American-Eurasian J. Agric. & Environ. Sci., 15 (8): 1679-1692, 2015
ISSN 1818-6769

DOI: 10.5829/idosi.aejaes.2015.15.8.95321

Ultrastructural Study of the Effect of Moringa Oleifera Lam on the Small Intestine of Adult Rats Treated by Different Doses of Diclofenac Sodium (Voltaren)

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Abstract: Forty five rats were divided into the following groups (15 each): Group I was served as a control group, Group II was subgrouped to IIa, b and c, that were administered oral 50, 100 and 150 mg/kg of Diclofenac Sodium (DS) respectively for 2 days after fasting for 20 hours. Group III was subgrouped to IIIa, b and c. These rats were maintained on oral Moringa Oleifera (MO) (500mg/kg) daily for one week and then they were administered the same doses as in the previous group. Transmission electron microscopy (TEM) showed several alterations in the villous absorptive cell epithelial cells. These changes were mainly separation between two adjacent cells, degeneration and mitochondrial damage. Moreover, plasma cells and eosinophils were observed in the lamina properia. Administration of MO resulted in organization of microvilli, increase in goblet cell numbers with extruding their content into the lumen, abundant mitochondria in the cytoplasm of absorptive cells. It is also noticed that the inflammatory cells appeared tightly contact with the lamina properia. Morphometric analysis showed significant increase in the numbers of goblet cells especially in the groups received voltaren and MO. In conclusion the current study showed that MO leaves might have a partial protective effect on the rat duodenal mucosal histological changes resulted from the administration of high doses of diclofenac sodium in rat.

Key words: Ultrastructure · Moringa Oleifera Lam · Voltaren · SEM · TEM

INTRODUCTION

Moringa oleifera lam (MO) (horse radish tree) (Moringaceae) is a small sized tree, Which is native to south Asia and also grows in tropical Africa [1]. Also, can be cultivated in Egypt. Various parts of MO were generally known for their multiple pharmacological effects including their anti-inflammatory effects [2]. MO is a rich source of antioxidant [3]. It has been reported that aqueous extracts of leaf, fruit and seed of MO act as an antioxidant [4]. The extract of MO had been found to have potent antioxidant action in vivo [5] and in vitro studies [6]. Accumulating evidence supported the protective effects of phenolic antioxidants from medicinal plants against oxidative stress-mediated disorders [7]. The antiulcer properties of *Moringa oleifera* extract and demonstrates that the extract preserves the histological integrity and dimensions of the various layers of pylorus and duodenum of wistar rats, thereby preventing a decrease gastrointestinal surface area following ethanol induced injury [8]. Pharmaceutical studies revealed that the methanol extract of the root of *Moringa oleifera* was reported to possess anti- inflammatory effect in rats [2]. Estrogenic and antiprogestational activities, -significant antispasmodic activity as inhibition of acetylcholine-induced contraction [2]. Hepatoprotective effect on liver damage and inhibition of the growth of *Pseudomonas aeruginosa* and *Staphylococcus aureus* -in vitro [9-11]. Diclofenac drug is affecting the permeability of the cell membranous system result in release of the mitochondrial protein which lead to the split of the cellular implications to enable its therapeutic effect, where they found that DS caused split of DNA of the mucous cells lining the intestines [12]. *Moringa leifera* removes oxygen free

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radicals in epithelial cells. It has been reported to promote healing and prevent relapse of intestinal ulcers, which is attributed to inflammatory cell migration into the intestinal mucosa [9-11].

The objective of this study is light microscope examination to evaluate the possible protective role of Moringa oleifera lam on adult rat duodenal mucosa then following administration of different high doses of Voltaren, (Diclofan sodium) by electron microscope (TEM and SEM) and assess the possible role goblet cells using morphometric indice.

MATERIALS AND METHODS

Chemicals: Moringa Oleifera (MO) leaves were purchased from Herbs & Seeds Co. Paranaque, Metro Manila, Philippines by mail. The dried leaves of the plant *Moringa oleifera* was grounded into fine powder and stored in a glass container at 4°C. The powdered sample was extracted with distilled water (DW) using reflux method. The crude aqueous extract was concentrated *in-vacuo,* properly labeled and stored in the refrigerator at 4°C [13].

Diclofenac Sodium (Voltaren Retard @)-Novartis) (100mg): The tablet packs were purchased from a local pharmacy (Jeddah). The purchased DS tablets were grounded into fine powder and stored in a glass container at 4°C. The required doses were calculated according to the weight of each animal. The powder was dissolved in a solution of combined physiological saline and 1% carboxymethylcellulose (CMC). The used doses in the current study were selected according to a pilot study on different doses of DS to select the highest minimal non lethal dose.

Experimental Animals: Forty five albino Wistar adult male rats weighing 200 to 250 g were purchased from animal house of KFRC (King Fahd research center) under the rules of Canadian ethical approval from the Local Biomedical ethical committee of King Abdulaziz University. The rats were housed in large cages environmentally controlled (25°, 12-h light /12-h dark cycles). Commercial food and tap water were supplied ad libitum. They were sacrificed under light ether anesthesia with neck dislocation.

They were divided into control group (n=15) and were maintained on the dissolving vehicle (physiologic saline + CMC). The second group (n=15 animals) were maintained on vehicle (physiologic saline + CMC) once daily for 1 week then were fasted for 24 h. Then, they were subdivided into 3 subgroups (5 animals each) namely are: Subgroup IIa, where animals administered DS (50 mg/kg) for 2 days then were sacrificed after 3h from the second dose. SubgroupII b, where animals were administered DS (100 mg/kg) for 2 days then were sacrificed after 3h from the second dose. Subgroup II c, where animals were administered DS (150 mg/kg) for 2 days then were sacrificed after 3h from the second dose.

The third group (n=15) were administered MO orally in a dose of (500 mg/kg) once daily for 7 days. The animals of this group were subgrouped to the followings (5 animals each) namely are, Subgroup IIIa, where animals were administered DS (50 mg/kg) for 2 days then were sacrificed after 3h from the second dose. Subgroup IIIb, where animals were administered DS (100 mg/kg) for 2 days then were sacrificed after 3h from the second dose. Subgroup IIIc, where animals were administered 150 mg/kg of DS for 2 days then were sacrificed after 3h from the second dose [14].

Preparation of Tissues for Light Microscope Examination (LM): Tissue samples were taken from the duodenum after good washing by injection of saline followed by buffered formalin (BF) and then fixed in BF, processed through graded alcohols and xylene and embedded in paraffin blocks in automatic processor of the pathology lab of King Abdulaziz university hospital. Serial sections of 4-6 µ were made on longitudinally and transversely oriented specimens. Sections were routinely stained with hematoxylin and eosin and histochemically by PAS- Alcian blue stain to identify the type of the secreted mucin.

