

# Effect of genetically modified corn on the jejunal mucosa of adult male albino rat



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## ABSTRACT

Genetically modified (GM) plants expressing insecticidal traits offer a new strategy for crop protection. GM-corn contains *Bacillus thuringiensis* (Bt) genes producing delta endotoxins in the whole plant. Diet can influence the characteristics of the gastrointestinal tract altering its function and structure. The aim of this study was to evaluate the effect of GM-corn on the histological structure of jejunal mucosa of adult male albino rat using different histological, immunohistochemical and morphometrical methods. Twenty adult male albino rats were divided into two equal groups; control and GM-corn fed group administered with 30% GM-corn for 90 days. Specimens from the jejunum were processed for light and electron microscopy. Immunohistochemical study was carried out using antibody against proliferating cell nuclear antigen (PCNA). Different morphometrical parameters were assessed. Specimens from GM-corn fed group showed different forms of structural changes. Focal destruction and loss of the villi leaving denuded mucosal surface alternating with stratified areas were observed, while some crypts appeared totally disrupted. Congested blood capillaries and focal infiltration with mononuclear cells were detected. Significant upregulation of PCNA expression, increase in number of goblet cells and a significant increase in both villous height and crypt depth were detected. Marked ultrastructural changes of some enterocytes with focal loss of the microvillous border were observed. Some enterocytes had vacuolated cytoplasm, swollen mitochondria with disrupted cristae and dilated rough endoplasmic reticulum (rER). Some cells had dark irregular nuclei with abnormally clumped chromatin. It could be concluded that consumption of GM-corn profoundly alters the jejunal histological structure.

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## 1. Introduction

Genetically modified (GM) or transgenic crops have been grown for human and animal consumption since the 1990s (James and Krattiger, 1996). There are currently over 200 different GM-crops with various traits approved for consumption in many countries (Zdziarski et al., 2014). GM plants expressing insecticidal traits offer a new strategy for crop protection, but at the same time, present a challenge in terms of food safety assessment (Yanfeng et al., 2013), these plant products are becoming increasingly common in the human food-chain, despite this, feeding studies examining the effects of GM-crops on animal and human health are relatively scarce (Snell et al., 2012). The most widespread GM-plant materials with the highest importance at the feed market are

MON810 corn and MON-40-3-2, RR soybean meal (Reichert et al., 2012).

“MON810: Ajeeb YG” is a GM-corn that has resistance to borers, and this variety was produced by incorporating the MON810, produced by Monsanto company, in the Egyptian conventional corn “Ajeeb” (Rayan et al., 2012). MON810 variety contains Cry1Ab genes from *Bacillus thuringiensis*, and these genes produce delta endotoxins in the whole plant. These endotoxins activate in the alkaline environment of insects’ gut, and then the insects die within 24–48 h (Tenuta et al., 2011).

Within the next few years, crops that have been genetically engineered for *Bacillus thuringiensis* resistance could dramatically lower production costs and provide farmers with new insect control options (Ibrahim and Shawer, 2014). With respect to safety of GM foods, there are conflicting opinions, some studies reported that GM foods had potentially toxic properties, which could provoke unintended effects of genetic modification and others reported that it is safe for use (Tyshko et al., 2007; Abdo et al., 2013).

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Diet can influence the characteristics of the gastrointestinal tract since the intestinal mucous membrane is directly in contact with food and absorbs the substances produced by digestion, also digestive tract is the first site of contact for any ingested compound. Furthermore, since the stomach and the intestines are the sites of longest residence for any ingested product, these should become the most important sites for the evaluation of an ingested compound's toxicity (Montagne et al., 2003). In particular, it has been reported that the diet may affect both small and large intestine in terms of mucosal architecture, villous height and crypt depth, epithelial cell proliferation and other features (Serolini et al., 2007; Tralbalza-Marinucci et al., 2008). Moreover, it is known that diet and the histochemical characteristics of goblet cell mucins and/or mucous membrane are strictly correlated (Morini and Grandi, 2010).

Histomorphological changes have been widely used to assess the effects of GM ingredients on the diets of mice and rats (Hartke et al., 2005; Hedemann et al., 2006). So this work was performed to study the effect of GM corn on the histological structure of jejunal mucosa of adult male albino rat using different histological, immunohistochemical and morphometrical methods.

## 2. Materials and methods

The present study was carried out on twenty adult male albino rats, weighing 150–200 g. The animals were kept in adequate ventilation and temperature, where food and water were consumed freely throughout the experimental period. The experiment was approved by the Local Ethics Committee of Faculty of Medicine, Tanta University (Egypt). After a one-week acclimatization period, animals were randomly divided into two equal groups: **Group I** (Control group): received a diet of grain conventional corn meal (non-GM) for 90 days. **Group II** received GM-corn (Ajeeb YG; Bt MON810) obtained from the agricultural administration, Sakha, Kafr Elsheikh governorate, Egypt. Flours from GM-corn grains were formulated into the animals' diet at a concentration of 30% and administered for 90 days (El-Shamei et al., 2012).

Animals were closely observed for their general health and behavior. Animals' feed consumption and total body weight were recorded throughout the experiment. At the end of the experiment, animals were anesthetized using intraperitoneal injection of pentobarbital (40 mg/kg) (Gaertner et al., 2008). The jejunal specimens were dissected, rinsed with phosphate buffered saline (PBS) and prepared for light and electron microscopic examination.

### 2.1. For examination by light microscopy

Cross-sectioned jejunal specimens were fixed in 10% neutral buffered formalin, washed, dehydrated, cleared and embedded in paraffin. Sections of 5  $\mu$ m thickness were stained with haematoxylin and eosin (H&E) for the study of general histological features and Periodic Acid Schiff reagent (PAS) for detection of neutral mucopolysaccharide (Bancroft and Gamble, 2008).

