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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 (Yanfang 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 (Seralini et al., 2007; Trabalza-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 μm 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 μm 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 gluteraldehyde (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 μm 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 gluteraldehyde (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_2 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

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