Preparation of Tissues for Electron Microscopic Study (EM)

Transmission Electron Microscopy (TEM): Duodenum specimens were immersed in phosphate-buffered fixative containing glutaraldehyde (GH) at pH 7.4. All steps were done in the electron microscopic unit (EMU) at King Fahed Medical Research Center (KFMRC). Sections were cut and mounted on copper grids and stained with an 8% solution of uranyl acetate followed by lead citrate [15-16] and photographed by Philips Model CM 100 electron microscope.

Scanning Electron Microscopy (SEM): Duodenum specimens represented Group I, Group IIc and Group IIIc) were placed immersed in phosphate-buffered fixative containing glutaraldehyde (GH) at pH 7.4. Then, the

1x1mm specimens were immersed in primary fixative (GH) overnight, dehydrated, air-dried and coated with gold and examined by SEM.

Morphometric Study

Goblet Cells of the Crypts: The number of Goblet cells in each section from all strips on each section from each group was performed on PAS-alcian Blue stained sections.

Statistical Study: The collected data will expressed as mean ± SD. The statistical analysis of collected data was performed by using the SPSS program using the student T test to compare each group with the control group. The significant difference was considered at $P<0.05$.

RESULTS

Control Group I: Examination of H&E stained sections showed the different Layers of the duodenum. The layers appeared as mucosa, submucosa, musculosa and serosa (Fig. 1A). Most of the duodenal villi had a Leaf like appearance (Fig.1B). The mucosa was lined with columnar absorbing cells with few cup shaped goblet cells in between (Fig.1C). The lamina propria of the villous core of villi showed smooth muscle fibers, blood and lacteal capillaries. The cells lining of the crypts showed frequent mitotic figures especially of the upper region of the crypts. The submucosa had studded with groups of mucous secreting acini of Brunner's gland (Fig. 1D). In PAS-alcian blue stained sections of duodenal villi showed the lining goblet cells with different grades of alcian blue-PAS positive reaction. The PAS positive apical brush border appeared continuous (Fig. 1E). The predominance of positive combined PAS-alcian blue positive reaction of goblet cells was seen between the cells lining the crypt. The basement membrane appeared as a thin continuous PAS positive membrane (Fig. 1F). The apical borders of the cells lining PAS positive rea ction of the lining epithelium the acini of Brunner's gland in the submucosa. (Fig. 1G).

The TEM examination of absorptive cells in this study showed that absorptive cells as high columnar cells with apical microvilli. The nucleus appeared elongated with one or two nucleoli. The nuclear sap appeared electron lucent with peripheral accumulations of irregular heterochromatin along the inner nuclear membrane. The goblet cells appeared imprisoned between the columnar absorptive cell with bulbous extension of its apical region mainly by electron lucent mucin granules with few electron dense granules (Fig. 2a). A higher magnification of its luminal surface showed uniform, closely packed microvilli that embedded in the Mat like surface coat (the glyocalyx). Each microvillus in anchored in the underlying terminal web (Tw) by bundles of actin filaments. The cytoplasm of terminal web had no cellular organelles except few endocytic vesicles and high electronic density of fibrillar network of Tw. Laterally, the plasmalemmae of adjacent cells showed the junctional complexes (Fig.2b). Under the Tw, there were vesicles of the smooth endoplasmic cisternae followed by oval mitochondria surrounded by the rough endoplasmic reticulum (RER) with their electron dense ribosomes (Fig.2c).

Subgroup IIa and Subgroup IIIa

DS (50 mg/kg) and (MO + DS 50 mg/kg): After administration of the DS (V50) subgroup IIa, the TEM examination of the villous absorptive cell epithelial cells of the showed some affected cells. The goblet cell had multiple heterogenous electron lucent and dense granules enclosed between the apices of 2 absorptive cells. The wide separation between the cells was evident (Fig. 3A). The apical part of an absorptive cell had marked disorganization of the microvilli with shortness and separation. Some cells showed unclear differentiation between the terminal web and the underlying cytoplasm (Fig. 3B). The mononuclear cells in the lamina propria appeared activated as plasma cell with its characteristic appearance (Fig. 2C). In subgroup IIIb (V50+M), the specimens from animals protected by Moringa showed goblet cell with multiple homogenous electron lucent mucin granules (Fig. 3D). The apical part of an absorptive cell showed apparently short well organized microvilli with occasional small buds. The terminal web appeared similar to control (Fig. 3E). The basal lamina appeared intact with the telopodes of talocyte underneath the crypt lining epithelial cell. The lamina propria mononuclear cells were closely related and had made junctional contact with the processes of a talocyte (Fig. 3F).

Subgroup IIb and Subgroup IIIb

DS (100 mg/kg) and (MO + DS 100 mg/kg): The TEM examination of the villous absorptive cell epithelial cells of after the administration of the DS (100 mg/kg) (subgroup IIb), showed goblet cell with multiple heterogeneous electron dense granules enclosed. The apical part of an absorptive cell with degenerated empty mitochondria and clumped filamentous appearance of cytoplasm. The apical part of an absorptive cell showed short and separation.

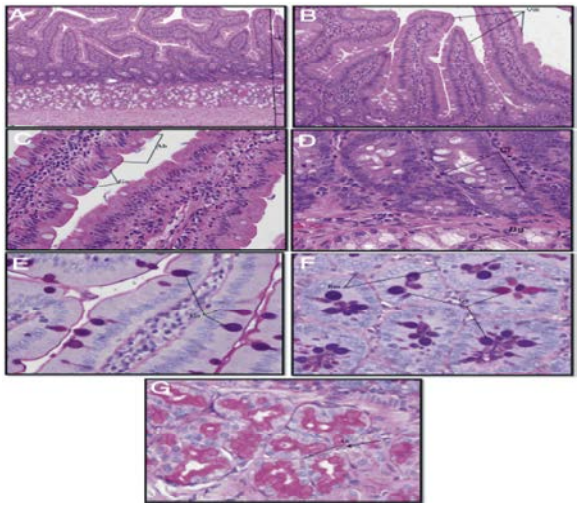

Fig. 1A-G: (1A): duodenum of control rats showing different Layers of duodenum of a control rat as mucosa (Mu), submucosa (Sm), muscularis (Mu) and serosa. H&E. X100.(1B): the Leaf like appearance of the duodenal villi. Notice the high columnar absorbing cell with oval nuclei occupying the middle third of their cytoplasm. H&E X200. (1C): the lining columnar absorbing cells of the mucosa and few goblet cells (GO) in between. Notice the lamina propria of the villous core with smooth muscle fibers (f) and blood lacteal capillaries (c). H&E X400. (1D): the crypts and part of Brunner's gland (Bg). Notice mitotic figures of upper part of cell Lining the crypt (Cr). H&E X600. (1E): duodenal villi, showing goblet cells (Go) with different grades alcian blue-PAS positive reaction. Notice the continuous PAS positive apical brush border. PAS-alcian blue. X600. (1F): A photomicrograph of the crypts, showing the predominance of combined PAS-alcian blue positive reaction of goblet cells between the cells of the crypt. Notice the thin continuous PAS positive basement membrane. PAS-alcian blue. X600. (1G): A higher magnification of the acini of Brunner's gland in the submucosa, showing the apical PAS positive reaction of the lining epithelium. The basement membrane has a thin continuous PAS positive (Ab). PAS-alcian blue. X600