### 2.2. For immunostaining with proliferating cell nuclear antigen (PCNA)

For detection of proliferating crypt cells, 5  $\mu$ m thick sections were dewaxed, rehydrated, and washed with phosphate buffered saline (PBS) and then incubated with PBS containing 10% normal goat serum. Sections were incubated with the mouse monoclonal antibody PC10 against PCNA (sc-56, Santa Cruz Biotech, Santa Cruz, USA) (1:100) overnight in a humid chamber at 4 °C and then incubated with biotinylated rabbit anti-mouse Ig (1:200) for 60 min at room temperature. Sections were incubated with a

streptavidin–biotin–horseradish peroxidase complex (1:100) prepared 30 min in advance and mixed shortly before use with an equal volume of PBS. The immunoreactivity was visualized using 3,3'-diaminobenzidine (DAB) hydrogen peroxide as a chromogen and sections were counterstained with Mayer's haematoxylin. The negative control sections were prepared by excluding the primary antibodies (Ramos-Vara et al., 2008).

### 2.3. For examination by transmission electron microscopy

Cross-sectioned jejunal specimens were divided into small pieces and fixed in 4% phosphate buffered glutaraldehyde (0.1 M, pH 7.3), post-fixed with 1% phosphate-buffered osmium tetroxide, then dehydrated in ascending grades of ethanol. After immersion in propylene oxide, the specimens were embedded in epoxy resin mixture. Semithin sections (1  $\mu$ m thick) were stained with 1% toluidine blue and examined by light microscope for proper orientation (Bozzola and Russell, 1999). Ultrathin sections (80–90 nm) were stained with uranyl acetate and lead citrate, to be examined by JEOL-JEM-100 transmission electron microscope (Tokyo, Japan) at the Electron Microscopic Unit, Faculty of Medicine, Tanta University, Egypt.

### 2.4. For examination by scanning electron microscopy

Longitudinal jejunal specimens were cut open to expose the lumen, rinsed with phosphate buffered saline (PBS) and fixed in 4% phosphate buffered glutaraldehyde (0.1 M, pH 7.4), then in phosphate-buffered 1% osmium tetroxide, dehydrated in graded alcohol series, put into amyl acetate, dried with liquid CO<sub>2</sub> under pressure with critical point dryer (E 3000) and coated with gold particles (Rau et al., 2001). These samples were observed under Jeol JSM scanning electron microscope (SEM), at the Electron Microscopic Unit, Faculty of Medicine, Tanta University, Egypt.

### 2.5. Morphometric study

The images were acquired using a Leica microscope (DM3000, Leica, Germany) coupled to a digital camera (DFC-290, leica, Germany). The image analysis was done using Leica Qwin 500C Image analyzer computer system (Leica Imaging System LTD., Cambridge, England) at Central Research Lab, Faculty of Medicine, Tanta University, Egypt. Images were analyzed for:

#### 2.5.1. The mean height of jejunal villi

The height of jejunal villi (from the tip of the villus to the villus-crypt junction) were measured in H&E stained sections. Ten randomly-selected non-overlapping microscopic fields for each specimen were measured at a magnification power of 100.

#### 2.5.2. The mean jejunal crypt depth

The crypt depth was measured in H&E stained sections. Ten randomly-selected non-overlapping microscopic fields for each specimen were measured at a magnification power of 100.

#### 2.5.3. The mean number of goblet cells

Goblet cells in PAS-stained sections were counted in both villi and crypts. Ten randomly-selected non-overlapping microscopic fields for each specimen were measured at a magnification power of 200.

#### 2.5.4. The mean percentage of PCNA-immunopositive cells

This was calculated to quantitatively evaluate the number of PCNA-positive immunostained nuclei at a magnification power of 400. The results were expressed as a percentage of the total

number of cells counted (number of labeled nuclei  $\times$  100/total cell number).

## 2.6. Statistical analysis

The data were analyzed by student *t*-test using statistical package for social sciences statistical analysis software (version 11.5; SPSS Inc., Chicago, Illinois, USA). All values were expressed as mean  $\pm$  standard deviation. Differences were regarded as significant or highly significant if probability value  $P < 0.05$  or  $P < 0.001$  respectively (Dawson-Saunders and Trapp, 2001).

## 3. Results

No animals expressed any sign of ill health throughout the experiment and no deaths were reported. There were no observable alterations in the animals' behavior, feed consumption or average weight gain as there was a non-significant difference in total body weights between the two dietary groups at the start and at the end of the experiment (Initial weights in gm; control group  $166.12 \pm 12.10$ , GM-corn fed group  $167.65 \pm 12.42$ , and final weights in gm; control group  $251.83 \pm 13.31$  GM-corn fed group  $256.31 \pm 14.55$ ).

### 3.1. Light microscopic results

#### 3.1.1. Haematoxylin and eosin (H&E) stain

Examination of H&E-stained sections obtained from the control group (group I) showed the normal histological features of the jejunal mucosa with long finger like villi covered with simple columnar epithelial cells (enterocytes) and goblet cells. The villi had a core of loose connective tissue extending from the lamina propria containing some connective tissue cells and lymphocytes. Intestinal crypts of Lieberkuhn appeared as tubular invaginations extending from the bases of villi into the lamina propria (Fig. 1a). The enterocytes had eosinophilic cytoplasm and basal oval nuclei. The luminal surface of the enterocytes was covered by a regular continuous striated (brush) border. Goblet cells appeared as empty spaces between the enterocytes (Fig. 1b).

In the GM-corn fed group (group II), the jejunal mucosa showed different forms of structural changes such as distortion, shortening, flattening and fusion of some villi as well as deepening of the crypts. In addition, shedding of some epithelial cells in the intestinal lumen with subsequent erosion was observed in focal areas, on the other hand, stratification of enterocytes and numerous goblet cells were detected in other areas (Fig. 1c–f). Enterocytes with vacuolated or clear cytoplasm and pyknotic nuclei were observed at the basal aspect of the some villi (Fig. 1c and d). Focal mononuclear cellular infiltration and congestion of some blood capillaries were also detected (Fig. 1d and g). Moreover, marked disruption, disorganization, distortion and shedding of jejunal mucosa were observed at certain areas (Fig. 1h).

