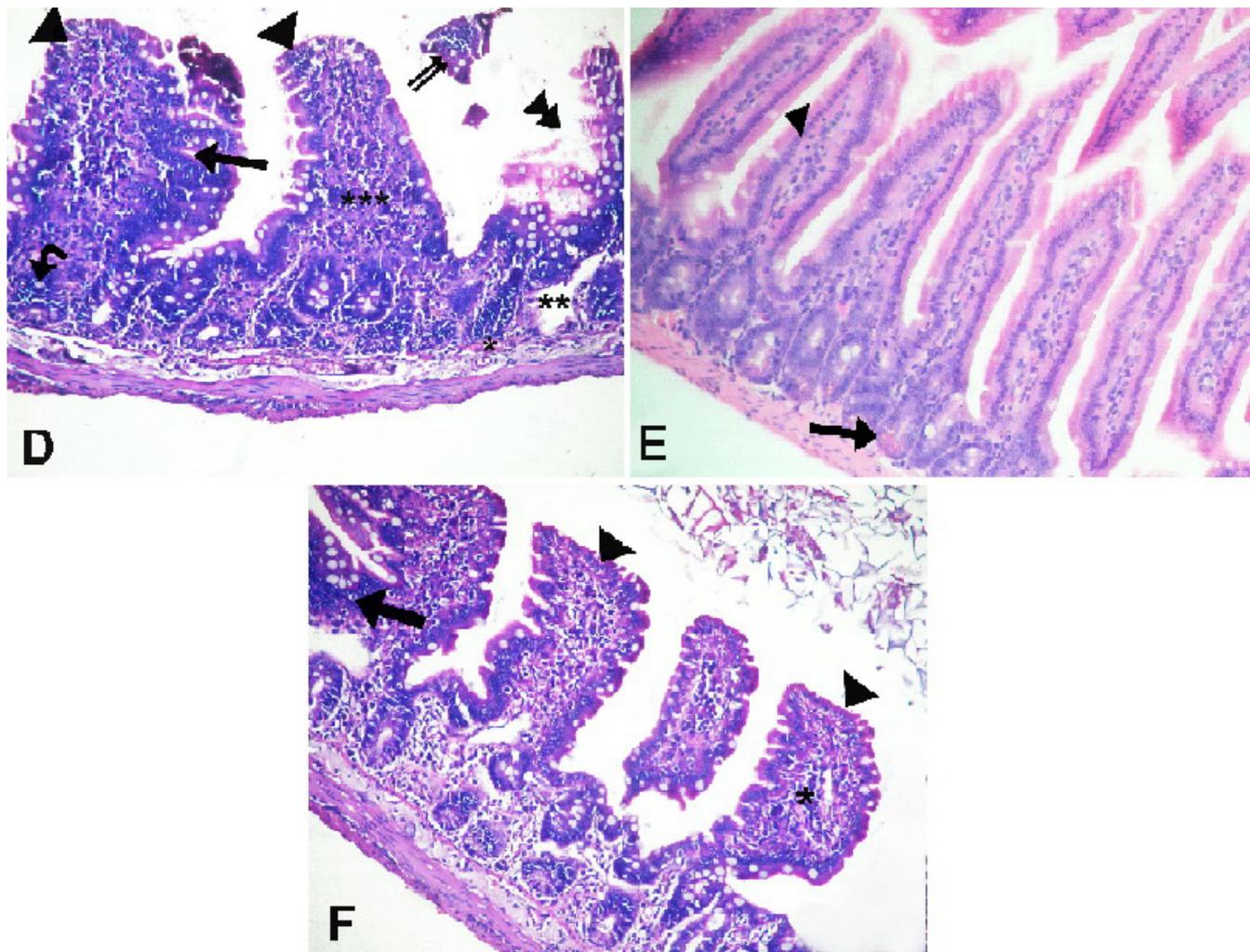
Fig. 2: Effect of manuka honey on tartrazine-induced injury of the jejunal mucosa of rats stained by H & E. D) Group 2 (TZ): short villi with broad apices (►), atrophic villi with areas of lost epithelium (►►), cuboidal desquamated enterocytes (double arrow), atypical aggregation of epithelial cells (→), congested blood capillaries (*), inflammatory cellular infiltrations (***), and degenerated crypts (curved arrow) with wide spaces (**) (H&E X 400). E) Group 3 (TZ+ MH): finger-like villi covered by enterocytes and goblet cells (►), and paneth cells (→) (H&E X 200). F) Group 4 (TZ Recovery): short blunt villi (►), degenerated atypical epithelium (→) and inflammatory cellular infiltrations (*) (H&E X 400).
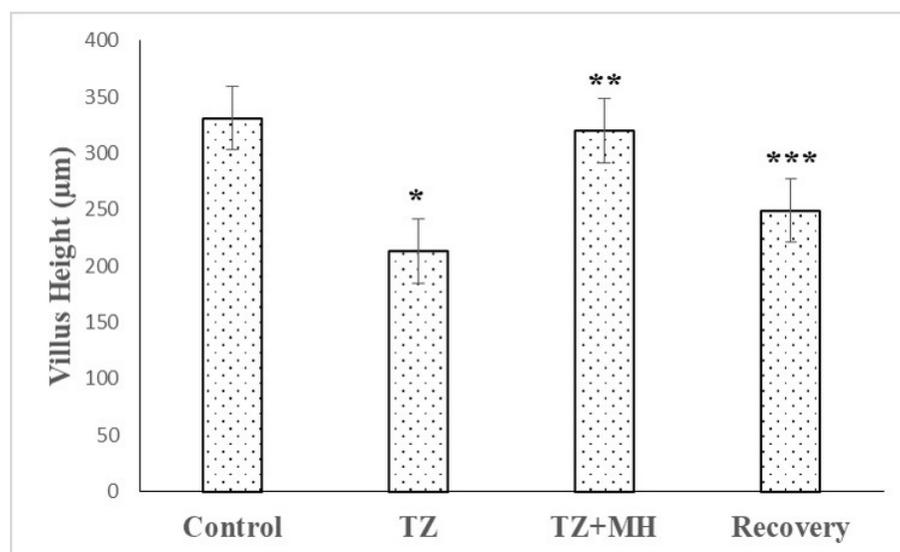