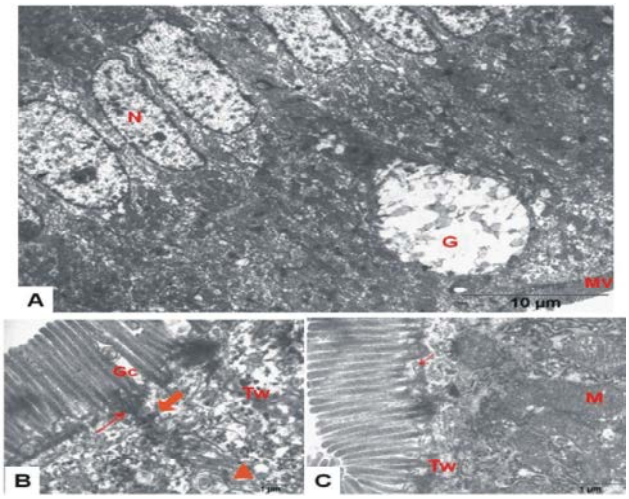

Fig. 2: A TEM micrographs of duodenum from control rat: (A): showing a group of absorptive cells (A) with their basal oval nuclei (N), microvilli (MV). Notice Goblet cell (G) in between the absorptive cells. bar 10µm. (B) A higher magnification of the luminal border showing uniform, closely packed microvilli the glyocalyx (Gc). Notice the actin filaments (thick arrow), terminal web (Tw) with fine fibrils (F), tight junction (→), adherent junction (thick arrow) and desmosomes (arrow head. Bar 1µm. (C): showing the apical region of an absorptive cell with area under the terminal web where oval mitochondria (M), rough endoplasmic reticulum (RER), smooth endoplasmic reticulum (SER) and ribosomes. Bar 1µm.

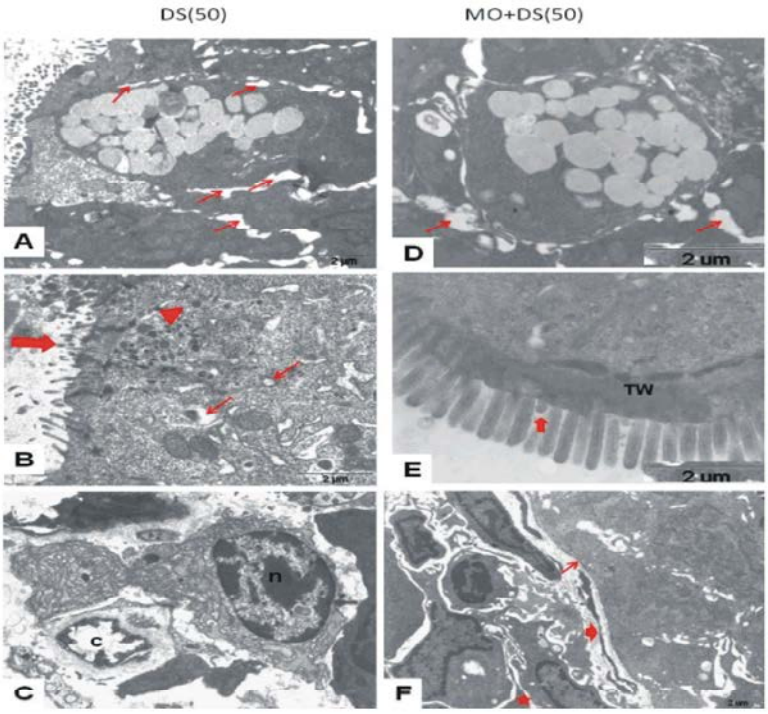

Fig. 3: TEM micrographs of duodenum from rat from subgroup IIa (A, B, C) and IIIa (D, E, F) (A): showing a goblet cell with multiple heterogenous electron lucent granules enclosed between the apices of 2 absorptive cells. Notice the wide separation between the cells and wide tight junction area (◂). Bar 5µm. (B) The apical part of an absorptive cell has marked disorganization of the microvilli (thick arrow) with shortness and separation. It is difficult to discriminate the terminal web from the underlying cytoplasm. (C) showing cells in the lamina propria, an active plasma cell can be seen. (c). Notice the associated telopodes (→) around a blood capillary. Bar 5mm. (D) showing a goblet cell with multiple homogenous electron lucent mucin granules. Bar 5mm. (E) showing apparently short well organized microvilli with occasional small buds (thick arrow). Bar 5mm. (F) showing the cells under the basal lamina (→) with the telopodes of talocyte underneath the crypt lining epithelial cell. Notice the close contact between the mononuclear cells and processes of a talocyte that make junction with them (*)

It is difficult to discriminate the terminal web from the underlying cytoplasm. Most of the cells in the lamina propria showed the ultrastructure feature of a plasma cell with the characteristic cartwheel appearing nucleus with disrupted membrane and eosinophils with their characteristic crystalloid granules (Figs. 4A, B, C).

In subgroup IIIb (MO+DS 100), the specimens from animals protected by Moringa showed goblet cell extruding their content into the lumen and surrounded by 2 absorptive cells with their cytoplasm had elongated mitochondria (Fig. 4D). The apical part of an absorptive cell showed a linearly arranged microvilli with few degenerated ones. The terminal web appeared similar to control (Fig. 4E). The cells in the lamina propria showed plasma cells and eosinophils with their intact membranes. There was an evident junction between the lamina propria cells (Fig. 4F).