#### 3.1.2. Periodic acid Schiff reagent (PAS) stain

Examination of the PAS-stained sections from the control group revealed intact PAS-positive brush border of the villi and goblet cells inbetween the epithelial cells lining jejunal mucosa (villi and crypts) (Fig. 2a). On the other hand, sections from the GM corn-fed group showed an apparent increase in the number of goblet cells lining jejunal mucosa with an increased intensity of PAS reaction at the brush border. Interruption of brush border at certain sites was also observed (Fig. 2b).

#### 3.1.3. PCNA immunohistochemical staining

Examination of PCNA-immunostained sections of the control group showed that only a few number of immunopositive

epithelial cells lining the crypts expressed as brown nuclear coloration (Fig. 3a). On the other hand, sections from GM-corn fed group showed an apparent increase in the number of PCNA-immunopositive epithelial cells lining the crypts as well as in the inflammatory cells infiltrating the lamina propria (Fig. 3b and c).

### 3.2. Electron microscopic results

#### 3.2.1. Transmission electron microscopy

Examination of ultrathin sections from control animals showed that the enterocytes appeared regularly arranged and closely packed, containing basal oval euchromatic nuclei with prominent nucleoli, many mitochondria, and showed regular continuous microvillous borders. Goblet cells were observed in between the enterocytes with the characteristic mucin granules (Fig. 4a and b).

Ultrathin sections from the GM-fed group showed marked ultrastructural changes in some enterocytes, with focal loss of the microvillous border and partial shedding of some cells. Some enterocytes contained vacuolated cytoplasm, swollen and degenerated mitochondria with disrupted cristae and dilated rough endoplasmic reticulum (rER). Other cells contained dark irregular nuclei with abnormally clumped chromatin (Fig. 4c–f). Goblet cells expressed vacuolated cytoplasm and dilated rER (Fig. 4d and e).

#### 3.2.2. Scanning electron microscopy

SEM examination of jejunum from the control animals revealed the typical leaf-shaped villi of the rat small intestine (Fig. 5a). Goblet cells distended with mucus were clearly observed in between the enterocytes (Fig. 5b).

Examination of group II fed with GM-corn revealed apparent erosions and fissures at the tips of the jejunal villi, they were frequently surrounded by enterocytes of normal appearance (Fig. 5c). In other areas, stratified cells were observed at the villous surface together with some exfoliated sheets of cells (Fig. 5d). A number of apically swollen cells clearly demarcated from each other and several other cells which had lost their brush border were observed (Fig. 5e). Occasional extravasations of red blood cells were observed at certain sites of the villous surface (Fig. 5f).

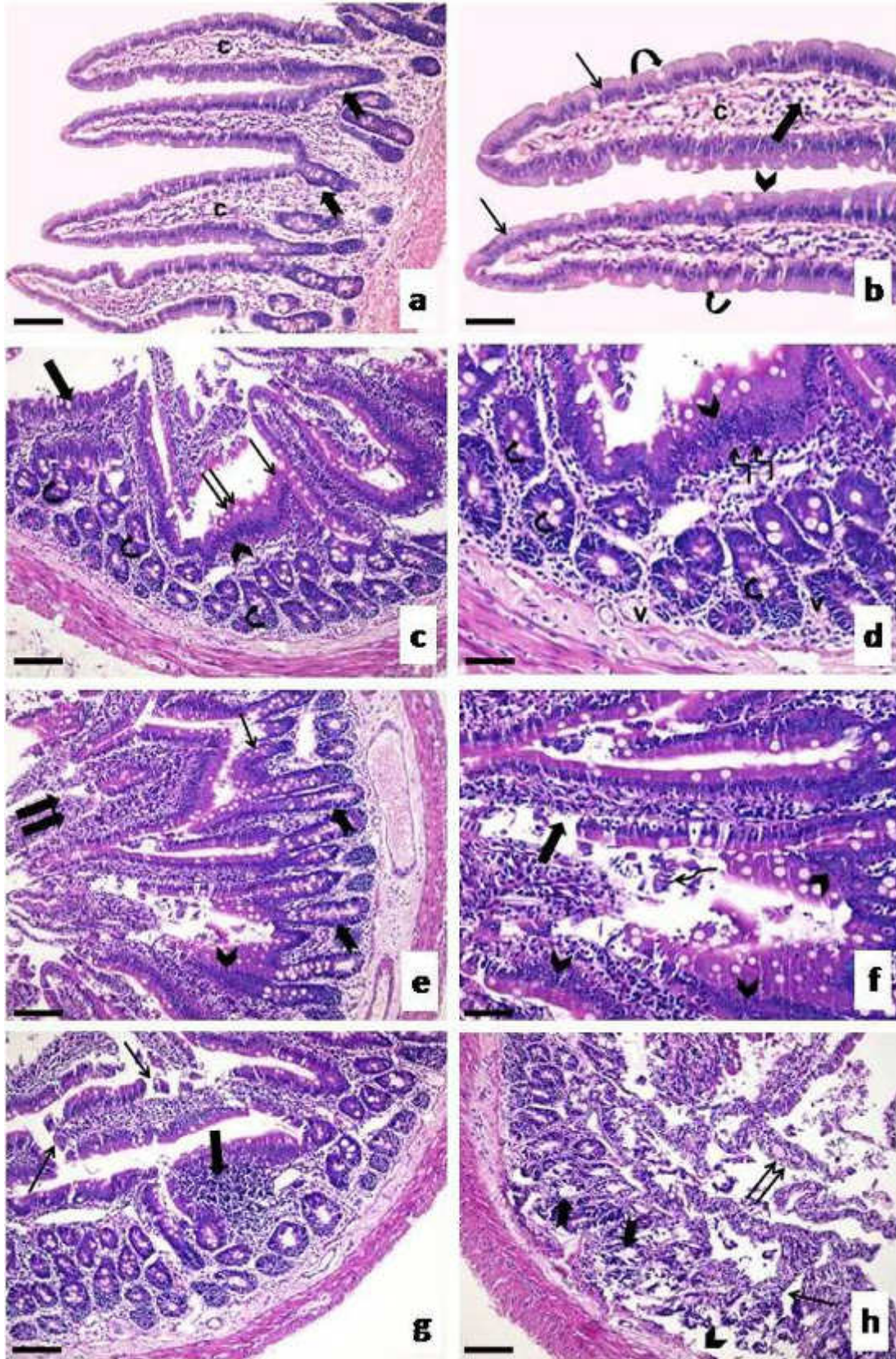
### 3.3. Morphometric and statistical analysis

