

THE EFFECTS OF DIETARY SODIUM NITRITE SUPPLEMENTATION ON JEJUNAL MUCOSA AND POSSIBLE PROTECTIVE EFFECT OF VITAMIN A IN ALBINO RATS

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ABSTRACT

Nitrate (NO₃⁻) and nitrite (NO₂⁻) are naturally-occurring oxidation products of nitrogen which widely used in the food industry. The goal of this study was to illustrate the histopathological effects of sub-lethal dose of sodium nitrite on jejunal mucosa and the possible protective effect of vitamin A. Thirty adult male Albino rats were divided into 3 groups as follows: Group I (control) received distilled water, Group II (Nitrite-treated) which received intra-gastric daily dose of 50 mg/kg NaNO₂ and Group III (Nitrite & vit-A treated group) which received intra-gastric daily dose of 50 mg/kg NaNO₂ +10,000 I.U/ rat vitamin A. Animals were sacrificed 8 weeks after treatment. Blood samples were collected and examined. Specimens from jejunal mucosa of rats were collected for the optical and electron microscope study. The blood samples showed significant reduction in all blood cells counting and albumin level with elevated methemoglobin level in the nitrite treated group. Light microscopic examination revealed that; Sodium nitrite cause jejunal mucosa damage represented by abnormal shape and length of jejunal mucosa villi, necrosis with marked loss of covering epithelium and excessive cellular infiltration in its core of lamina. PAS stained sections exhibited weak or negative PAS reaction of goblet cells of brush border of villi and crypts. The enterocytes and goblet cells showed ultrastructural changes. Vitamin A administration resulted in marked regression of the previously mentioned jejunal mucosal effects. Results of the current study revealed that dietary supplementation of nitrite cause multiple jejunal mucosal injury but the co-administration of Vitamin A greatly reduce the toxic effects of nitrite supplementation on jejunal mucosa.

KEY WORD: Sodium nitrite, Vitamin-A, Jejunal mucosa, Histopathology, Ultra-structure.

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Access this Article online	Journal Information
Quick Response code 	International Journal of Anatomy and Research ISSN (E) 2321-4287 ISSN (P) 2321-8967 https://www.ijmhr.org/ijar.htm DOI-Prefix: https://dx.doi.org/10.16965/ijar 
	Article Information
	Received: 27 Nov 2019 Peer Review: 28 Nov 2019 Revised: None
	Accepted: 12 Dec 2019 Published (O): 05 Jan 2020 Published (P): 05 Jan 2020
DOI: 10.16965/ijar.2019.355	

INTRODUCTION

Gastrointestinal disorders such as constipation, irritable bowel syndrome, ulcers, hyperacidity, inflammation, hemorrhoids, perianal abscesses, diverticular diseases, colitis and cancer are very common causing enormous human suffering. They are mostly due to an imbalance between damaging factors within the gastrointestinal lumen and protective mechanisms of the

mucosa [1].

Sodium nitrite (NaNO₂) is widely used in the food industry as color fixative and preservative of fish and meat products. It acts as a flavor-enhancer and inhibit rancidity by preventing fat oxidation. It also inhibits the growth of microorganisms, especially Clostridium botulinum. Nitrogenous fertilizers; especially nitrates and nitrites are of great importance and concern to

man and animals because they possess mutagenic, carcinogenic, teratogenic and embryo toxic agents [5]. Accidental or intentional acute exposure to high levels of nitrite has been reported to cause death, mainly due to methemoglobinemia [6].

Chronic exposure to lower doses of nitrite causes adverse health effects, which includes birth defects, respiratory tract complaint and nervous system affection. Prolonged exposure to nitrite can also cause carcinogenicity and mutagenicity [7]. The part of population that is more prone to nitrite toxicity includes anemic and glucose 6-phosphate dehydrogenase deficient individuals, pregnant women and infants [8]. Human exposure to nitrite mainly occurs through the oral route through contaminated drinking water or food, primarily affecting the gastrointestinal tract and small intestine [9]. Complete absorption of nitrite occurred through the small intestine [10].

The ability of the body to resist the toxic effects of any environmental agents is dependent upon the detoxication and antioxidant systems. Vitamins are known to be strong antioxidants. Thus, their administration may increase the function of endogenous free radical scavengers and, so, decreasing the unfavorable effects of nitrates and nitrites on the body cells [11].

Vitamin A is essential for normal growth and differentiation of epithelial tissues. It regulates intestinal epithelial cell proliferation and regeneration. Vitamin A participates in the important metabolic steps that are involved in immune competence and mucosal integrity. It has potential to prevent chemotherapy-induced mucosal lesions. Vitamin A also plays an important role in the regulation of cell division in the small intestine of the rats [12].

Therefore, the aim of the present study was to investigate the effect of sub-lethal dose of sodium nitrite on jejunum mucosa of albino rats. In addition, the role of vitamin A as an antioxidant to prevent the toxic effect of nitrites was taken into consideration.

MATERIALS AND METHODS

Chemicals and drugs:

Sodium Nitrite 98% pure (NaNO_2) M.W. 84.99 was provided by the British Drug Houses LTD.

Vitamin A: Vitamin "A" capsules (A-Viton); were supplied by Kahira Pharm. and Chem. Ind. Co. Cairo-Egypt (each contains 50,000 I.U.). Each capsule was emptied and dissolved in 25 ml of sunflower oil [13], so each 0.5 ml of the prepared solution contained about 10000 IU for each rat was given.

Experimental Animals and groups: The study was conducted at the Human Anatomy and Embryology Department, Faculty of Medicine, Suez Canal University. A total of 30 male healthy Sprague-Dawley rats, 2-3 months of age, weighing 200-250 gm, were used throughout the study. They were obtained from the Animal house of the Faculty of Veterinary Medicine, Suez Canal University. They were housed individually for a 2-weeks of acclimatization period prior to the experiment. Rats were fed ad libitum by standard laboratory pellet and tap water. A 12-hr light, 12-hr dark cycle was maintained. Room temperature was at $23 \pm 2^\circ\text{C}$ with a relative humidity of 45-55%. All experimental procedures and animal maintenance were conducted in accordance with the institutional standards of animal care and approved by the local ethics committee. After two weeks of acclimatization period, animals were divided into 3 experimental groups which were treated as follows:

Group I: (Control) where animals were received distilled water.

Group II: (Nitrite-treated), where rats received a daily oral dose of 1/10 of the LD50 of Sodium Nitrite (10%) which is equal to 500 mg/kg b. wt prepared by dissolving in distilled water and given via intra-gastric intubation for 8 weeks [14].

Group III: (Nitrite-treated plus Vitamin-A), where rats received a daily oral dose of sodium nitrite (500 mg/kg b. wt) plus vitamin - A (10.000 I.U= 0.5 ml for each rat/day) [15] via intra-gastric intubation for 8 weeks.

Animal's weight of each group was reported at the beginning and at the end of experiment as an indicators for effect of nitrites on rat's weight. Animal were scarified after 8 weeks of treatment and blood samples were taken, centrifuged and examined for detection of changes in all hematological indices, albumin and methemoglobin levels. Animals behavior, or

any clinical signs were carefully observed, and any deaths were recorded during the experimental period.