Subgroup IIc and Subgroup IIIc

DS (150 mg/kg) and (MO + DS 150 mg/kg): The TEM examination of the apical part of an absorptive cell showed marked disorganization of the microvilli. The tight junction between the 2 cells was deformed and no junctional complex underneath with different sized and decrease number of mitochondria. The cytoplasm showed autophagic vacuoles and lysosomes. The mitochondria appeared globular with ballooned cristae (Fig. 5A). The goblet cells was packed with multiple fused mucin granules and remnants of the granular membrane in between the granules. Some tight junctions were evident and underneath membranes were folded and interdigitated (Fig. 5B). The cells in the lamina propria under the basal lamina had disturbed membranes commonly of an eosinophil with its characteristic crystalline granules. Some cells showed

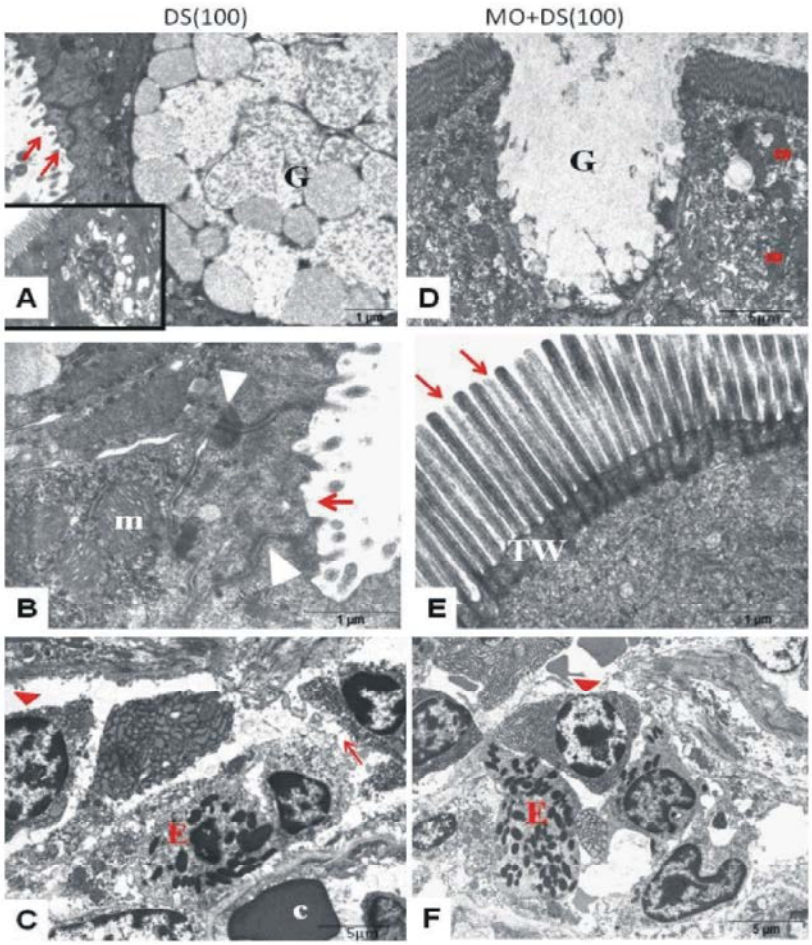

Fig. 4: Electron micrographs of duodenum from rat from V100 (A, B, C) and V100+M (D, E, F) (A): showing a goblet cell with multiple heterogeneous electron lucent granules enclosed between the apices of 2 absorptive cells. Notice the wide tight junction area (◂) and increased filamentous electron density of some of them. Bar 1□m. (B) The apical part of an absorptive cell has marked disorganization of the microvilli (thick arrow) with shortness and separation. It is difficult to discriminate the terminal web from the underlying cytoplasm. Bar 1□m (C) showing cells in the lamina propria, an active plasma cell can be seen (◂). Junctions between connective tissue cells (→) is unclear around a blood capillary (c). Bar 5mm. (D) showing a goblet cell with mucin granules poring mucin between 2 absorptive cells with elongated mitochondria (m). Bar 5□m. (E) showing well organized microvilli with degeneration of some of them (→). Notice well organized terminal web (TW). Bar 1mm. (F) showing intact cell membrane of an active plasma cell can be seen (◂) and an eosinophil (E). Notice the evident junctional contact between cells in the lamina propria cells (→).

marked cytoplasmic changes. Some blood capillaries appeared with narrow closed lumena (Fig. 5C). In subgroup IIIc, the ultrathin sections showed group of epithelial cells with uneven microvilli and their nuclei appears as hyperchromatic (Fig. 5D). The goblet cells showed well defined mucin granules together with areas of electron lucent fused granules. The cytoplasm of the absorptive cells around the goblet cells had abundant mitochondria (Fig. 5E). The cells in the lamina propria showed predominance of the eosinophils with their intact membrane. Some blood capillaries had a narrow lumen. Some were packed by neutrophil obliterating its lumen (Fig. 5F).

Scanning Electron Microscopy (SEM)

Group I (Control): The scanning electron microscopic examination of the control duodenal wall showed the regular arrangement of the duodenal villi.