There was a highly significant decrease in the mean height of jejunal villi in the GM-corn fed group ( $482.09 \pm 9.31$ ) compared to the control group ( $507.01 \pm 10.10$ ). Additionally, a highly significant increase in the mean depth of jejunal crypts in the GM-corn fed group ( $163.92 \pm 7.38$ ) compared to the control group ( $133.60 \pm 5.02$ ) was detected (Table 1, Fig. 6a and b).

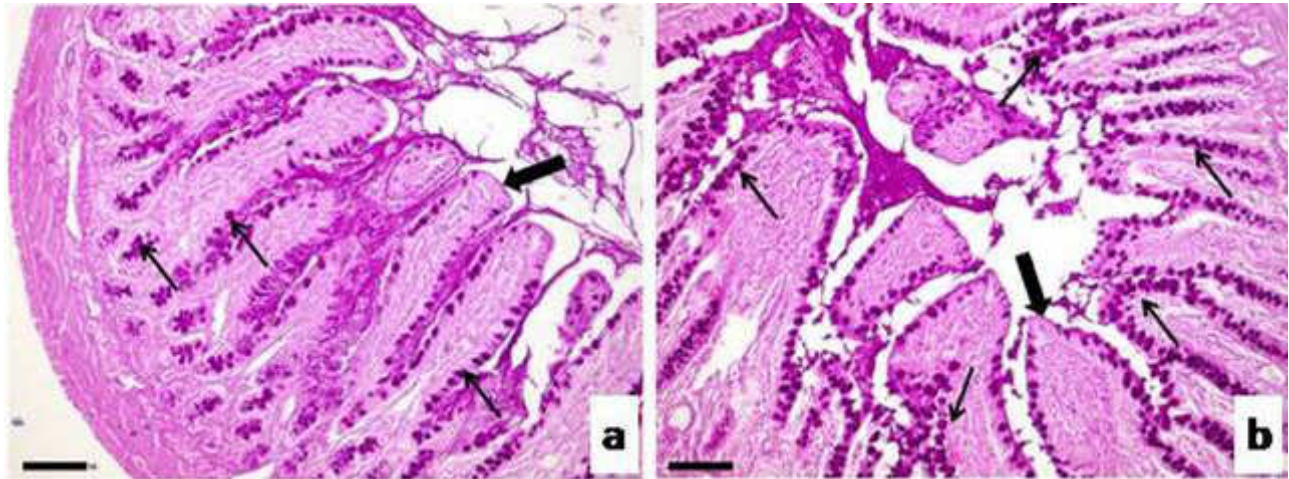
Moreover, the mean number of goblet cells counted in both villi and crypts in the GM-fed group ( $76.38 \pm 3.22$ ) showed a highly significantly increase compared to the control ( $50.31 \pm 3.76$ ). Additionally, a highly significant increase ( $P < 0.001$ ) in the number of PCNA-immunopositive cells in the epithelial cells of the GM-corn fed group ( $23.11 \pm 2.13$ ) compared with the control group ( $8.34 \pm 1.17$ ) was detected (Table 1, Fig. 6c and d).

## 4. Discussion

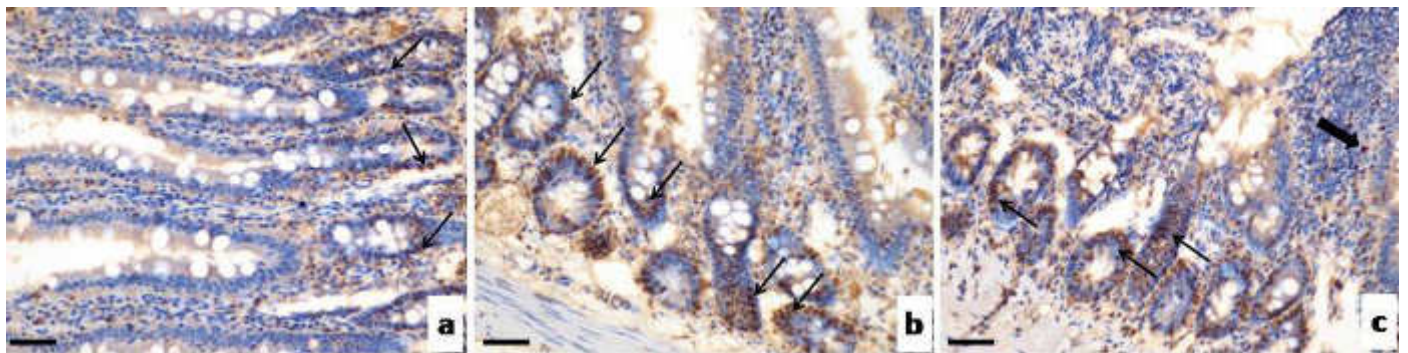
Using genetically modified crops in human food or animal feed raises a considerable amount of public concern about the possible impact of GM crops on the environment, food safety and both animal and human health, taking in consideration the importance of ethical, political, and economical aspects. Conflicting reports and contradicting opinions continuously rise about the possible hazards of GM crops on human health in particular. In addition, too little is known about the validity of the different safety