Fig. 3: Effect of manuka honey on the villus height of tartrazine-induced injury of the jejunal mucosa of rats. Values presented as means ± SD. Control: group 1, TZ: tartrazine (Group 2) (* $P< 0.05$ compared to the control group). TZ+MH: tartrazine and manuka honey (Group 3) (** $P< 0.05$ as compared to groups 2 and 4). Recovery: TZ recovery (Group 4) (*** $P> 0.05$ in comparison to group 2).
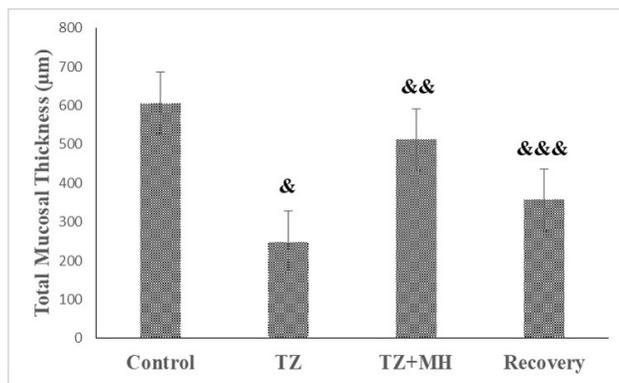
Fig. 4: Effect of manuka honey on the total mucosal thickness of tartrazine-induced injury of the jejunal mucosa of rats. Values presented as means ± SD. Control: group 1, TZ: group 2 (& *P*< 0.05 compared to the control group). TZ+MH: group 3 (&& *P*< 0.05 as compared to the groups 2& 4). Recovery: group 4 (&&& *P*> 0.05 in comparison to group 2).
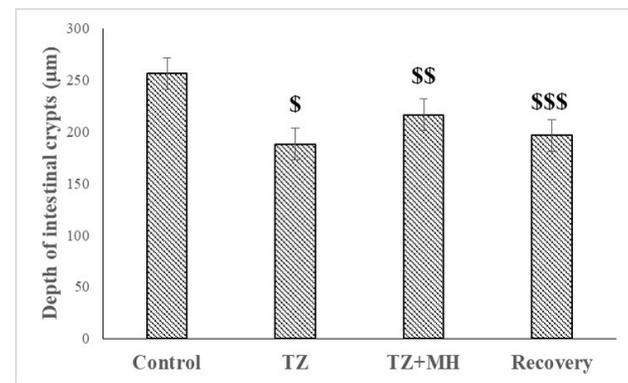
Fig. 5: Effect of manuka honey on the depths of the intestinal crypts of tartrazine-induced injury of the jejunal mucosa of rats. Values presented as means ± SD. Control: group 1, TZ: group 2 ($ *P*< 0.05 compared to the control group). TZ+MH: group 3 ($$ *P*< 0.05 as compared to the groups 2& 4). Recovery: group 4 ($$$ *P*> 0.05 in comparison to the TZ group).
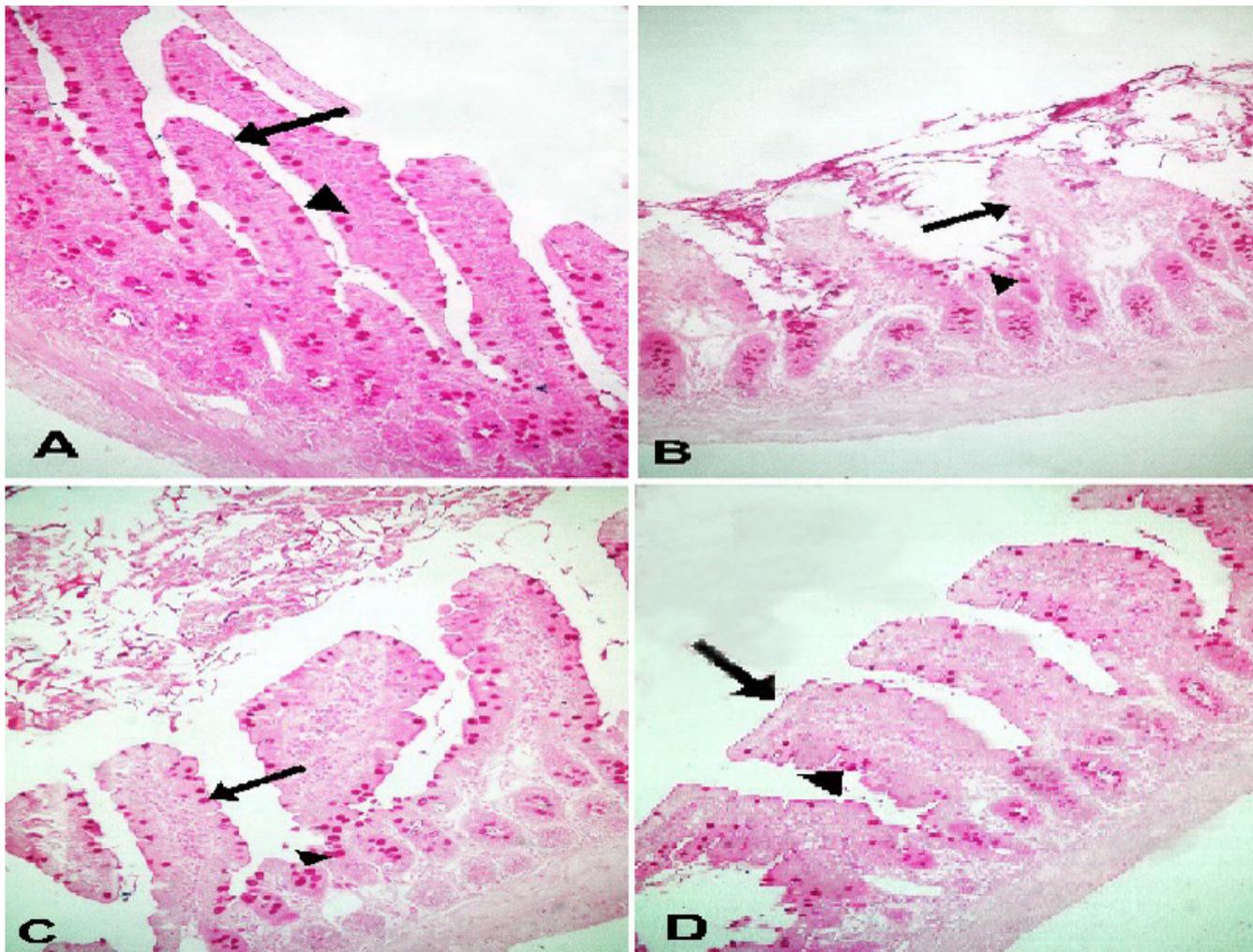