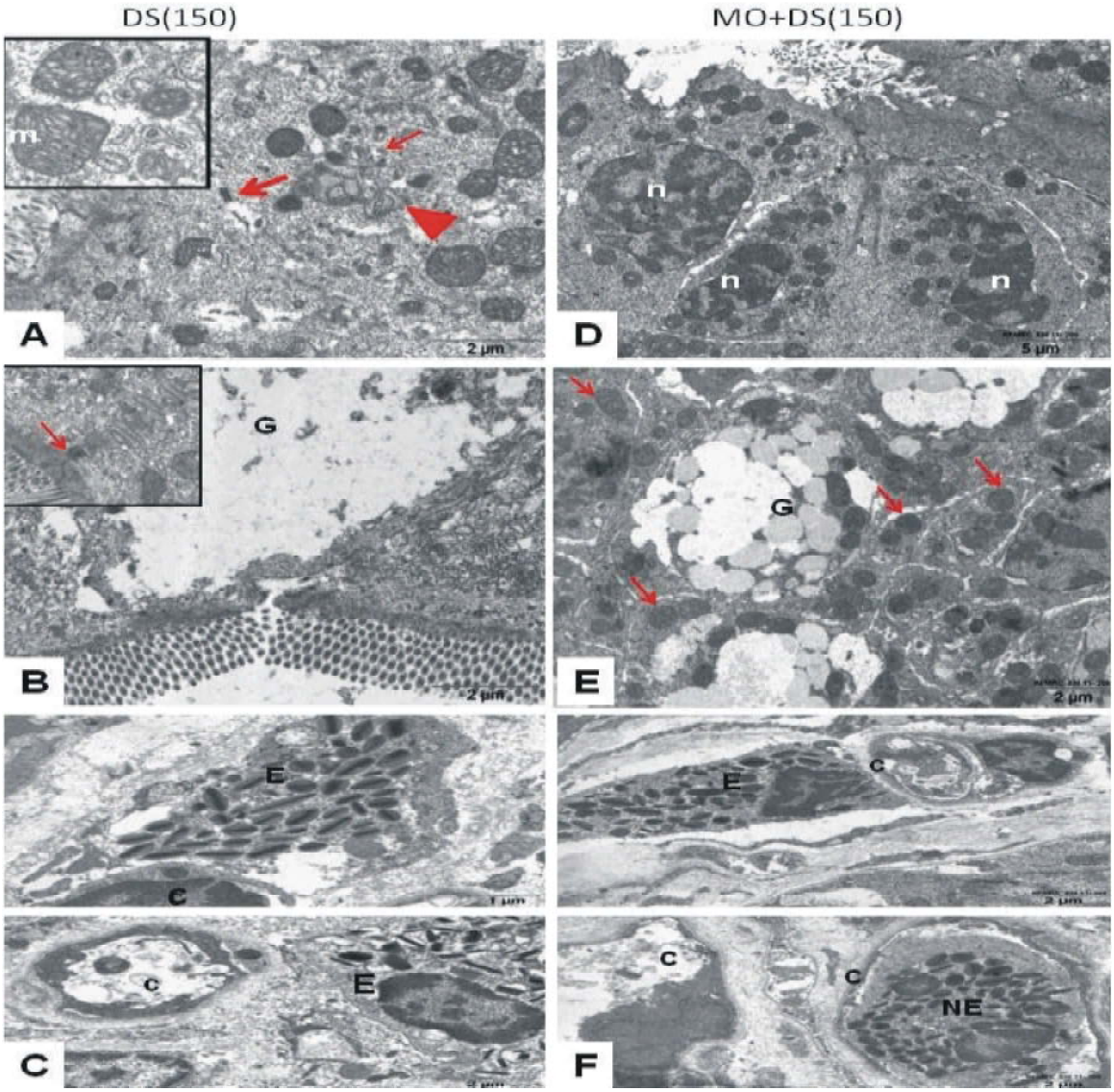

Fig. 5: TEM micrographs of duodenum from rat from V150 (A, B, C) and V150+M (D, E, F), (A): showing the apical part of an absorptive cell has marked disorganization of the microvilli. The tight junction (thick arrow) between the 2 cells is deformed and no junctional complex underneath with variable size and decrease number of mitochondria. Notice the autophagic vacuoles (head arrow) and group of lysosomes (thin arrow).Inset: showing the globular mitochondria with ballooned cristae. Bar 2µm (B) showing goblet cell (G) with multiple fused mucin granules and remnants of the granular membrane in between granules. Inset: shows persistence of some tight junctions (→) and underneath interdigitating membranes. Bar 2mm. (C) showing in the upper part of the photo, cells in the lamina propria, has disturbed membrane of an eosinophil with its characteristic crystalline granules, while in the lower part shows marked cytoplasmic changes. Blood capillary (c) has closed lumen in the upper photo. Bar 1&2µm. (D) showing group of epithelial cells with uneven microvilli and their nuclei appears as hyperchromatic (n). Bar 5µm. (E) showing a goblet cell with combined well defined mucin granules and areas with electron lucent fused granules (*). Notice the cytoplasm of the cells around with abundant mitochondria (→). Bar 2µm. (F) showing in the upper part cells in the lamina propria, with intact membrane of an eosinophil. (E) in relation to a narrow lumen capillary, while in the lower part shows a blood capillary (c) packed by a neutrophil (NE) obliterating its lumen. Bar 2µm.

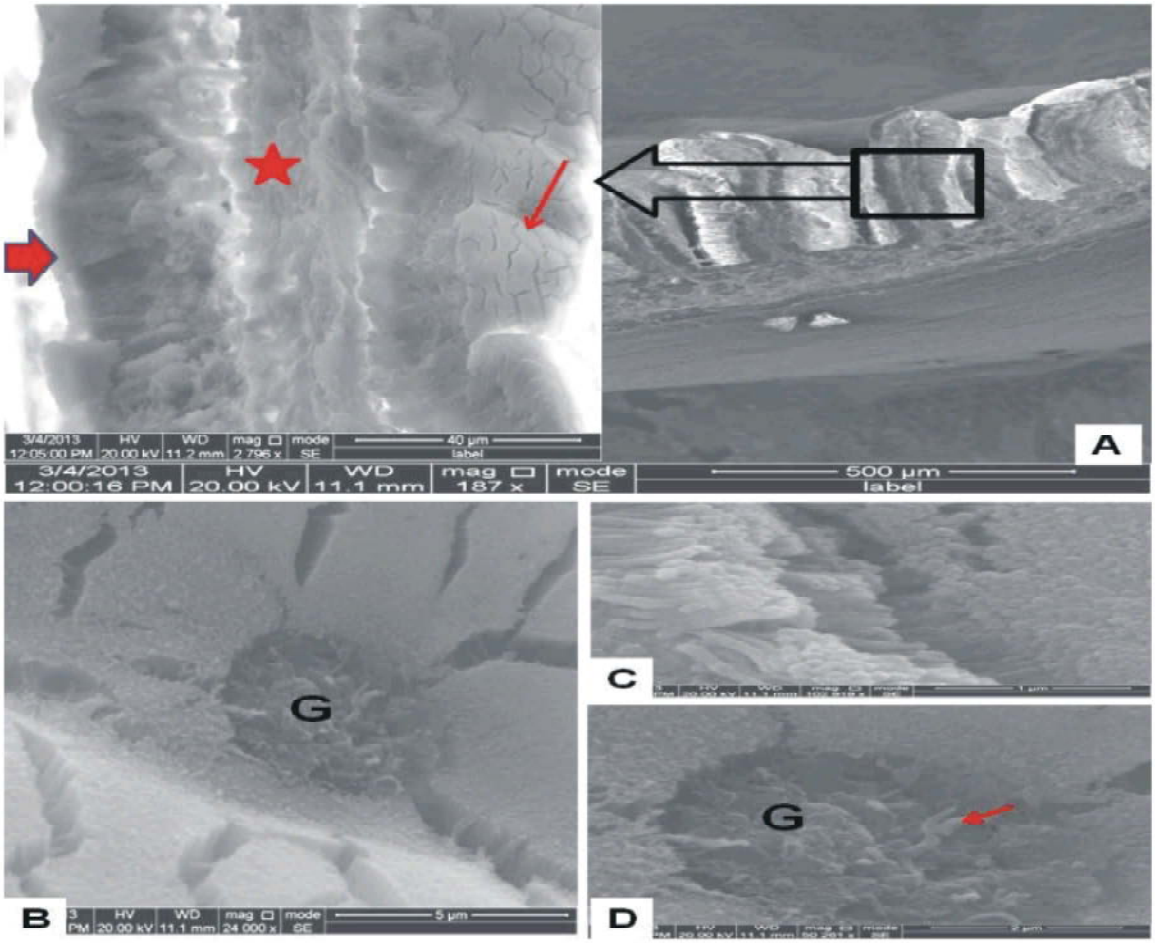

Fig. 6: Scanning electron micrographs of the control group, A. Cross sectional of duodenal wall showing the regular arrangement of the duodenal villi. bar 500µ. Inset shows a single villous with a regular central core (*). On the right side the hexagonal top appearance of cells (a) and on left side regularly arranges cells with apical brush border (thick arrow). Bar 40µ. B. showing the top surface of absorptive cells and in the middle the surface appearance of a goblet cell with the normal separation between cells (G). Bar 5µ. C. Higher magnification of top surface shows the compact regularly arranged microvilli with uniform diameter. Bar 1µ. D. Higher magnification of the top surface of imprisoned goblet cell (G) between microvilli of the columnar absorptive cells has few irregular microvilli (à) between the mucin material (*). Bar 2µm.