**Fig. 1.** Light microscopic examination (H&E): a,b control group showing long finger-like villi with a core of connective tissue (c) and covered with enterocytes (thin arrows) and goblet cells (curved arrows), invaginated crypts (notched arrows) are between the bases of villi. The enterocytes have eosinophilic cytoplasm and basal oval nuclei. Notice the regular striated border (arrow head) of the luminal surface of enterocytes. Some lymphocytes are seen (thick arrow). c–d GM-corn fed group showing distortion (thick arrow), shortening (thin arrow) and flattening of some villi (double arrows) with focal stratification of the enterocytes (arrow heads) and apparent increased number of goblet cells (curved arrows), pyknotic cells are observed in the basal aspect of villous lining (angular arrows). Notice congested blood capillaries (V). e–f GM-corn fed group showing shortening (thin arrow) and fusion of some villi (double thick arrows), deepening of the crypts (notched arrows) and disruption of the villous covering epithelium with the



**Fig. 2.** PAS staining: a) control group showing PAS positive reaction in goblet cells (thin arrows) and intact brush border of jejunal mucosa (thick arrow). b) GM-corn fed group showing numerous goblet cells with strong positive reaction (thin arrows). Notice focal interruption of the brush border (thick arrow). [Magnification  $\times 400$ , scale bar =  $50 \mu\text{m}$ ].



**Fig. 3.** PCNA-immunostaining a) control group showing some PCNA immunostained positive cells lining the crypts expressing brown coloration (thin arrows). b, c) GM-corn fed group showing numerous PCNA immunostained positive cells lining the crypts (thin arrows) as well as in the inflammatory cells infiltrating the lamina propria (thick arrows). [Magnification  $\times 400$ , scale bar =  $50 \mu\text{m}$ ].

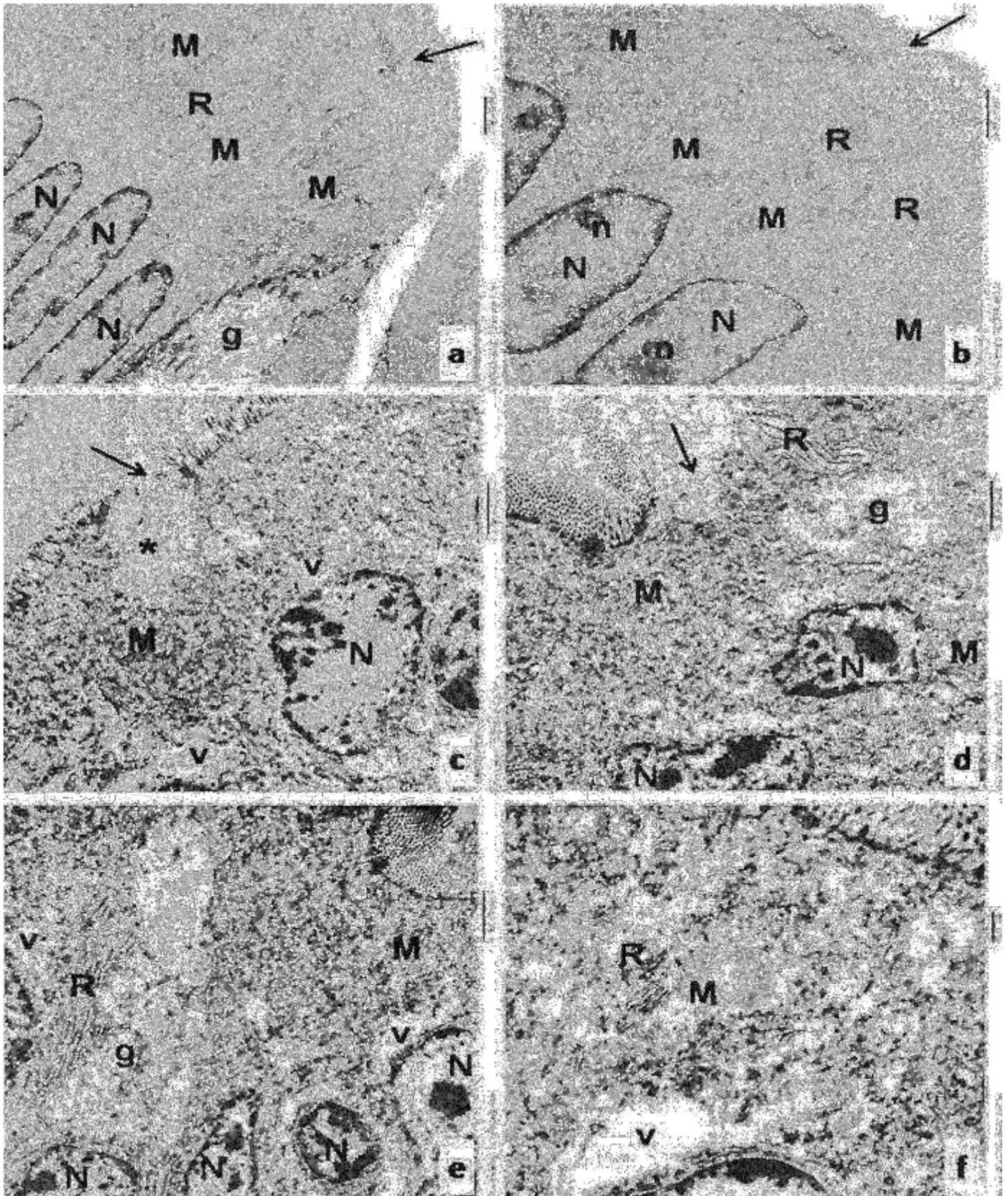
assessments tests that were undertaken for these crops. The need for deeper insight into the influence of one of the most common GM crops in Egypt (MON810: Ajeeb YG) has encouraged the design of this work to assess the impact of its long term consumption on the jejunal mucosa of adult male albino rat employing different histological, immunohistochemical and morphometrical techniques.