Light Microscopic Study: At the end of each period of the experiment, the jejunum specimens were taken 5 cm far from the end of duodenum and were fixed in aqueous Bouin's fixative, dehydrated in ascending grades of ethyl alcohol, cleared in xylene and then impregnated in paraffin wax. Sections of 5 m in thickness were cut by the microtome for histological study and then stained by the following methods [16];-

1-Hematoxylin and eosin stain (H&E); sections were examined and photographed under light microscope for the studying of histopathological features.

2- Periodic Acid Schiff reagent stain (PAS); sections were examined and photographed under light microscope for detection of presence of neutral mucopolysaccharides.

Transmission Electron Microscopic Study: The jejunal specimens were cut in small pieces of 1 mm² size and fixed in 2.5% glutaraldehyde for 24 h. Specimens were washed in 0.1 M phosphate buffer at 4 c, then post fixed in 1% osmium tetroxide at room temperature. Specimens were dehydrated in ascending grades of ethyl alcohol, then embedded in Epon resin. Semithin sections (1 m) were stained with toluidine blue in borax and examined with light microscope. Ultrathin sections (50 nm) were cut, mounted on copper grids and stained with uranyl acetate and lead citrate [17]. Specimens were examined and photographed with Joel 100SX transmission electron microscope at EM unit Faculty of Medicine Ain Shams University.

Morphometric study: The morphometric measures were performed by the touch count method using a computer assisted image analyzer (soft imaging system –An Olympus Company) at National Research Center-Pathology Department of national research center. The measurements were performed using a ×40 objective lens in five non-overlapping fields in ten randomly chosen sections from six different animals for each group. For each case, at least 15 villi and crypts were assessed. The following parameters were studied [18]:

1-Villus height (um); measured as the distance

from the tip of the villus to the villus- crypt junction.

2-Villus width (um); measured as the distance from the border of the villus to another border of the same villus at its crypt junction.

3- Crypt depth(um); represented by the difference between the total mucosal thickness and the villus height.

4-Villus height per Crypt depth ratio.

5- Villus surface area (mm²); measured as the area within the villous outer boundary and base line.

Statistical analysis: The analysis was done with SPSS19.0. The morphometric data of each animal group were statistically analyzed and the ANOVA test was employed to compare the studied animal groups. Data were expressed as the mean (±) SD. Significance of the data was determined by P values where a P< 0.05 was considered significant.

RESULTS

Weight changes in the studied groups: The present work revealed a statistically significant reduction in the rat's body weight after 8 weeks of the experiment in nitrites treated group (Table-1) in comparison with control group, but no significant reduction in other experiment groups.

Table 1: Mean ± SD of rat's body weight changes among the different groups.

Groups	Weight at beginning of the study	Weight at the end of study
Control	250.34±30.12	253.24±41.17
Nitrite	233.25±25.46	140.42±20.67* ^a
Nitrite + vit-A	240.58±20.74	220.27±15.72

* P < 0.001 in comparing to control, ^a P < 0.005 in comparing to nitrite & vit-A group

Hematological results: There were statistically significant changes in all hematological indices and methemoglobin level in the nitrite treated group (group II) in comparison with other study group (Table-2). More reduction was noticed in the hemoglobin level in this group (P < 0.001). A significant increase in the number of both basophiles and esinophiles were noticed. Albumin level significantly decreased in nitrite group in comparing to other groups. Methemoglobin level was significantly increased in

nitrite group but its level was significantly decrease in group treated by vitamin -A with NaNO₂.

Table 2: Means ± SD deviations of hematological variables including methemoglobin level & albumin level in different study groups.

CBC indices	Control	Nitrite group	Nitrite & vit-A group
RBCs × 10 ⁹ /L	5.1±1.03	2.8±1.2*	4.4±2.07
Hemoglobin(g/dL)	13.55±1.09	7.44±2.1* ^a	10.2±1.03
Hematocrit (%)	38.8±1.8	30.7±1.78	34.2±.9
Mean corpuscular volume (MCV)	82.1±6.09	66.8±16.09*	76.8±12.3
Mean Corpuscular Hemoglobin Concentration (MCHC)	34.9±9.9	22.3±5.6*	30.7±5.9
Platelet count (10 ⁹ /L)	310.4±34.3	266.6±39.3*	280.4±77.2
White Blood Cells			
Neutrophils (%)	53.74±1.19	35.3±18.4	48.5±7.2
Lymphocytes (%)	37.2±8.5	23.9±7.9*	33.23±4.6
Basophiles (%)	1.33±0.6	13.4±13.2* ^a	7.77±2.4
Esinophiles (%)	3.4±0.9	22.3±15.7* ^a	5.65±1.6
Monocytes (%)	7.4±3.2	6.2±2.6	8.7±1.5
Methemoglobin level (%)	1.33 % ±0.3	22.21±6.04%* ^a	7.24±4.04%
Albumin level	4.03±0.6	3.04±0.09*	3.08±1.02

* P < 0.005 in comparing the nitrite group with control,

^a P < 0.005 in comparing the nitrite group with nitrite & vit-A group

^c P < 0.005 in comparing the nitrite & vit-A group with control

Light Microscopic results: Light microscopic examination of the H&E stained sections of the rat jejunum of the control group revealed that; the wall of the jejunum was formed of mucosa, submucosa, muscularis externa and serosa (Fig. 1-A). The mucosa consist of villi, crypts, submucosa and muscularis mucosa. The intestinal villi; which was tongue like projection, tall, cylindrical and protruding into the lumen. The villi were covered by columnar absorbing epithelial cells (enterocytes) with basally located

nuclei. The goblet cells were oval in shape, mucins secreting and interspersed between the enterocytes. The villi have a connective tissue cores of the lamina propria consisting of mononuclear cells, smooth muscle fibers, central lacteals and blood capillaries (Fig. 1-B). The lamina propria surrounded the crypts and extended upward to form the core of the villi. The deep cavities; the crypts of Lieberkühn were found between the villi (Fig. 1-C).

Fig. 1: Photomicrograph of sections of the jejunal mucosa of control rats. **A.** The wall of jejunum consists of mucosa, submucosa (SM), muscularis externa (ME) and serosa (S). The mucosa forms fingerlike projections villi (V) which project into the lumen of jejunum (L). The villus comprises columnar epithelial cells (EP). **B.** The villi are lined by columnar epithelial cells (EP), a connective tissue core of lamina propria (CO) containing mononuclear cells (arrow), smooth muscle fiber (elbow arrow) and blood vessels (arrow head). The goblet cells (g) are located between the columnar absorbing cells. **C.** The crypts of Lieberkühn (C) are located between the villi. The crypts are lined with columnar absorbing cells (detached arrow) and goblet cells (g). SM: Submucosa, ME. Muscularis Externa (H&E A, X10, B&CX40).