Fig. 6: Effect of manuka honey on tartrazine-induced injury of the jejunal mucosa of rats stained by PAS stain X 200. A) Control: strong PAS positive reaction of the brush border of enterocytes (→) and magenta red colorations of goblet cells (►). B) Group 2: interruption of the PAS stained brush border (→) and decreased number of goblet cells (►). C) Group 3: strong PAS positive reaction of the brush border of enterocytes (→) and goblet cells (►). D) Group 4: moderate PAS positive reaction of the brush border of enterocytes (→) and moderate number of goblet cells (►).
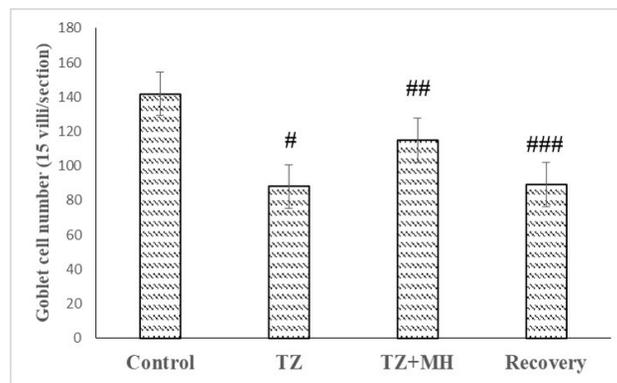
Fig. 7: Effect of manuka honey on the number of goblet cells of tartrazine-induced injury of the jejunal mucosa of rats. Values presented as means ± SD. Control: group 1, TZ: group 2 (# $P< 0.05$ compared to the control group). TZ+MH: group 3 (## $P< 0.05$ as compared to the groups 2& 4). Recovery: group 4 (### $P> 0.05$ in comparison group 2).
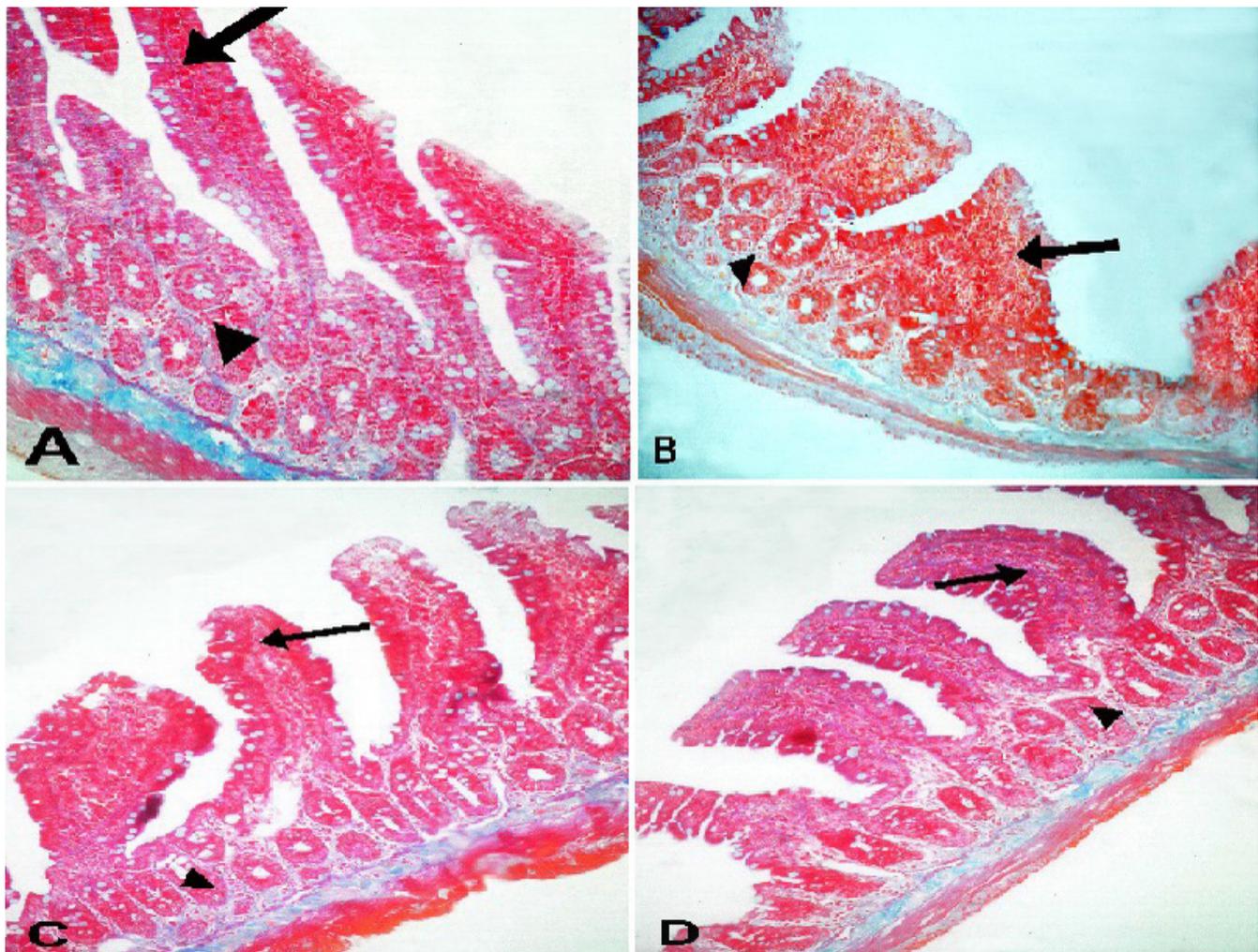