In the current work, focal structural changes including distortion, shortening, flattening and fusion of some jejunal villi were observed in GM-corn fed group. In addition, stratification alternating with shedding of the jejunal surface epithelium were detected as was similarly reported (Fares & El-Sayed 1998). These changes could be observed as early as after only 45 days of GM-corn consumption (El-Shamei et al., 2012). Moreover, some researchers have suggested that significant GM-maize linked effects were generally detected either after 14 weeks of consumption or at a high GM feed dose in the diet (de Vendômois et al., 2009). Additionally, stratification and shedding of epithelium of the villi in this study came in association with a significantly increased crypt proliferation as detected using immunohistochemical staining against PCNA. Some authors provided evidence of proliferative activation of basal epithelial cells of alimentary

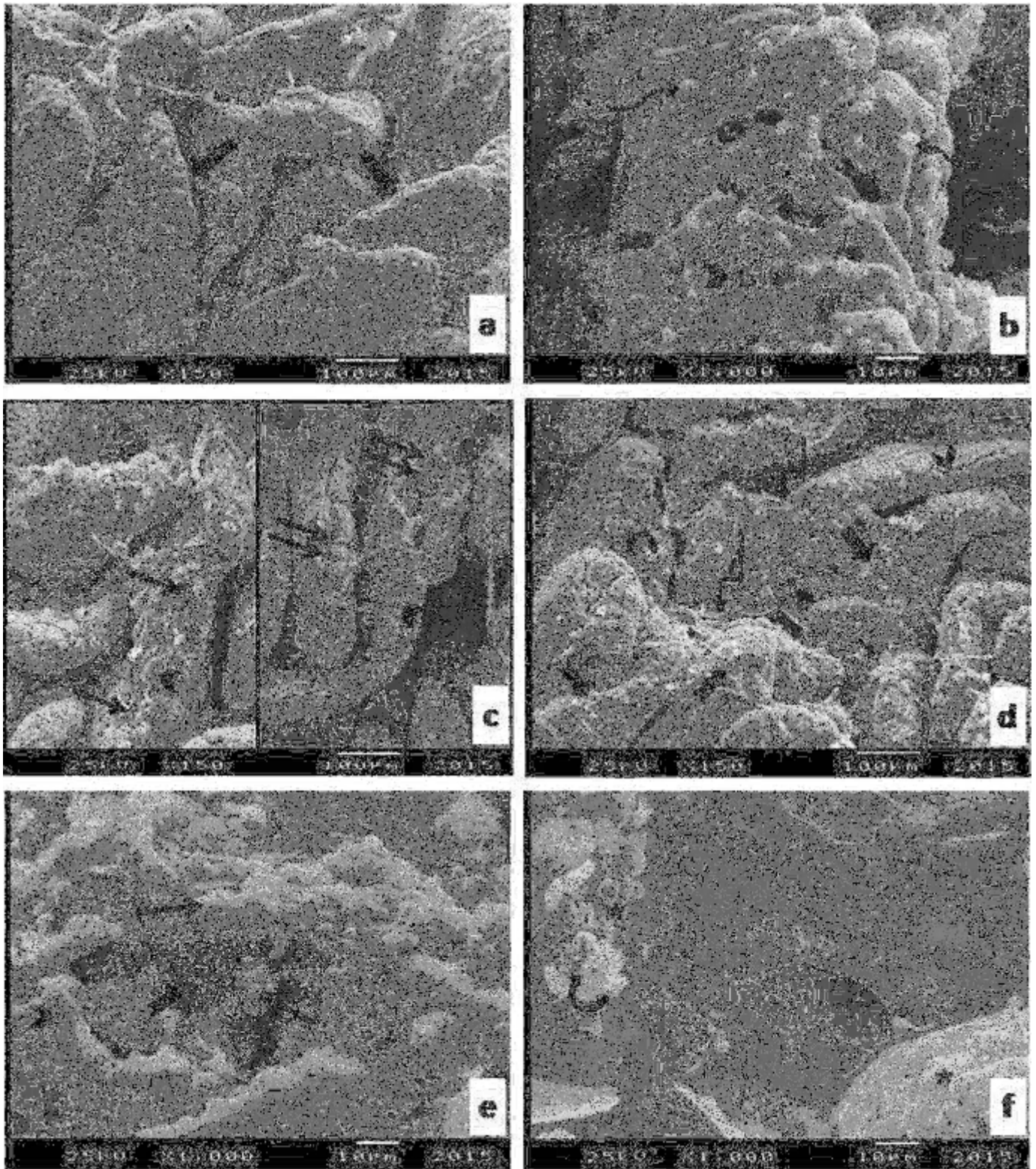
canal in all GM-corn fed animals indicating an increased turnover rate (Trabalza-Marinucci et al., 2008).

Erosions in the villi and denuded mucosal surface were also evident as observed on both light and electron microscopic levels in GM-corn fed group. Erosion is a very alarming finding as it could lead to occasional hemorrhage especially in more vulnerable groups. Similar erosions were observed in rat jejunum upon chronic intake of dietary fibers (Cassidy et al., 1981). They observed as well large denuded areas where the cell demarcations were very evident. Although they suggested a possible correlation with bile acids sequestration, they did not give much of an explanation for such changes. Yet, it could be suggested that these surface erosions are most likely attributed to the inflammatory changes observed during the current study, where inflammatory signs in the jejunum of GM-corn fed group were detected in the form of dilated congested blood vessels and mononuclear cellular infiltration in the lamina propria, in addition to cellular and hemorrhagic debris observed with the scanning electron microscopy. This coincides with the observations of a previous work, where a higher rate of severe inflammation in the stomach of pigs upon mixed GM maize and soyabean feed consumption was documented (Carman et al., 2013). One explanation for the inflammation could be the action of

enterocytes shed into the lumen (wavy arrow) leaving eroded villous surface (thick arrow). Notice focal stratification of the enterocytes (arrow heads). g–h) GM-corn fed group showing area of focal mononuclear cellular infiltration in lamina propria (thick arrow). Notice areas of disrupted mucosa (thin arrows) with sloughing of the villi (double arrows) and the crypts (notched arrows) leaving denuded areas (arrow head). [Magnification; (a, c, e, g and h)  $\times 200$ , scale bar =  $100 \mu\text{m}$  (b, d and f)  $\times 400$ , scale bar =  $50 \mu\text{m}$ ].