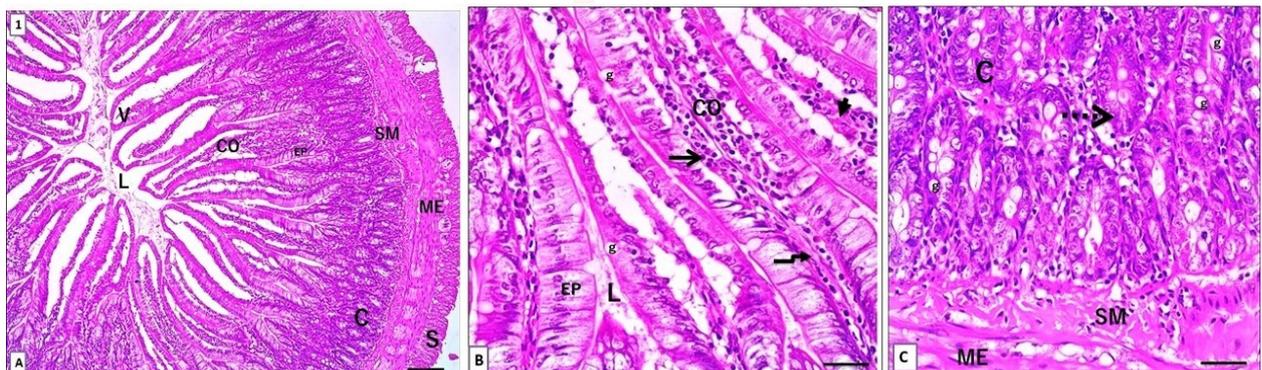
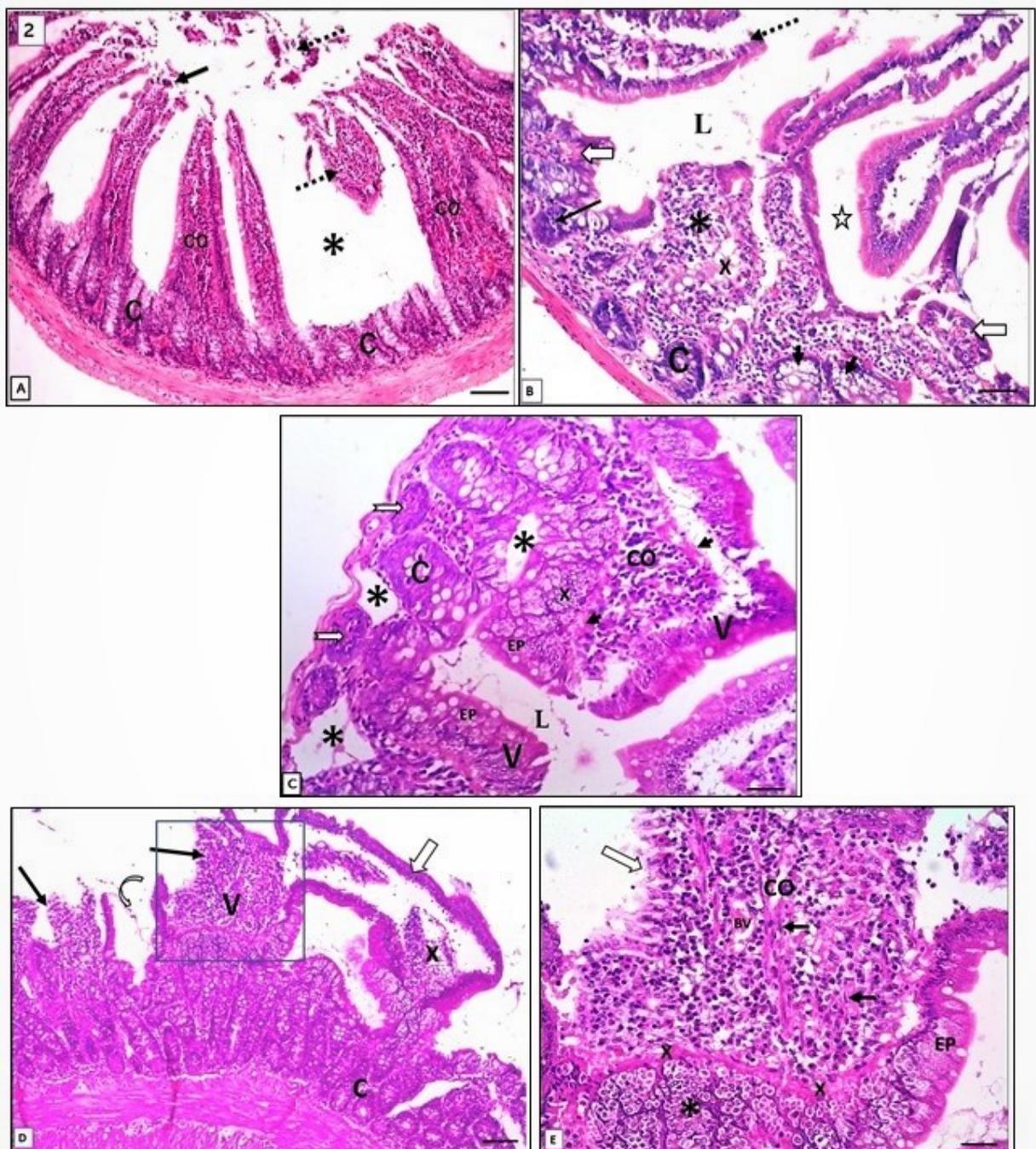


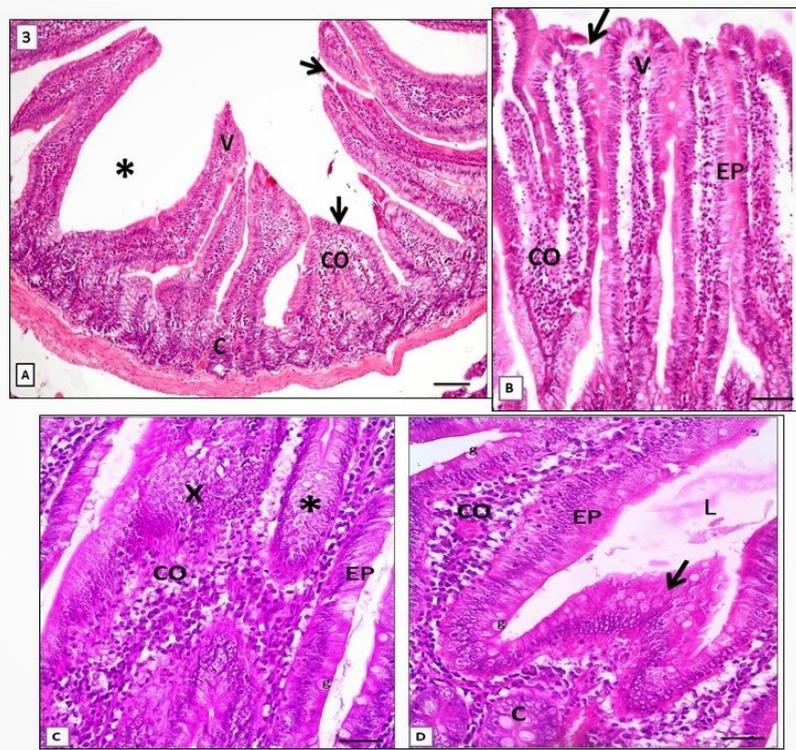
Fig. 2: Photomicrograph of sections of jejunal mucosa of nitrite treated rats showed; **A.** Area of complete loss of villi (*) with loss of epithelium into the lumen (detached arrows). Notice losing of surface epithelium (arrow). The core of lamina propria (CO) showed increased cellularity. The crypts (C) were disrupted. **B.** Abnormally shaped villi projecting into the lumen (L) with losing of surface epithelium (detached arrow), inverted blunt shaped villi which surrounded by large space (star). The villi showed loss of surface epithelium with excess inflammatory cells (*) and edema in its core (X). The crypts are distorted and atrophied (black arrow), some are lined by abnormally enlarged & vacuolated cells (arrows heads). Excess Paneth cells inside the crypts (wide white arrows). **C.** The jejunal mucosa showed abnormally short, wide and blunted villi (V). Its surface epithelial cells (EP) are enlarged and have pale cytoplasm with excessive goblet cells. The core of villi (CO) showed hemorrhage (arrow heads) with accumulation of groups of cells having vacuolated cytoplasm (X). There are abnormal spaces between villi and crypts (*). The crypts (C) are short, distorted and lined by abnormal cells (white arrows). **D.** The jejunal mucosa showing sever damage of the villi (V) with excessive losing of surface epithelium (black arrows) with disruption of space between villi (curved arrow). Abnormal shaped villi (white arrow). There are area of collection of sloughed cells (X). The crypts (C) showed abnormal shapes. Figure E is magnification of the square in fig D; cut section of a villi showed damage of surface columnar epithelium (white arrow) with exposure of core of lamina propria (CO) which showed increased inflammatory cells, excess myofibroblast cells (black arrows), dilated and congested blood vessels (BV). The epithelial cells (EP) showed abnormal based line (X) and collections of groups of enlarged cells with pale vacuolated cytoplasm and pale nuclei (*).