Fig. 8: Effect of manuka honey on tartrazine-induced injury of the jejunal mucosa of rats stained by Mallory's trichrome X 400. Control group: few collagen fibers in the lamina propria of the intestinal villi (→) and around the intestinal crypts (►). B) Group 2: increased amount of collagen fibers in the villi (→), and around the degenerated glands (►). C) Group 3: normal collagen content in both villi (→) and around the crypts (►). D) Group 4: moderate amount of collagen fibers in both villi (→) and crypts (►).
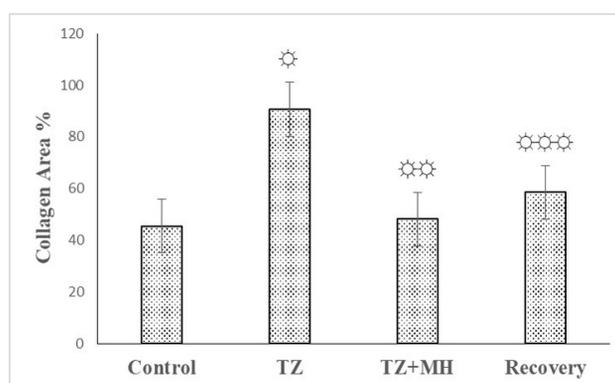
Fig. 9: Effect of manuka honey on the area % of mucosal collagen fibers of tartrazine-induced injury of the jejunal mucosa of rats. Values presented as means ± SD. Control: group 1, TZ: Group 2 (☼ *P*< 0.05 compared to the control group). TZ+MH: group 3 (☼☼☼ *P*< 0.05 as compared to the groups 2& 4). Recovery: group 4 (☼☼☼☼ *P*> 0.05 in comparison to group 2).
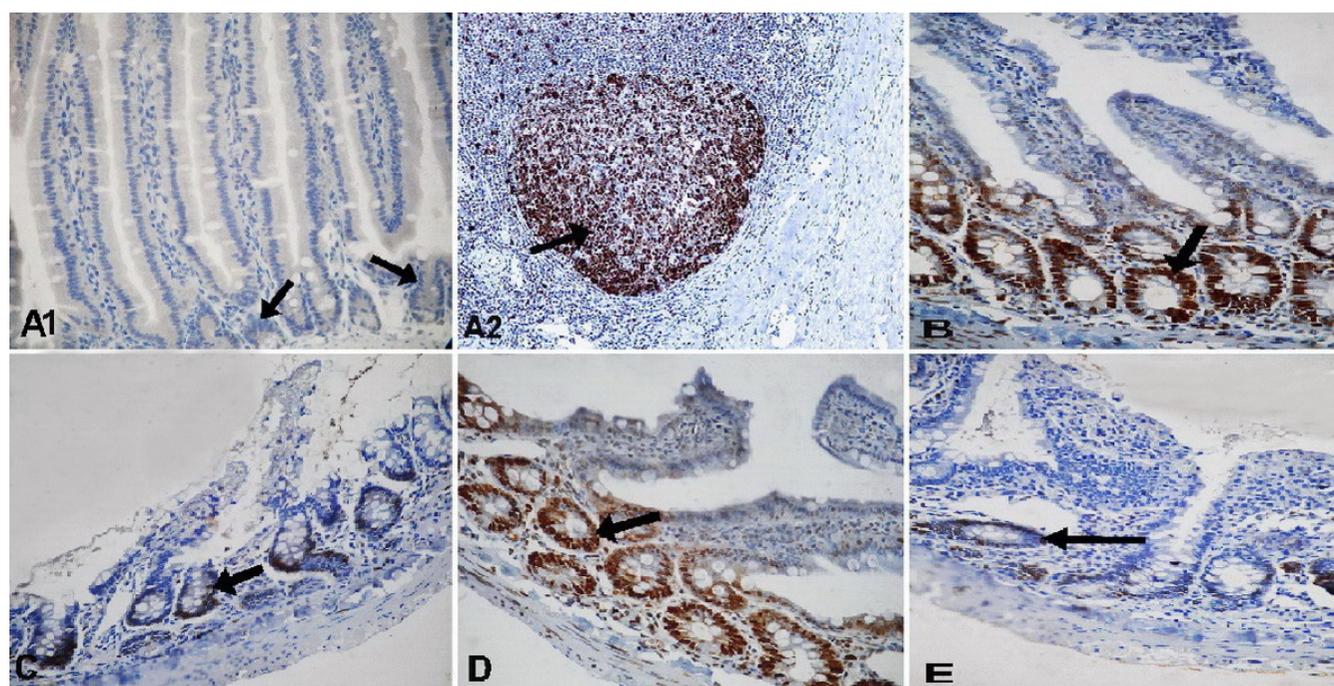

Fig. 10: Effect of manuka honey on tartrazine-induced injury of the jejunal mucosa of rats stained by PCNA X 400. A1) Negative control: negative PCNA immunohistochemical reaction of the nuclei of the intestinal glands (→). A2) Tonsils positive control for PCNA expression (→). B) Control group: many PCNA positive nuclei of the intestinal glands (→). C) Group 2: decreased number of PCNA positive nuclei in the intestinal glands (→). D) Group 3: intestinal glands with increased number of PCNA positive nuclei. E) Group 4: decrease in the number of PCNA positive nuclei of the intestinal glands (→).
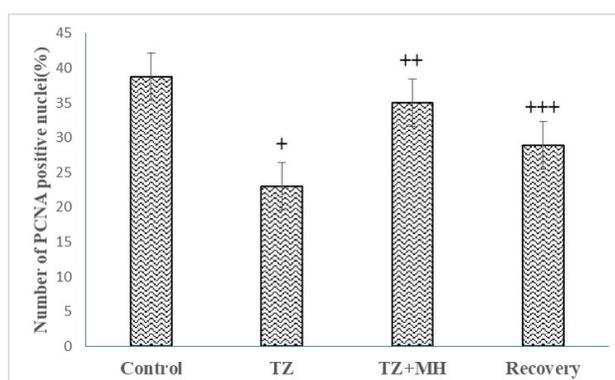