Each villous had a central core. Their top surface has a hexagonal appearance of cells with a regularly arranged cells and apical brush border (Fig. 6A). Higher magnification of the top surface of absorptive cells around the top surface of a goblet cell showed the normal separation between cells. Higher magnification of top surface showed the compact arranged microvilli with its regular uniform diameter. Higher magnification of the top surface of imprisoned goblet cell between microvilli of the columnar absorptive cells showed its few irregularly arranged microvilli between the mucin material (Figs. 6 B, 6C, 6D).

Subgroup IIc : (DS 150 mg/kg): The scanning electron microscopic examination of duodenal wall of Group IIc (DS150) showed the lateral surface of the duodenal mucosa with a collapsed appearance of villi and irregular cracks of the tip its surface. Close examination of the lateral surface of the villous showed uneven irregular appearance with loss of the hexagonal appearance of cells that was seen in control specimens (Fig. 7 A and inset). Higher magnification of the top surface of a group of absorptive cells showed the abnormal appearance of the top surface of some cells between microvilli of some cells. Higher magnification of top surface of this abnormal

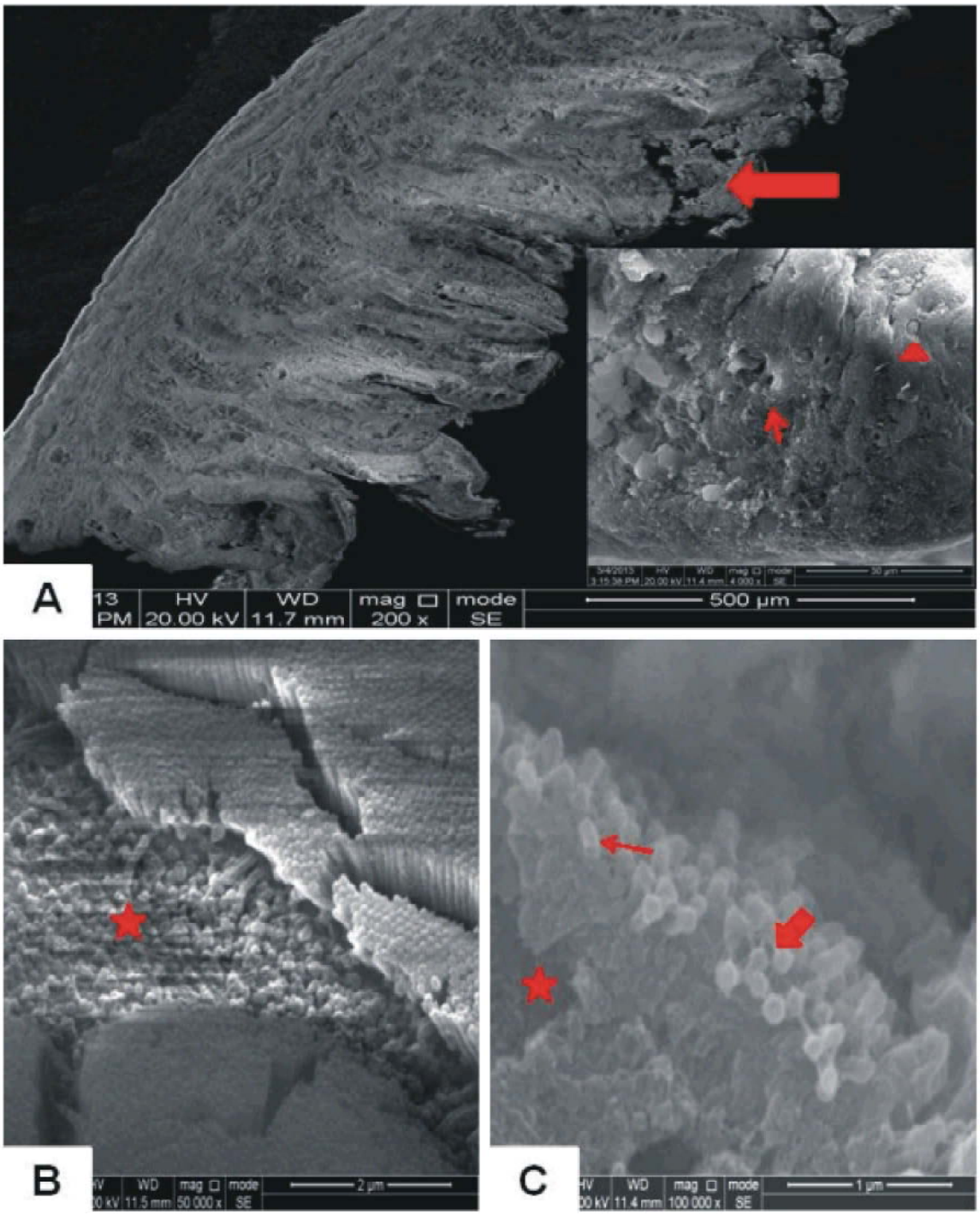

Fig. 7: Scanning electron micrographs of the Group IIc, A. showed a the collapsed appearance of villi. Notice the top surface of a villous with irregular cracks of the tip its surface (thick arrow). Bar 500µ. Inset: shows a single villous with an irregular top surface and the lateral surface of a villous has an uneven irregular appearance with loss of the hexagonal appearance. Bar 30µ. B. showing the top surface of a group of absorptive cells, some has a normal microvillous appearance and others has an abnormal appearing microvilli (*). Bar 2µ. C. Higher magnification of top surface of the abnormally appearing cells shows their short microvilli (arrow) and areas of loss of microvilli (*). Notice the accumulation of rounded cells (thick arrow). Bar 1µ