The jejunal sections of the nitrite treated rats (Group II) showed a great pathological changes of the villi of jejunal mucosa. There were areas of complete loss of villi. When present; they appeared in abnormally shapes, width and height. Some appeared as inverted blunt shaped and surrounded by large space. Most of the villi showed loss of its surface epithelium and sloughing into the lumen leading to exposure of the core of lamina propria which showed increased inflammatory cells, excess myofibroblast cells, dilated and congested blood vessels. In another villi the surface epithelial cells were enlarged and have pale cytoplasm with excessive goblet cells. The core of lamina propria of most of the villi showed increased cellularity, edema, hemorrhage with accumulation of groups of large cells with vacuolated cytoplasm. The core of some villi showed abnormal tissue band with collections of groups of enlarged cells with pale vacuolated cytoplasm and pale nuclei beneath it. There were

wide abnormal spaces between villi and crypts. The crypts of jejunal mucosa were short, distorted and lined by abnormally enlarged cells & vacuolated cytoplasm. Distorted and atrophied crypts were seen. Excess Paneth cells were appeared inside the crypts (Fig. 2 A, B, C, D & E). Concerning the nitrite plus vitamin -A treated rats (group III); the histological examination of H&E stained sections of jejunal mucosa showed a relatively picture that mimic the control group. Most of the villi became intact and completely covered with columnar epithelium as normal. Some villi showed foci of epithelial sloughing but had normal core of connective tissues, but others showed foci of epithelial hyperplasia projecting towards the lumen. Fused villi were seen with wide intervillous spaces. Crypts appeared with normal epithelial lining and normal goblet cells in between. Some sections showed increase cellularity in connective tissues core with aggregation of group of large vacuolated cells (Fig. 3 A, B, C & D).

Fig. 3: Photomicrograph of sections of the jejunum of nitrite plus vitamin - A treated group. **A;** Most of the villi (V) are intact and completely covered with epithelium as normal with normal core of connective tissues (CO). Some villi are fused (arrow). Notice wide intervillous space (*). Normal Crypts (C) with normal epithelial lining. **B:** Cut section showing intact villi (V) which completely covered with normal epithelium cells (EP) with normal core of connective tissues (CO) with foci of epithelial separation (arrow). **C.** The villi are lined by normal epithelial cells (EP) and goblet cells in between (g). The core (CO) showed increase cellularity (X) and aggregation of groups of large vacuolated cells (*). **D:** Normal villi with normal epithelial lining and goblet cells (g) in between, Normal core of connective tissues (CO). Foci of epithelial hyperplasia (arrow) which get toward the lumen (L). There are normal crypts (C). (H & E; A & B X20. C & D X 40)



PAS - stained sections of jejunal mucosa of control rats showed strong positive reaction in the brush border of the columnar absorptive cells and numerous goblet cells which appeared magenta red (Fig. 4. A&B).

PAS staining of sections of control group showed strong positive reactions in both brush border & goblet cells of all villi and inside crypts (Fig.4A&B).

Concerning the nitrite treated rats; the PAS stain sections of jejunal mucosa showed interrupted brush border of surface epithelium and exhibited weak PAS reaction or even lost in some areas. Marked loss in goblet cell between the enterocytes of the villi and crypts was noticed. Most of the goblet cells contained few mucous granules and others were completely depleted and appeared negative PAS stain (Fig. 5. A & B).

Concerning examination of **PAS** stained sections of nitrite plus vitamin –A group showed a moderate to strong PAS positive reaction which was observed in the well-defined brush border of villi. The goblet cells of crypts and villi also were strongly positive and distended with mucous granules. Some crypt appeared slightly shrunken with little secretion. Vacuolation appeared between villi (Fig. 6. A, B &C).

Electron Microscopic results: Ultrathin sections of jejunal mucosa of control group showed the columnar absorbing cells covering the villi and crypts which were regularly arranged and possesses closely backed microvilli on their luminal surface with basal oval euchromatic nuclei. Their cytoplasm was rich in elongated mitochondria, rER cisternae and contain excess lysosomes. The goblet cells had few microvilli and were formed of an apical part distended with mucous globules of variable electron density. Some mucous globules had electron dense cores. Goblet cells were readily distinguishable from other types of epithelial cell by their many mucous granules, which often fill the cytoplasm

between the basally located nucleus and the apical membrane. All cell types were morphologically distinguishable (Fig. 7. A &B).

Ultrathin sections of jejunal mucosa of nitrite treated group showing; the columnar absorbing cells covering the villi (the enterocytes) have abnormal shape with variant shapes euchromatic nuclei which were loosely attached and abnormally arranged with losing of their microvilli. The goblet cells situated between the enterocytes showed aged nuclei with thick and distended granular secretion with vacuolated cytoplasm (Fig 8, A&B). Some villous enterocytes showed downwards displacement of their small nuclei into the base of core of villi or situated abnormally to the side of the villi. Some showed foci of losing of brush border with erosion of in the underlying core. The eroded area contain vacuoles, hemorrhage, swollen lysosomes, plasma cell and an apoptotic cell with a pyknotic elongated nucleus (Fig 8, C&D). Some enterocyte showed large vacuoles and surrounded by excess inflammatory cells especially eosinophiles. Another enterocytes appeared shrunken with aged nuclei and with irregular cytoplasm filled with excess inflammatory cells (Fig 8, E&F).

The ultra-thin sections of jejunum mucosa of nitrite plus vitamin-A treated group showed; the villus epithelium (enterocytes) appeared completely as normal group with little inflammatory infiltrates and plasma cells in their core. The enterocytes had intact brush microvilli with basal rounded euchromatic nuclei. The goblet cells were distended with mucous globules (Fig. 9, A, B, C &D).

Fig. 4: Photomicrographs of rat jejunum of control group stained with PAS Stain showing; **A.** Strong PAS positive stain in brush border (elbow arrows) of all villi (V) and goblet cells (black arrows) of villi and inside crypts (C). (PAS Mic. Mag. × 200). **B;** Strong PAS positive stain in brush border (elbow arrows) and goblet cells (black arrows) of villi (V) (PAS Mic. Mag. × 400).