Fig. 11: Effect of manuka honey on the number of PCNA positive nuclei (%) of tartrazine-induced injury of the jejunal mucosa of rats. Values presented as means ± SD. Control: group 1, TZ: group 2 (+ *P*< 0.05 compared to the control group). TZ+MH: group 3 (++ *P*< 0.05 as compared to the groups 2& 4). Recovery: group 4 (+++ *P*> 0.05 in comparison to group 2).
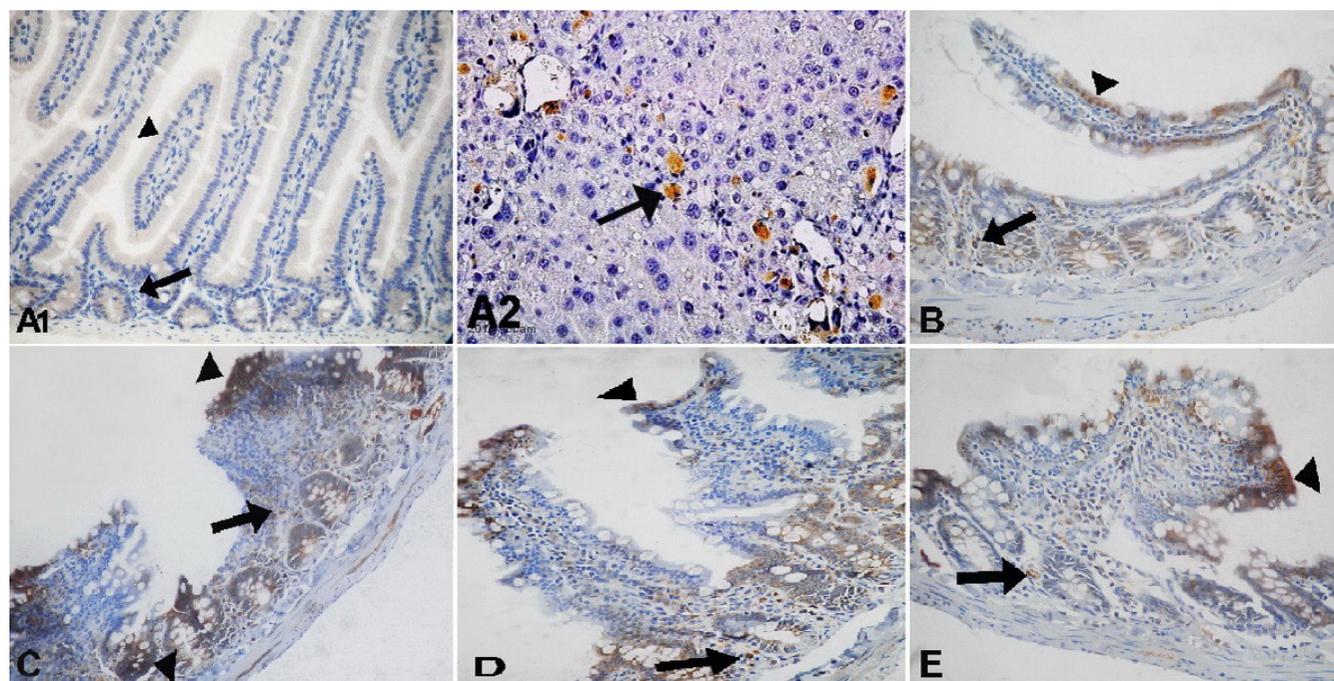
Fig. 12: Effect of manuka honey on tartrazine-induced injury of the jejunal mucosa of rats stained by TNF-α X 400. A1) Negative control: negative TNF-α immunohistochemical reaction of the epithelial cell (▶) and macrophages (→). A2) Brain positive control for TNF-α cytoplasmic expression (→). B) Control group: few epithelial (▶) and macrophage cells (→) with low TNF-α immunohistochemical reactive. C) Group 2: increased number and intensity of TNF-α-immunohistochemical of both epithelial (▶) and macrophage cells (→). D) Group 3: decreased number and intensity of TNF-α-immunohistochemical reaction of epithelial (▶) and macrophage cells (→). E) Group 4: moderate increased number and intensity of TNF-α-immunohistochemical reactive epithelial (▶) and macrophage cells (→)
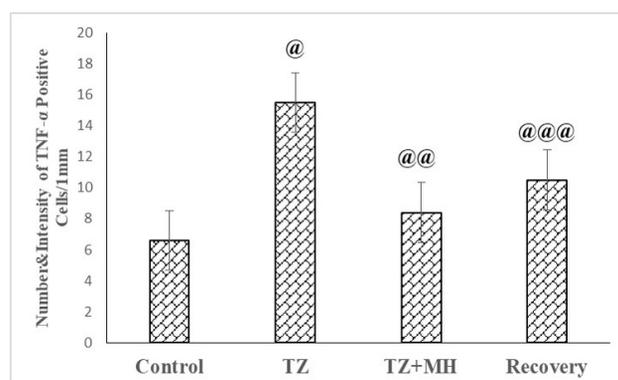

Fig. 13: Effect of manuka honey on the number& intensity of TNF-α positive cells of tartrazine-induced injury of the jejunal mucosa of rats. Values presented as means ± SD. Control: group 1, TZ: group 2 (@ $P < 0.05$ compared to the control group). TZ+MH: group 3 (@@ $P < 0.05$ as compared to groups 2& 4). Recovery: group 4 (@@@ $P > 0.05$ in comparison to group 2).