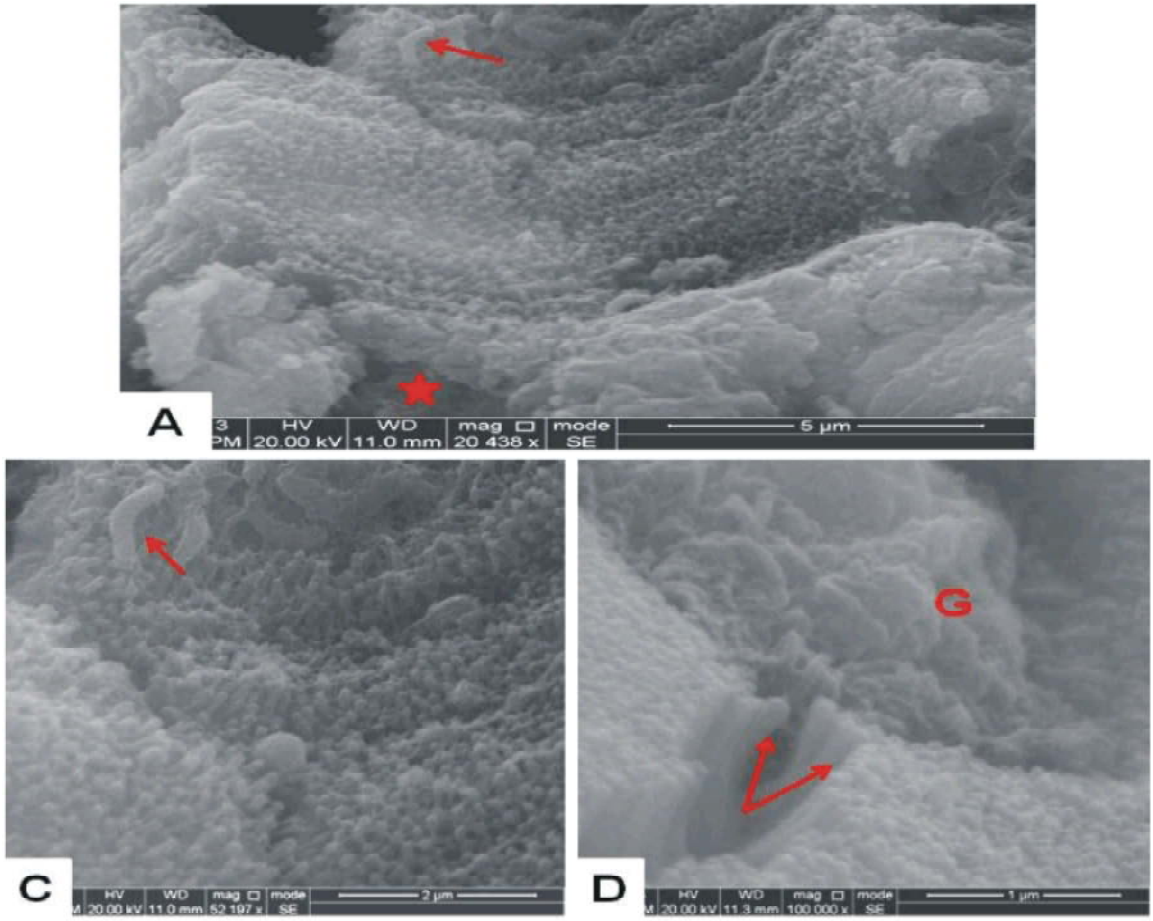

Fig. 8: Scanning electron micrographs of the Group IIIc, A. showed the top surface of a group of absorptive cells with a velvety appearance with cracked areas (*). Notice a goblet cell with few irregular microvilli (à). Bar 5µ. B. Higher magnification of the mentioned group of cells shows their short microvilli with individual tall microvilli (thin arrow). Notice the large emerging rounded cell between microvilli (headarrow). Bar 2µm. C. showing the top surface of a nearby goblet cell (G) and the surrounding absorptive cells with uniform regularly arranged microvilli (arrows). Bar 1µm

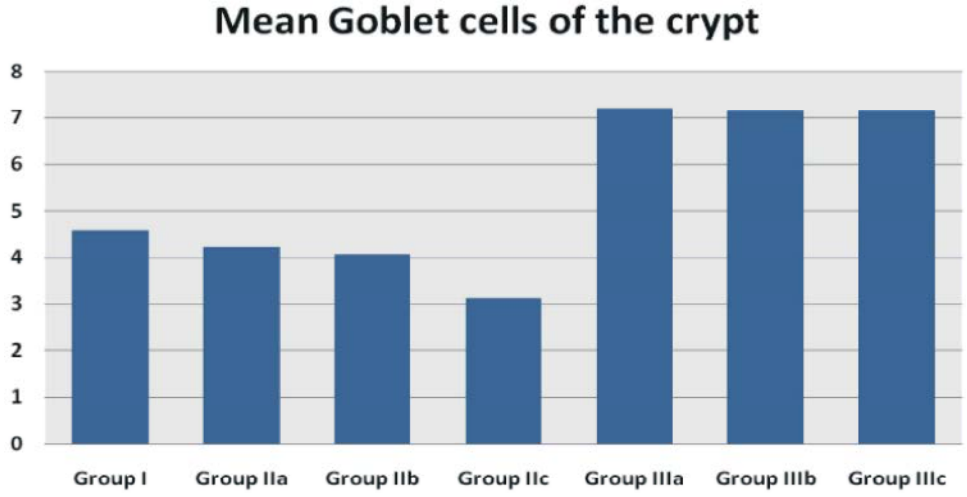

Fig. 8: Illustrate the changes in the number of goblet cell of the crypts in the different groups

Table 1: Changes in Goblet cell percentage in different groups.

Groups	Goblet cells of the crypt (%) (mean + SD)	T
Control	5±2	
G IIa (V50)	4±2	0.731
G IIb (V100)	4±2	1.061
G IIc (V150)	3±1	3.655
G IIIa (V50 +M)	7±4*	3.354-
G IIIb (V100 +M)	7±4*	3.185-
G IIIc (V150 +M)	7±3*	4.394-

Significant = $p < 0.05$ =*

microvilli revealed the cracks in top surface together with short individual microvilli and groups of rounded inflammatory cells (Figs. 7 B, C).

Subgroup IIIc :(MO+ DS 150 mg/kg): The scanning electron microscopic examination of duodenal wall of Group IIIc (MO+DS150) showed the top surface of a group of absorptive cells with a velvety appearance. A higher magnification of cells showed the short microvilli around the goblet cell. The emerging of rounded cells from the surface was a frequent finding (Figs. 8A, B). Higher magnification of top surface of a nearby cells showed the uniform regularly arranged microvilli of absorptive cell around a goblet cells that appeared covered by mucin (Fig. 8 C).

Morphometric Results: The changes in the mean crypt goblet cells was recorded in Table (1) (Fig. 8). The significant increase in the goblet cell number was observed in group III.

DISCUSSION

Light and electron microscopes examination of absorptive cells of the control group showed that absorptive cells as high columnar cells with apical microvilli. The nucleus appeared elongated with one or two nucleoli. The nuclear sap appeared electron lucent with peripheral accumulations of irregular heterochromatin along the inner nuclear membrane. The goblet cells appeared imprisoned between the columnar absorptive cell with bulbous extension of its apical region mainly by electron lucent mucin granules with few electron dense granules. Moreover, each microvillus was anchored in the underlying terminal web (Tw) by bundles of actin filaments. These observation was in accordance with the description of the duodenal mucosa [17].

The gastrointestinal epithelium is covered by a protective mucus gel. Changes in goblet cell functions and in the chemical composition of intestinal mucus were detected in response to a broad range of luminal insults, including alterations of the normal microbiota. Goblet cells may have broad biomedical applications because mucus alter-ations appear to characterize most diseases of mucosal tissues [18] After administration of the DS (50mg/kg) subgroup IIa, TEM examination showed that goblet cell had multiple heterogeneous electron lucent and dense granules enclosed between the apices of two absorptive cells. The wide separation between the cells was evident. The apical part of an absorptive cell had marked disorganization of the microvilli with shortness and separation. The mononuclear cells in the lamina propria appeared activated as plasma cell with its characteristic appearance.