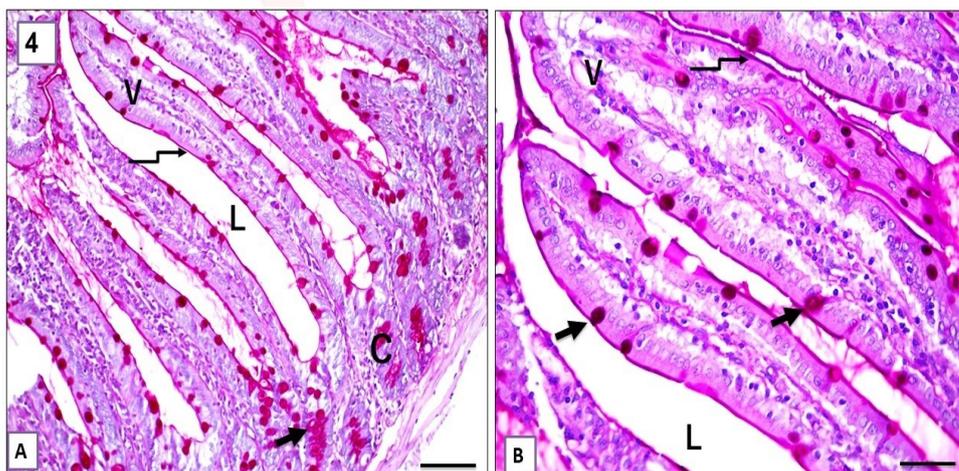


Fig. 5: Photomicrographs of rat jejunum of nitrite treated group stained with PAS Stain showing: **A:** Great loss of epithelial cells inside lumen (*). Great loss of villi (X) with abnormally shaped villi (V). Core of villi (CO). The most of villi (V) are negatively stained with PAS brush border (arrow head) or weak stain. The goblet cells of villi and inside crypts (White arrow) showed weak stain (PAS Mic. Mag. × 200). **B:** Cut section of the villi showed foci of complete loss of brush borders of epithelium (white arrows) with negative PAS stain. The core of villi shows excess vacuolation (*) and areas of hemorrhage (crossed arrows) (PAS Mic. Mag. × 400).

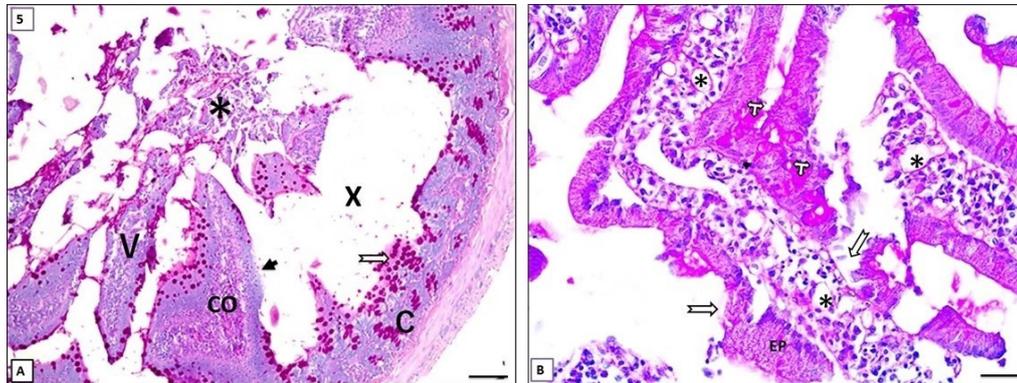


Fig. 6: Photomicrographs of rat jejunum of nitrite treated group stained with PAS Stain showing: **A:** Moderate to strong PAS positive reaction observed in the well-defined brush border of villi (V) (zigzag arrows). The goblet cells of crypts (arrows) are strongly positive and distended with mucous granules. Some villi are fused (*). (PAS x10). **B:** Strong PAS stain (zigzag arrow) of brush border of villi (V) to moderates PAS stain (white arrow). The goblet cells of crypts (C) are distended with secretions (arrow head), but some crypt slightly shrunken with little secretion (X). Wide area of valuation (*). (PAS x20). **C:** A strong PAS positive reaction is observed in the well-defined brush border of villi (V) (zigzag arrows). There are areas of loss of surface epithelium (white arrows). The core shows hypercellularity (X) (PAS X 40).

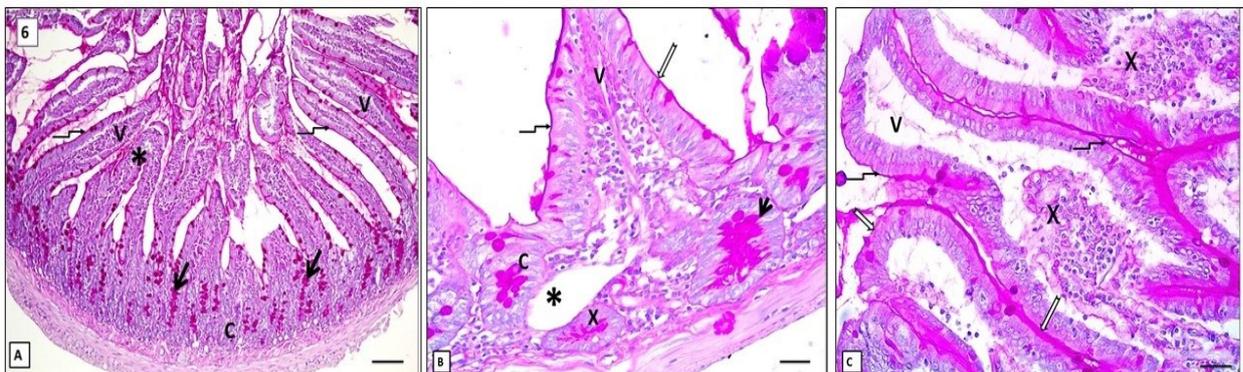


Fig. 7: An electron micrograph of the control rat jejunum showing: **A:** the columnar absorbing cells (CC) of the villi having microvilli (thick arrow) on their luminal surface with basal oval nuclei (N). Their cytoplasm are rich in mitochondria (M) and rER. The Goblet Cell (G) containing large rounded mucous globules (elbow arrow) and bearing few microvilli (arrow head). It is located between the columnar cells and opens into the lumen of jejunum (X1500). **B:** A large columnar absorbing cells (CC) with basal oval nuclei (N) with rER and excess lysosomes (L) in its cytoplasm (X3000).