DISCUSSION

Food dyes are main components that enter in many food and drink industries in which peoples consume every day. The uncontrolled consumption of azo dyes in food and drinks such as TZ gain a high concern because of its associated health hazards as well as tissue toxicity, which is due to the reduction of its azo bond into toxic metabolites like sulfanilic acid and aminopyrazolone by the intestinal flora. These are further oxidized into N-hydroxy derivatives by P450 enzymes[19, 20].

The histological, immunohistochemical, and morphometric results of the present research revealed that; the oral intake of 10 mg/kg/day of TZ for 12 weeks induced jejunal mucosal degenerative and atrophic changes

seen in H&E stained sections. There were significantly decreased villus height, mucosal thickness as well as crypt depth in addition to congested blood capillaries. Moreover, a decreased PAS staining affinity of the mucosal goblet cells with significant decrease in its number were noticed. Also, there was fibrosis seen by Mallory's trichrome stain in addition to decreased cell proliferation with significantly decreased PCNA immunohistochemical expression. Additionally, the jejunal mucosal inflammation was detected in the form of significantly increased expression of TNF-α in addition to mucosal mononuclear cellular infiltrations.

These findings were in agreement with previous studies of Al-Seeni *et al*., (2018)[21] who documented the degenerative and atrophic effect of TZ on GIT. They attributed the

TZ-induced tissue damage as a result of the induction of oxidative stress with the generation of ROS. This also proved by Bhatt *et al.*, (2018)[22] who mentioned that TZ-induced oxidative stress associated with elevated levels of malondialdehyde (MDA); the end product of lipid peroxidation besides elevated nitric oxide (NO) levels with the inhibition of the endogenous antioxidant defense enzymes.

In this work, the degenerative changes were seen in H&E, as well as PAS stained sections, could be explained by the fact that TZ was degraded into toxic metabolites. Consequently, a decrease in the gamma-glutamyl-cyteineyl-glycine (GSH) levels, the most important intracellular thiol antioxidant that protects tissues against oxidative damage and boosts the normal development and function of the intestinal mucosa[23]. Moreover, TZ is proved to have a mutagenic effect with subsequent DNA damage which may be due to its direct action on the cell nuclear DNA. Moreover, the elevated levels of TNF-α seen in group 2 could explain the loss of mucin-producing goblet cells. Hence, decreasing the mucosal thickness and exposing it to luminal antigens with more mucosal inflammation and injury[24].

The present research revealed a significant increase in the amount of mucosal collagen fibers detected in group 2 especially around the degenerated glands and the congested blood vessels. The associated TZ- tissue injury and the associated oxidative stress lead to tissue inflammation and cytokine dysregulations then followed by fibroblasts stimulation and collagen deposition[25]. This was confirmed also in the present study by the significantly increased expression of TNF-α; a pro-inflammatory cytokine produced mainly by macrophages concerned with tissue inflammation, proliferation as well as fibrosis. This leads to the release of the TNF-α -inflammatory signals by the damaged tissue stimulates the differentiation of fibroblasts into fibrocytes ending with tissue fibrosis[26].

In the present work, group 2 showed a significant decrease in the percentage of PCNA positive nuclei of the cells lining the intestinal glands in comparison to the control group. PCNA is a cell replication marker that directly involved in DNA synthesis and plays a vital role in the replication as well as repair of the damaged DNA[26]. It was previously mentioned that TZ has antiproliferative effects by inducing DNA damage consequently affecting cell proliferation[24]. Additionally, the TZ associated increased levels of the TNF-α lead to the arrest of the cell cycle and cell differentiation with subsequently decreased cell proliferation[27].

The current research revealed the presence of inflammatory cellular infiltrations in the lamina propria of the jejunal mucosa with a significant increase in the TNF-α expression in both epithelial cells and macrophages compared to the control group. Other studies have shown that TZ could elicit pro-inflammatory responses with the recruitment of inflammatory cells in tissues besides the TZ associated synthesis of leukotriene[27]. Furthermore, TNF-α stimulates the activation of NF-κB in the epithelial cells with the production of different inflammatory chemokines consequently, recruitment of the different immune cells initiating an inflammatory response[28]. On the other hand, the released TNF-α initiates activation of the ICAM-1 besides other adhesion molecules that bring inflammatory cells into the jejunal mucosa[29].

The current study revealed that MH ameliorates the previously mentioned degenerative changes of the jejunal mucosa of rats treated by TZ. Also, MH induces its protective effect better than the TZ withdrawal of the recovery group, which in agreement to other studies made on GIT like gastritis, peptic ulcer, and colitis[30].

Manuka honey has been used in the field of medicine as an antibacterial agent due to its MGO content (methylglyoxal). Then, was proved to have potent wound healing properties. MH also has an antioxidant effect due to its high methyl syringate content which can neutralize the superoxide radicals as well as binding to iron. Consequently, it prevents the formation of hydroxide radicals from hydrogen peroxide that is a very damaging substance[31,32]. Furthermore, the polyphenolic components of MH (pinocembrin or pinobanksin) can cross the cell membrane and scavenge the intracellular free radicals and triggering the 5' AMP-activated protein kinase (AMPK) phosphorylation. So, the antioxidant enzyme expression will be increased[11].

The anti-inflammatory effect of MH can be indirect through its antimicrobial effect with subsequent removal of the dead tissues, reduction of the exudates, decreased edema as well as prevention of microbial contamination of the injured mucosa. It may has a direct anti-inflammatory effect through the reduction of the release of cytokines such as TNF-α (as shown by the significant decrease of its expression in our present study), IL-1β, and IL-6, as well as the reduction of NO, and leukocytes levels. Moreover, MH can increase the release of heme-oxygenase-1 which catalyzes heme degradation[13,31]. Furthermore, MH can inhibit the NF-κB pathway (IκBα phosphorylation) with subsequent decreased inflammation and promotion of the cell proliferation[11].

In the current study, the possibility of recovery from the damaging effects of TZ on rat's jejunal mucosa was examined after 12 weeks from the ceasing of TZ intake. The histological sections revealed partial improvement in the structure of jejunal mucosa, regarding the epithelial appearance, villus structure and height, total mucosal thickness as well as the depths of the intestinal crypts. Moreover, the morphometric results revealed a non-significant difference in the number of goblet cells, amount of mucosal collagen fibers, PCNA positive reaction and the number of TNF-α +ve cells as compared to group 3.