In subgroup (IIb) (DS 100), the goblet cell had heterogeneous electron dense granules enclosed. The apical part of an absorptive cell showed degenerated empty mitochondria and clumped filamentous appearance of cytoplasm. The apical part of an absorptive cell showed short and separation. In subgroup IIc (DS 150), the apical part of an absorptive cell showed marked disorganization of the microvilli. The tight junction between the two cells was deformed and no junctional complex underneath, while others were evident and underneath membranes were folded and interdigitated. The mitochondria had different sizes and decrease number with globular and ballooned cristae. The cytoplasm showed autophagic vacuoles and lysosomes. The goblet cells was packed with multiple fused mucin granules and remnants of the granular membrane. The cells in the lamina propria under the basal lamina had disturbed membranes commonly of an eosinophil with its characteristic crystalline granules. [19] mentioned that paracetamol with a high dose resulted in destruction and distortion of the nuclei and accumulation of chromatin around the nuclear envelope. Diclofenac drug is affecting the permeability of the cell membranous system result in release of the mitochondrial protein which lead to the split of the cellular implications to enable its therapeutic effect, where they found that DS caused split of DNA of the mucous cells lining the intestines. Furthermore, the present study is in accordance with the results concluded by Triebskorn *et al.,* (2004) that increase in the size of Golgi bodies where vesicles filled with secreted substances.

In subgroup IIIb (MO + DS 50), the specimens taken from animals administered Moringa showed goblet cells with multiple homogenous electron lucent mucin granules. The terminal web appeared similar to control. The basal lamina appeared intact with the telopodes of talocyte underneath the crypt lining epithelial cell. The lamina

propria mononuclear cells were closely related and had made junctional contact with the processes of a talocyte, this relation had suggested its role the protection of the mucosa. [20] stated that talocytes are involved in immune response.

In subgroup IIIb (MO+DS 100), the specimens from animals administered Moringa showed goblet cell and surrounded by two absorptive cells with their cytoplasm had elongated mitochondria. The terminal web appeared similar to control. The cells in the lamina propria showed plasma cells and eosinophils with their intact membranes. In the present study, subgroup IIIc (MO+DS150), goblet cells showed well defined mucin granules together with areas of electron lucent fused granules. The cytoplasm of the absorptive cells around the goblet cells had abundant mitochondria. The cells in the lamina propria showed predominance of the eosinophils with their intact membrane. [21] explained the ameliorating effect of moringa that can act through disruption of inflammatory signal transduction pathways.

A study on human intestinal (Caco-2) cell monolayers, it was reported that MOL prevented oxidation of actin and led to the protection of actin cytoskeleton and intestinal barrier integrity against oxidant insult. These two reports show that MOL acts in the management of tight junctions in small intestinal mucosa. It was suggested that MOL improved intestinal mucosa integrity by reducing intestinal [22].

The shortening and uneven microvilli, ballooned mitochondria, SER proliferation and deformity in nuclei were dose dependant. These results were in agreement with [23]. In contrary to our results, It was explained that most of the mitochondrial changes in mice treated by indomethacin were attributed to the lack of the prescribe the less effective dose and for the shortest phosphoric oxidization in mitochondria which result in period possible and make periodic intestinal examination dilation, vacuolation and destruction of RER [24].. However, the changes in the mitochondria of absorptive cells may be a response to the damage resulting from the treatment by the drug. As other studies reported that voltaren affects the membrane enzymes and causes an increase the permeability of the mitochondrial membrane [25- 26]. *Moringa leifera* removes oxygen free radicals in epithelial cells. It has been reported to promote healing and prevent relapse of intestinal ulcers, which is attributed to inflammatory cell migration into the intestinal mucosa.

[27 - 28] reported that gastrointestinal mucosal damage induced by NSAIDs in a randomized controlled trial carried out in healthy volunteers. Different electron densities of mucoid granules suggested the different chemical nature of mucin. This suggestion was based on the fact that the various types of glycoprotein are identified histochemically as natural acid and sulfated glycoproteins. These may be present in varying proportions in different mucous-secreting cells of the body causing the difference in the ultrastructural appearance of secretory granules. In addition, the pleomorphism of granules suggests that they have a malleable semisolid content. The granules may be spherical or if closely packed, polygonal [29].

The importance of acidic mucin was explained by many studies. They mentioned that the intestinal mucus is considered as a protective layer between the lining epithelial cells and the enzymatic activity of the intestinal chyme. They added that mucus has this property of resisting enzymatic hydrolysis due to its carbohydrate moieties, especially the acidic groups. Other investigators suggested that acidic mucin has a fundamental role in protecting the pit cells against HCl during its passage. The alternating layers of neutral and acidic mucins in the surface coat act as a safeguard against HCl and digestive enzymes in the lumen [30-31].

In the present study, scanning electron microscopic examination of the control duodenal wall showed the regular arrangement of the duodenal villi with central core. Their top surface had a hexagonal appearance of cells with a regularly arranged cells and apical brush border. Its top surface showed the compact arranged microvilli with its regular uniform diameter. While, the duodenal wall of Group IIc (DS150) showed the lateral surface of the duodenal mucosa with a collapsed appearance of villi and irregular cracks of the tip of its surface. The absorptive cells showed the abnormal appearance of the top surface of some cells between microvilli of some cells. Meanwhile, Group IIIc (MO+DS150) showed the top surface of a group of absorptive cells with a velvety appearance. The cells showed the short microvilli around the goblet cell. The emerging of rounded cells from the surface was a frequent finding. Similar observations were record [32].

Morphometric study in the present study showed an increased number of goblet cells in all groups administered DS and MO compared to the control. Goblet cells are responsible for mucus production. It is known that mucus plays an important role in immunity [32]. Moreover, the intestinal mucus is considered as a protective layer between the lining epithelial cells and the enzymatic activity of the intestinal chyme. They added that mucus has this property of resisting enzymatic hydrolysis due to its carbohydrate moieties, especially the

acidic groups. Other investigators suggested that acidic mucin has a fundamental role in protecting the pit cells against HCl during its passage. The alternating layers of neutral and acidic mucin in the surface coat act as a safeguard against HCl and digestive enzymes in the lumen [30-31].

CONCLUSION

The current study concluded that MO leaves might have a partial protective effect on the rat duodenal mucosal histological changes, that resulted from the administration of high doses of diclofenac sodium.

ACKNOWLEDGEMENT

The authors are thankful Dr. siham K.M. Abunasef for her assistance in manuscript preparation. We gratefully acknowledge the work of Tagreed E. Y. Natto for her assistance in experimentally preparation. This work was supported the funds provided by King Abduaziz City Science and Techbology (KACST).

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