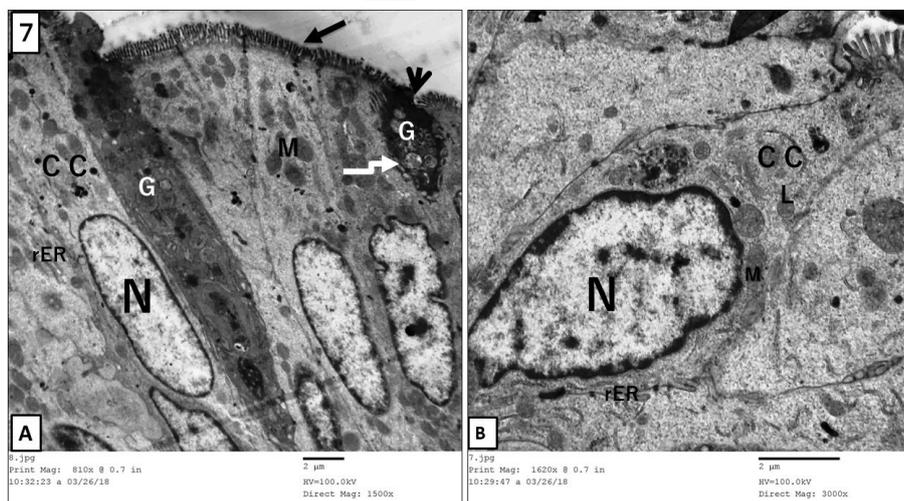


Fig. 8: An electron micrograph of the rat's jejunum treated with nitrite showing. **A:** The enterocytes showed loosely attached and not arranged microvilli with loss of most of them (arrow head). The enterocytes have abnormal shape with long and thin nucleus (N) or small and rounded nuclei (n). The goblet cells situated between the enterocytes; one shows aged nuclei with thick and dark granular secretion (white arrow) but another distended with mucinous secretion (long arrow) (X1500). **B:** An irregular enterocytes with vacuolation of their cytoplasm (*). The goblet cell (G) has basal aged with thick wall nucleus. Its secretions are thick and show dark and spherical granules (X). There are losing of microvilli (white arrow) (X3000). **C:** The enterocytes (EP) showing displacing of their large nuclei downward into base of core (CO) of villi (white arrow), small nuclei (white arrow) or situated abnormally to the side of the villi (n). Foci of losing of brush borders (arrow head) (X1000). **D:** A wide area of losing the brush border (thick white arrow) with erosion of in the underlying core. This eroded area contain vacuoles (*) hemorrhage (X), swollen lysosomes (crossed arrow), plasma cell (arrow head) and an apoptotic cell with a pyknotic elongated nucleus (thin arrow) (X750). **E:** Enterocytes of the villi with slightly irregular, dark thick & dense euchromatic nuclei (thick white arrow) surrounded by large homogenous cytoplasm from one side and excess dark granules (black arrow) from one side. One enterocyte shows large vacuoles (*) which surrounded by excess inflammatory cells; Eosinophiles (X) (X1500). **F:** Abnormal enterocyte (thick black arrow) with rounded aged nuclei (N) with irregular cytoplasm filled with excess inflammatory cells (long arrow). Another enterocyte is surrounded by excess Eosinophiles (X). An enterocyte was degenerated with shrunken cytoplasm (thick white arrow) (X1500).

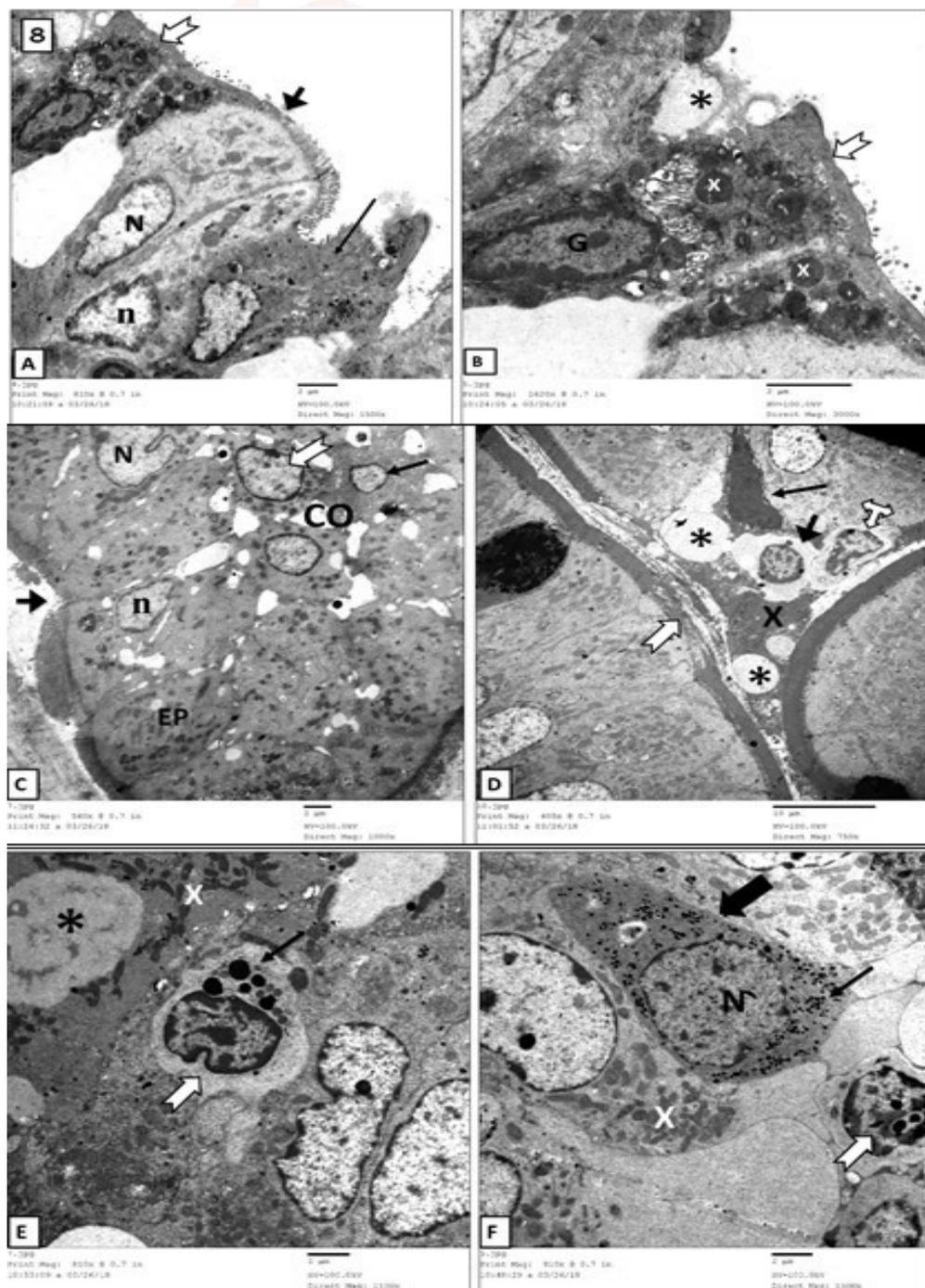


Fig. 9: An electron micrograph of the villus epithelium of the jejunum of nitrite plus vitamin A treated group. **A:** The villus enterocytes (EP) appeared completely as control with intact brush microvilli (arrow) and having normal elongated nuclei (N). The goblet cells (G) are distended with mucous globules (X1500). **B:** The absorbing cells (EP) have intact brush border (arrow), basal rounded nuclei (N) with some inflammatory infiltrates (X) in their core (X1000). **C:** The absorbing cells (EP) have intact brush microvilli (arrow) and having normal euchromatic nuclei (N). The goblet cells (G) are distended with mucous globules (X1000). **D:** The absorbing cells (EP) have basal rounded nuclei (N) and normal goblet cells in between (G). **D:** Enterocytes (EP) have basal rounded nuclei (N) and normal goblet cells in between (G) but showing excess inflammatory infiltrates (X) and large plasma cell (white arrow) in their core (X750).