CONCLUSION

From the previous data, we can conclude that oxidative stress and release of TNF-α are the main mechanisms of TZ -induced injury of the jejunal mucosa of rats. Additionally, the ameliorative effect of MH to these injurious effects (through its antioxidant and anti-inflammatory actions) was better than TZ withdrawal from the tissues.

CONFLICT OF INTERESTS

There are no conflicts of interest.

REFERENCES

1. Ghonimi WA and Elbaz A: Histological changes of selected Westar rat tissues following the ingestion of tartrazine with special emphasis on the protective effect of royal jelly and cod liveroil. J Cytol Histol. (2015) 6(4): 1-6.

2. El Rabey HA, Al-Seeni MN, Al-Sieni AI, Al-Hamed AM, Zamzami MA and Almutairi FM: Honey attenuates the toxic effects of the low dose of tartrazine in male rats. J Food Biochem. (2019) 43(4): e12780-e12786.

3. Abdel-Aziz H, Alazouny Z, Abdelfadee K and Abohashem A: Effect of tartrazine on thyroid gland of male rat and ameliorating role of curcumin (histological and immunohistochemical study). J Biochem Cell Biol. (2019) 2(111): 2-8.

4. El-sakhawy MA, Mohamed DW and Ahmed YH: Histological and immunohistochemical evaluation of the effect of tartrazine on the cerebellum, submandibular glands, and kidneys of adult male albino rats. Environ SciPollut R. (2019) 26(10): 9574-9584.

5. 5.   Balta I, Sevastre B, Mireşan V, Taulescu M, Raducu C, Longodor AL, Marchiş Z, Mariş CS and Coroian A: Protective effect of blackthorn fruits (Prunus spinosa) against tartrazine toxicity development in albino Wistar rats. BMC chem. (2019) 13(1): 104-110.

6. Ali FA, Abdelgayed SS, EL-Tawil SO and Bakeer MA: Toxicological and histopathological studies on the effect of tartrazine in male albino rats. Int J Biol Biomol Agr Food Biotechnol Eng. (2016) 10(8): 513-518.

7. Mpountoukas P, Pantazaki A, Kostareli E, Christodoulou P, Kareli D, Poliliou S, Mourelatos C, Lambropoulou V and Lialiaris T: Cytogenetic evaluation and DNA interaction studies of the food colorants amaranth, erythrosine and tartrazine. Food Chem Toxicol. (2010) 48(10): 2934-2944.

8. Moutinho I, Bertges L and Assis R: Prolonged use of the food dye tartrazine (FD&C yellow n° 5) and its effects on the gastric mucosa of Wistar rats. Braz J Biol. (2007) 67(1): 141-145.

9. Himri I, Bellahcen S, Souna F, Belmekki F, Aziz M, Bnouham M, Zoeir J, Berkia Z, Mekhfi H and Saalaoui E: A 90-day oral toxicity study of tartrazine, a synthetic food dye, in wistar rats. Int J Pharm Pharmaceut Sci. (2011) 3(3): 159-169.

10. Almasaudi SB, Abbas AT, Al-Hindi RR, El-Shitany NA, Abdel-dayem UA, Ali SS, Saleh RM, Al Jaouni SK, Kamal MA and Harakeh SM: Manuka honey exerts antioxidant and anti-inflammatory activities that promote healing of acetic acid-induced gastric ulcer in rats. Evid Based Complement Alternat Med. (2017) 2017:1-12.

11. Minden-Birkenmaier BA, Cherukuri K, Smith RA, Radic MZ and Bowlin GL: Manuka honey modulates the inflammatory behavior of a dHL-60 neutrophil model under the cytotoxic limit. Int J biomater. (2019) 2019: 1-14.

12. Elbanna K, Sarhan OM, Khider M, Elmogy M, Abulreesh HH and Shaaban MR: Microbiological, histological, and biochemical evidence for the adverse effects of food azo dyes on rats. J Food Drug Anal. (2017) 25(3): 667-680.

13. Almasaudi SB, El-Shitany NA, Abbas AT, Abdel-dayem UA, Ali SS, Al Jaouni SK and Harakeh S: Antioxidant, anti-inflammatory, and antiulcer potential of manuka honey against gastric ulcer in rats. Oxid medcell longev. (2016) 2016: 1-10.

14. Fish R, Danneman PJ, Brown M and Karas A: Anesthesia and analgesia in laboratory animals. 2nd edition., Academic press. Elsevier Inc. London. UK. (2008)pp: 239-297.

15. Suvarna KS, Layton C and Bancroft JD: Bancroft's theory and practice of histological techniques E-Book. 8th edition., Elsevier Health Sciences. UK. (2018)pp: 73-150.

16. Buchwalow IB and Böcker W: Immunohistochemistry. Basics and Methods 1st edition., Springer. (2010)pp: 1-149.

17. Wang L, Li J, Li Q, Zhang J and Duan X-L: Morphological changes of cell proliferation and apoptosis in rat jejunal mucosa at different ages. World J Gastroenterol. (2003) 9(9): 2060-2064.

18. Lee JH, Lee T-K, Kim IH, Lee JC, Won M-H, Park JH, Ahn JH, Shin MC, Ohk TG and Moon JB: Changes in histopathology and tumor necrosis factor-α levels in the hearts of rats following asphyxial cardiac arrest. Clin Exp Emerg Med. (2017) 4(3): 160-167.

19. Bawazir A: Effects of food colour allura red (No. 129) on some neurotransmitter, antioxidant functions and bioelement contents of kidney and brain tissues in male albino rats. Life Sci J. (2016) 13(12): 10-17.

20. Hashem MM, Abd-Elhakim YM, Abo-EL-Sooud K and Eleiwa MM: Embryotoxic and teratogenic effects of tartrazine in rats. Toxicol Res. (2019) 35(1): 75-81.