**Fig. 4.** Transmission electron microscopy: a, b) control group showing regularly arranged closely packed enterocytes with basal oval euchromatic nuclei (N) and prominent nucleolus (n), mitochondria (M), rough endoplasmic reticulum (R) and an intact microvillous border (arrow). Notice goblet cell with mucin granules (g). [Magnification  $\times 8780$  and  $11,700$  respectively, scale bar =  $2 \mu\text{m}$ ]. c–f) GM-corn fed group showing enterocytes containing nuclei with abnormally clumping chromatin (N), swollen degenerated mitochondria with disrupted cristae (M), rarefaction and vacuolation of the cytoplasm (v), dilated rER (R) and focal loss of the microvillous border (arrow) with loss of the apical part of the enterocyte (asterisk). Notice goblet cell with coalesced mucin granules (g) and dilated rER (R). [Magnification (c,d and e)  $\times 11,700$ , scale bar =  $2 \mu\text{m}$  and f  $\times 29,200$ , scale bar =  $500 \text{nm}$ ].



**Fig. 5.** Scanning electron microscopy: a, b) control group showing the typical leaf-shaped villi with smooth microvillous surface (thick arrows) and goblet cells distended with mucus (wavy arrow) inbetween the enterocytes (arrow head). c,d) GM-corn fed group showing apparent erosions (thin arrows), fissures (double thin arrows) at the tips of the jejunal villi and areas of apparent stratification (notched arrows) and exfoliated sheets of cells (angular arrows). Notice surrounding enterocytes of normal appearance (arrow head) and distorted and damaged cells (curved arrow). e,f) GM-corn fed group showing number of apically swollen cells clearly demarcated from each other (thin arrows) and several other cells which had lost their brush border (notched arrows) with areas of enterocytes of normal appearance inbetween (arrow head). Extravasations of red blood cells are observed at certain sites of the villous surface (asterisks). Notice distorted and damaged cells (curved arrow). [Magnification (a, c and d)  $\times 150$ , scale bar =  $100\ \mu\text{m}$ , (b, e and f)  $\times 1000$ , scale bar =  $10\ \mu\text{m}$ ].

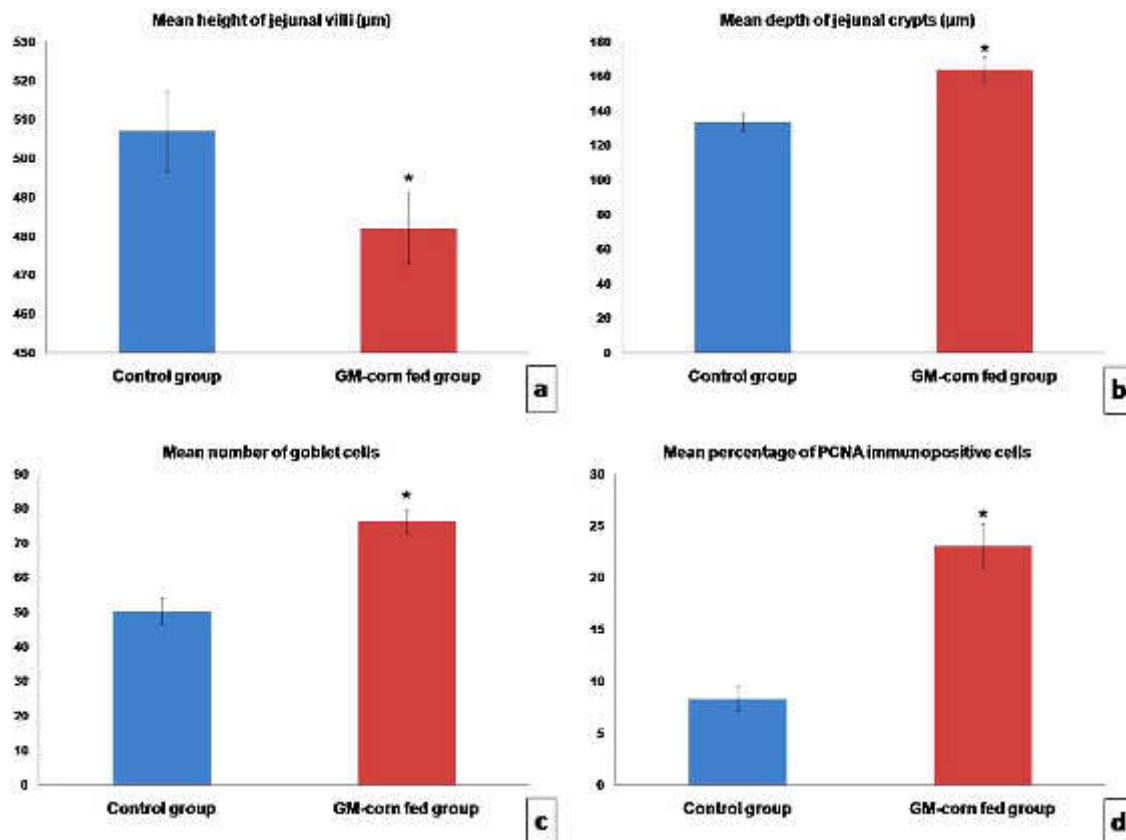
**Table 1**  
Morphometric analysis of jejunal specimens of all groups.