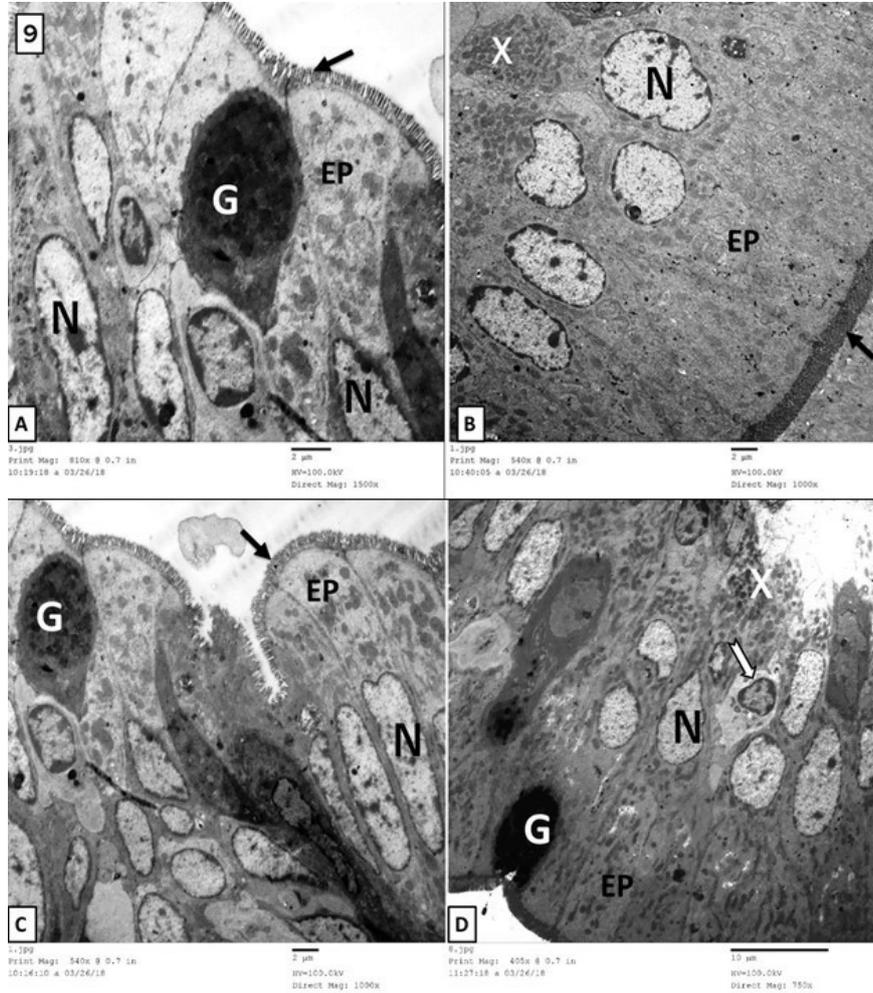


Table 3: Means \pm SD of the morphometric parameters of jejunal mucosa among the different groups.

* $P < 0.005$ in comparing the nitrite group with control.

^a $P < 0.005$ in comparing the nitrite group with nitrite & vit-A group.

^c $P < 0.005$ in comparing the nitrite & vit-A group with control.

Parameters	Control group	Nitrite group	Nitrite & vit -A group
Villus height (um)	657.54 \pm 55.43	297.52 \pm 45.22* ^a	554.54 \pm 35.33
Villus width (um)	129.32 \pm 33.4	79.22 \pm 13.4* ^a	97.32 \pm 23.6
Crypt depth (um)	194.65 \pm 22.5	77.35 \pm 12.5* ^a	163.75 \pm 27.3
Villus height: Crypt depth	5.21 \pm 0.67	2.1 \pm 0.37*	3.91 \pm 0.29
Villus surface area mm ²	0.094 \pm 0.012	0.034 \pm 0.03* ^a	0.084 \pm 0.012

Morphometric results: All morphometric parameters; the villus height, villus width, crypt depth, the ratio between villus height and crypt depth and villus surface area showed statistically significant reduction in nitrite treated group in comparison to control and nitrite plus vitamin-A treated groups. These parameters

were significantly improved when comparing with control group values after co-administration of vitamin-A with NaNO₂ (all data were presented in table 3).

DISCUSSION

Industrial progress in the manufacture and preservation of food leads to increasing the

human exposure to many harmful pollutants and toxicants. NaNO_2 is among one of these pernicious contaminants which affects human and animal health. Abundant use of nitrogenous fertilizers to increase crop yield and improper waste disposal has mainly contributed to the nitrite load of the environment. Overexposure to nitrite has been reported to cause various health problems including cancer with contaminated water being the main source [19].

In the present work, the rats treated with sodium nitrite showed significant decrease in their body weight and less weight gain than that of control group throughout the experiment periods. This reduction is due to direct toxic effect of sodium nitrite on gastrointestinal mucosa causing abdominal cramps, diarrhea and anemia or may due to inflammation of jejunal wall which leads to disruption in digestion and absorption causing nutritional deficiency and so weight loss [20]. There were no significant difference in the rat's body weight in the group treated with NaNO_2 and vitamin A in this study in comparing to control group. This correlated to effect of vitamin A which is essential for normal growth and differentiation of epithelial tissues. It regulates intestinal epithelial cell proliferation and regeneration [15]. Also vitamin A helps the maximal absorption of nutrients, resulting weight gain [21-24].

Regarding the hematological results of this work, the data showed a highly significant decrease in RBCs count, Hb concentration, Hct ratio and platelets count in group treated with sodium nitrite. This decrease may be attributed to microcytic and /or hypochromic anemia possibly as a consequence of the toxic effect of sodium nitrite on bone marrow, spleen and liver [25-27].

Counting of white blood cells results of this study showed significant decrease in both neutrophils & lymphocytes levels which were due to cytotoxic effect of nitrite on hematopoietic tissues and disruption of immune cells which play an important role in attacking and interacting with foreign antigens [28-29]. Also; data of this work showed significant increase in Basophiles and Eosinophiles percent in group treated by sodium nitrite due to release of nitric oxide which is a potent toxic mediator in chronic inflammation

either microbial or parasitic [30]. The higher level of Methemoglobin concentration in nitrite treated group of this work caused by oxidation effect of nitrite on the iron component of red blood cells (hemoglobin) that converts the ferrous ion of Hb to ferric ion rendering them unable to carry oxygen. The resulting condition is called methemoglobinemia (Met Hb) [31]. Unlike Hb, MetHb cannot exchange oxygen; hence, the presence of excess MetHb in the circulation proportionately reduces the ability of the blood to transfer oxygen [32-33].

The present results indicate a highly significant decrease in serum albumin levels throughout the experiment period in sodium nitrite treated group. Many explanations for this reduction of albumin level which may due to effect of nitric oxide (NO); the product of nitrite toxicity that reacts rapidly with superoxide to form highly reactive peroxynitrite (ONOO^-) such products may increase lipid peroxidation (LPO) which can be harmful to liver decreasing liver functions as so protein synthesis [10, 34-35].

Based on previous reports; the decrease in serum albumin level in their studies was due to trap of protein from the alimentary tract or due to hepatic necrosis as a result of sodium nitrite administration to male albino rats [36-37].