21. Al-Seeni MN, El Rabey HA, Al-Hamed AM and Zamazami MA: Nigella sativa oil protects against tartrazine toxicity in male rats. Toxicology reports. (2018) 5: 146-155.

22. Bhatt D, Vyas K, Singh S, John P and Soni I: Tartrazine induced neurobiochemical alterations in rat brain sub-regions. Food Chem Toxicol. (2018) 113: 322-327.

23. Ifemeje J, Ezeonyemalu U, Egbuna C, Olisah M and Ifemeje M: Effects of Four Different Food Additives on the Oxidative Stress Markers of Wistar Albino Rats. Int Ann Sc. (2020) 9(1): 46-51.

24. Khayyat L, Essawy A, Sorour J and Soffar A: Tartrazine induces structural and functional aberrations and genotoxic effects in *vivo*. PeerJ. (2017) 5: e3041-e3049.

25. Kalayarasan S, Sriram N and Sudhandiran G: Diallyl sulfide attenuates bleomycin-induced pulmonary fibrosis: Critical role of iNOS, NF-κB, TNF-α and IL-1β. Life Sci. (2008) 82(23-24): 1142-1153.

26. Şensoy E and Öznurlu Y: Determination of the changes on the small intestine of pregnant mice by histological, enzyme histochemical, and immunohistochemical methods. Turk J Gastroenterol. (2019) 30(10): 917-925.

27. Lau KS, Cortez-Retamozo V, Philips SR, Pittet MJ, Lauffenburger DA and Haigis KM: Multi-scale in *vivo* systems analysis reveals the influence of

28. immune cells on TNF-α-induced apoptosis in the intestinal epithelium. PLoS Biol. (2012) 10(9): 1-14.

29. Ruder B, Atreya R and Becker C: Tumour necrosis factor alpha in intestinal homeostasis and gut related diseases. int j mol sci. (2019) 20(8): 1887-1895.

29. Leo L, Loong C, Ho XL, Raman MFB, Suan MYT and Loke WM: Occurrence of azo food dyes and their effects on cellular inflammatory responses. Nutr j. (2018) 46: 36-40.

30. Prakash A, Medhi B, Avti P, Saikia U, Pandhi P and Khanduja K: Effect of different doses of Manuka honey in experimentally induced inflammatory bowel disease in rats. Phytother Res. (2008) 22(11): 1511-1519.

31. He J. The Antioxidant and Anticancer Potentials of New Zealand Manuka Honey and Thyme Honey. Auckland University of Technology. School of Aplied Sciences. New Zealand. (2016): 1-115.

32. Gill R, Poojar B, Bairy LK and Praveen KS: Comparative evaluation of wound healing potential of manuka and acacia honey in diabetic and nondiabetic rats. Jpharm bioallied sci. (2019) 11(2): 116-124.

الملخص العربى

التأثير التحسني المحتمل لعسل المانوكا على إصابة الغشاء المخاطي للصائم المستحثة بالتارترازين مع دور الإجهاد التأكسدي وعامل نخر الورم-ألفا: دراسة هستولوجية ومورفومترية

سماح قنديل وهبه السيد محمد شرف الدين

قسم الهستولوجي وبيولوجيا الخلية- كلية الطب- جامعة طنطا- مصر

مقدمة: التارترازين هي صبغة آزو الإصطناعية القابلة للذوبان في الماء مع لون أصفر ليمونى ساطع تستخدم لتحسين مظهر ومذاق ورائحة وجودة الطعام. تناوله لفترات طويلة يمكن أن يؤدي إلى مخاطر صحية خطيرة من خلال الحث علي الإجهاد التأكسدي وإلتهاب الأنسجة بوساطة عامل نخر الورم-ألفا. عسل المانوكا هو عسل داكن مشتق من شجرة المانوكا، له تأثيرات مضادة للأكسدة ومضادة للإلتهابات ومضادة للقرحة ومضادة للميكروبات.

الهدف: هدفت هذه الدراسة إلى التحقق من التأثير الضار للتارترازين على الغشاء المخاطي المبطن للصائم للفئران والتحسين المحتمل لعسل المانوكا مقارنة بسحب التارترازين من الأنسجة من خلال دراسة نسيجية ومورفومترية مع دور الإجهاد التأكسدي وعامل نخر الورم-ألفا.

المواد والطرق: تم إستخدام ٤٠ من ذكور فئران ويستار البالغة ١٨٠-٢٠٠ جرام ومقسمة إلى ٤ مجموعات (١٠ فئران لكل مجموعة): المجموعة ١ (المجموعة الضابطة)؛ المجموعة ٢ (مجموعة الترترازين): تلقت ١٠مجم / كجم / يوم من التارترازين عن طريق الفم لمدة ١٢ أسبوع؛ المجموعة ٣ (تارترازين + عسل المانوكا): تلقت ٢,٥ مجم / كجم / يوم من عسل المانوكا ساعة قبل التارترازين (١٠ مجم / كجم / يوم) عن طريق الفم لمدة ١٢ أسبوع؛ والمجموعة ٤ (مجموعة التعافي من التارترازين): تلقت ١٠ مجم / كجم / يوم من التارترازين يتم تناوله عن طريق الفم لمدة ١٢ أسبوع ثم يترك لمدة ١٢ أسبوعًا أخرى للتعافي.

النتائج: أظهرت المجموعة الثانية تغيرات تنكسية في الغشاء المخاطي للصائم، وإنخفاض كبير في إرتفاع الزغابات، وسمك الغشاء المخاطي وعمق المغاور. أيضا، إحتقان الشعيرات الدموية، إرتشاح للخلايا وحيد النواة، إنخفاض كبير في عدد الخلايا الكأسية، وزيادة كبيرة في ألياف الكولاجين. كما لوحظ إنخفاض كبير في (PCNA)، وزيادة كبيرة في TNF-α. أظهرت المجموعة ٣ تحسنًا ملحوظًا في النتائج السابقة أفضل من المجموعة ٤.

الخلاصة: عسل المانوكا يحسن من تلف الغشاء المخاطي للصائم المستحث بالتارترازين من خلال تأثيراته المضادة للأكسدة والمضادة للإلتهابات.