Parameters	Control group	GM-corn fed group	P value
Mean height of jejunal villi ( $\mu\text{m}$ )	507.01 $\pm$ 10.10	482.09 $\pm$ 9.31*	$P < 0.001$
Mean depth of jejunal crypts ( $\mu\text{m}$ )	133.60 $\pm$ 5.02	163.92 $\pm$ 7.38*	$P < 0.001$
Mean number of goblet cells	50.31 $\pm$ 3.76	76.38 $\pm$ 3.22*	$P < 0.001$
Mean% of PCNA immunopositive cells	8.34 $\pm$ 1.17	23.11 $\pm$ 2.13*	$P < 0.001$

Data are expressed as mean  $\pm$  standard deviation, \* indicates significance.

Cry 1Ab proteins produced by GM-corn that act as insecticides by inducing pore formation and disintegration of the gut tissue of certain borers that attack the corn plants (Fiuza et al., 2013). Additionally, some authors have collectively attributed the histopathological and biochemical changes observed upon GM-corn consumption to the possible role of the delta-endotoxin produced by the Bt corn (Abdo et al., 2014). In support of this hypothesis, six proteins in the mouse small intestine that could bind to a Cry protein (Cry 1Ac) were recognized (Vazquez-Padron et al., 2000), they added that when the Cry protein binds to these proteins, it results in hyperpolarisation of the intestine, which is consistent with the formation of cationic channels, as occurs in the insect gut. Moreover, persistence of Cry 1Ab proteins throughout the digestive tract of pigs was detected, indicating the resistance of these proteins to digestion (Chowdhury et al., 2003; Walsh et al., 2012b). On the contrary, Cry1Ab protein was indicated to bind at low levels to the cytoskeletal protein actin, which is a structural protein, but not to extracellular proteins that have been identified as receptors for Cry protein binding on target insect mid gastrointestinal tract epithelia (Shimada et al., 2006).

In the current study, several morphometrical parameters were assessed. Mean number of goblet cells was found to be significantly higher in GM-corn fed group compared to the control group. This came in accordance with the work of others studying 30% GM corn consumption in male albino rats after 45 days (El-Shamei et al., 2012). Goblet cells synthesize and secrete mucous layer that covers the gastrointestinal epithelium, providing protection against pathogens, lubrication for intestinal content and a medium to transport nutrients across the intestinal lumen to the epithelial cells. The mucous layer is composed of mucins that are secreted at a baseline rate, but in case of insult, bioactive compounds stimulates the goblet cells to increase mucin secretion either directly or by stimulating host cytokines such as TNF-alpha and IL-6 (Deplancke and Gaskins, 2001). Nevertheless, changes in the intestinal mucins are directly linked to the dynamic equilibrium between their biosynthesis by goblet cells and their degradation within the lumen by the microflora, thus the levels of mucins may vary in the epithelium according to the diet and to bacteria inhabiting the digestive tract (Montagne et al., 2003).



**Fig. 6.** Morphometrical and statistical analysis a) mean height of jejunal villi b) mean depth of jejunal crypts.c) mean number of goblet cells d) mean percentage of PCNA-immunopositive cells. \* $P < 0.05$  is significant versus control,  $n = 10$  in each group.



Similarly, an increase in the number of goblet cells/ $\mu\text{m}$  in jejunal villi was previously reported upon studying the effect of 38.9% of the very same GM-corn on male weanling pigs for 31 days (Walsh et al., 2012a), they attributed it to a possible change in gut microflora that enhanced inflammation and decreased integrity of the mucosal barrier. Intestinal commensal microflora have been reported to directly impact the intestinal epithelial functions including those of goblet cells (Kim and Ho, 2010) by producing mucin-degrading enzymes (Macfarlane et al., 2005) or by stimulation of mucin gene expression (Dohrman et al., 1998).

On the other hand, a significant decrease in mean villous height coupled with a significant increase in mean crypt depth was recorded during the current study. This could be indicative of the high enterocytes proliferation in the crypts and high exfoliation in the villi as similarly reported in response to different microflora in intestine (Shirkey et al., 2006). On the contrary, others reported a decrease in the villous height but did not comment on the crypt depth (El-Shamei et al., 2012), while an increase in both parameters throughout the small intestine could be detected by Walsh et al. (2012a). The discrepancies between different research works could be in part attributed to the variable doses of GM feed used in the different studies ranging between 11% and 38.9%, while the consumption durations ranged between 14 days and 3 years.

Transmission electron microscopic examination of specimens from the GM-corn fed group revealed marked ultrastructural changes mainly including nuclear irregularities with abnormal chromatin clumping, dilated rER and distorted mitochondria. Similarly, smaller cell nuclei containing increased amounts of heterochromatin were reported in the liver and pancreas of GM corn-fed animals (Trabalza-Marinucci et al., 2008). Moreover, nuclear alterations, mainly small, irregular and abnormal chromatin clumping in mice hepatocytes upon 90 days of GM soyabean consumption were as well recorded (Malatesta et al., 2005), they attributed these observations to a GM-induced modification in the metabolic activity of the cells although that the underlying mechanism remains elusive. Nevertheless, degenerated mitochondria and rER in association with delta endotoxin-treated mice were typically described (Fares and El-Sayed, 1998). Fine structural modifications of cellular components in relation to GM feed intake have already been described, yet, reports have assumed that there were no consequences on organ functions or animal health (Malatesta et al., 2002).

In summary, the current work presents a multi-approach histological assessment of a typical 90-days subchronic toxicity study through combining different histological, histochemical, immunohistochemical and morphometrical methods supported with both transmission and scanning electron microscopy for a full histological evaluation of the influence of GM-corn on the jejunum in a rat model. Results from the current study could show that in spite of the assuring reports on GM products, GM-corn has profoundly altered the histological structure of the jejunal mucosa at many levels and revealed several alarming signs as the proliferative and eroded hemorrhagic lesions in addition to several ultrastructural alterations described here for the first time for jejunum under GM-corn influence. Possible mechanisms have been proposed including inflammation associated with goblet cell overexpression and PCNA upregulation. This should motivate the conduction of a more extensive research to reveal the exact mechanism of such unintended effect and how to remodulate the GM crops to avoid their adverse effects.

#### Conflict of interest

The authors have no conflict of interest to declare.

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