All hematological parameters were greatly improved in group that treated by NaNO_2 and vitamin-A administration but still lower than control group. These results were due to effect of vitamin A as previously reported [38] as vitamin A is required for normal development of precursor cells (stem cells) into red blood cells. Additionally, vitamin A appears to facilitate the mobilization of iron from storage sites to the developing red blood cell for incorporation into hemoglobin; the oxygen carrier in red blood cells. Vitamin A supplementation has beneficial effects on iron deficiency (anemia), and improves the nutritional status of iron as it facilitate the mobilization of iron stores to developing red blood cells, where it is incorporated into the oxygen carrier hemoglobin [39-40]. The histopathological data of this work showed that the rats that were treated by sodium nitrite had a great morphological changes in jejunal mucosa. H & E stain showed

a lot of pathological changes in the jejunal villi as abnormal shape, height, disruption or shortening or complete loss. The covering epithelium of villi or enterocytes showed marked loss and sloughing into the lumen. Many explanations for these histopathological changes in jejunal mucosa in this study were previously reported; that the nitric oxide formed by NaNO₂ has been implicated in the pathogenesis of many inflammatory diseases of intestinal wall. Evidence that NO is a potent toxic mediator in chronic inflammation as the nitrite-generated ROS can cause oxidative modification of lipids and proteins which in turn will disrupt membrane integrity [41]. This might cause loss of enzyme molecules from the epithelial cell lining. The ROS of nitrite toxicity can directly oxidize enzyme molecules and decrease their activity mediating inflammatory reaction in epithelial cells [42-43].

The core of lamina propria showed hypercellularity, congestion of its blood vessels, edema and hemorrhage. This caused by effect of NaNO₂ which produces vasodilation by relaxing vascular smooth muscle and so edema and hemorrhage [44]. Same results were reported after founding higher protein oxidation in jejunal mucosa of NaNO₂-treated animals [45]. The jejunal crypts appeared fewer, disrupted, and shorter and were mostly lined by enlarged cells with pale vacuolated cytoplasm and pale disintegrated nuclei. Few goblet and Paneth cells were seen in the crypts. Researchers explain these findings as Reactive oxygen species (ROS), generated by intracellular redox reactions, together with the formation of harmful compounds such as nitryl chloride, results in cytotoxicity and tissue damage [46-47].

PAS is a staining method used to detect polysaccharides such as glycogen, and muco-substances such as glycoproteins, glycolipids and mucins in tissues. By PAS stained; results of this study showed the brush border of villi was interrupted and exhibited weak or negative PAS reaction. Marked reduction in goblet cell over the villi and crypts was noticed. Most of the goblets cells contained few mucous granules or were completely depleted and appeared negative PAS stain. This weak or negative stain was explained due to the toxic effect of ROS free radicals of NaNO₂ in damaging the goblet cells and

prevent its polysaccharides formation which give positive PAS reaction. Also; ROS free radicals affect the biosynthesis of glycoprotein layer covering the microvillar membranes that give PAS positive reaction. This glycoprotein layer is being highly sensitive to nutritional variations which were greatly decreased in NaNO₂ intake [48-49,36].

Multiple ultrastructural changes in the enterocytes of our results were seen in jejunal mucosa of rats treated by nitrite treated group as abnormal shape of cells with variant shapes euchromatic nuclei. Large vacuoles which surrounded by excess inflammatory cells as eosinophiles were noted in their cytoplasm. shrunken enterocytes with aged nuclei and with irregular cytoplasm also seen. Foci of erosion appeared containing vacuoles, hemorrhage, swollen lysosomes, plasma cell and an apoptotic cell with a pyknotic elongated nucleus. The goblet cells situated between the enterocytes showed aged nuclei with thick and distended granular secretion with vacuolated cytoplasm. It has been previously explained that these ultrastructural changes are due to that nitrite reacts with amines to produce nitrosamines and with amides to produce nitrosamides [50]. Nitrosamines and nitrosomides constitute the N-nitroso compounds (NNC) and the reaction with nitrite is called nitrosation, nitrosamines readily induced DNA interruption. Thus, NO and nitrite can interact directly with mitochondria to affect oxygen consumption, substrate oxidation, and generation of reactive oxygen species interrupting cell wall integrity and cytoplasm components [51]. The Reactive nitrogen species produced by exposure to nitrite is considered one of the most important causes of carcinogenesis through its reaction with body tissues and triggering lipid peroxidation, DNA lesions, enzyme inactivation and damage of different tissues organs [52-55].

The histological data of jejunal mucosa in this work were greatly different and to some extent resembles the normal picture when vitamin A coadministered with NaNO₂ (group III), that most of the villi became intact and completely covered with columnar epithelium as control with small area of epithelial separation but had normal core of connective tissues. Fused villi

with wide intervillous space were noticed. There was epithelial hyperplasia in some sections. Crypts appeared with normal epithelial lining and normal goblet cells. This improvement were as due to effect of vitamin A which has an essential role in cellular differentiation and proliferation, the latter being particularly important in growth, reproduction and immune response, and in maintaining epithelial integrity [56].

The critical function vitamin A plays an important role in regulating cellular differentiation provides a unique 'core' mechanism that would at least partly explain its influence on epithelial barriers, immune competence, healing, resistance and recovery [57-58].

The importance of vitamin A in differentiation process and promotion of nutritional state of goblet cells rendering them having moderate to strong PAS positive reaction as noted in well-defined brush border of villi and goblet cells of crypts of jejunal mucosa of rats treated with vitamin A with NaNO₂ of this study [59 - 60]. Ultra-thin sections of jejunum mucosa of nitrite plus vitamin A treated group showed great improvement when comparing with group treated by NaNO₂ only thus; the villus epithelium of the jejunum appeared completely as control group with little inflammatory infiltrates and plasma cells in their core. The enterocytes had intact brush microvilli with basal rounded euchromatic nuclei. The goblet cells were distended with mucous globules which indicate their good functions. This attributed to protective effect of vitamin A on the small intestine which may be explained by stimulation of DNA and RNA biosynthesis. Moreover, it was reported that vitamin A is one of antioxidants that may either block the formation of free radicals or scavenger them once they had formed [13]. Also; the protective effect of vitamin A could be also attributed to its role in regulation of differentiation of epithelial cells and goblet cells and maintenance of integrity of mucous membranes [61-63]. Regarding the morphometric parameters of this work, the villus height, villus width, crypt depth, the ratio between villus height and crypt depth and villus surface area showed significant reduction in nitrite group in comparison to another groups. This because the jejunal

mucosa is often greatly affected by malabsorption, autoimmune and inflammatory pathological processes induced by sodium nitrite [64]. So; this reduction noted were in response to jejunal destruction, inflammation and pathological changes caused by direct toxic effect of nitrite on the jejunal mucosa or generation of reactive oxygen species (ROS) which disrupt proliferation and normal function of mucosal cells [43]. Otherwise these morphometric parameters were greatly changed to resembles the control group values when vitamin-A was coadministered with NaNO₂. This could be attributed to proliferative, differentiation and anti-inflammatory effects of vitamin-A [65].

CONCLUSION

The results of the current study showed that, the sodium nitrite has a toxic effect on jejunal mucosa as proved by histopathological & ultrastructural changes of jejunal mucosa and most of these changes were reversible with concomitant administration of vitamin A. So; it is recommended that; the use of sodium nitrite should be limited and vitamin A must used as antioxidant to prevent the toxic effect of many agents present in daily food as sodium nitrite due to its anti-inflammatory and antioxidant properties.

Conflicts of Interests: None

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How to cite this article: Shimaa Anter Fareed, Eman Mohamed Kamel, Nassar Ayoub. THE EFFECTS OF DIETARY SODIUM NITRITE SUPPLEMENTATION ON JEJUNAL MUCOSA AND POSSIBLE PROTECTIVE EFFECT OF VITAMIN A IN ALBINO RATS. *Int J Anat Res* 2020;8(1.1):7256-7270. DOI: 10.16965/ijar.2019.355