Original Article

# Structural changes in pancreatic acinar cells and β-cells of rat fed with genetically modified corn

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## Abstract

**Background:** Genetically modified (GM) organisms have been an issue of intense public concern. Among the different GM organisms, GM plants have attracted a large amount of media attention. Corn used for food has been GM to resist glyphosate herbicide, insect resistance (Bt-corn), and vitamin-enriched corn with increase in β carotene, Vitamin C, and folate. As GM foods are starting to be present in our diet, concerns have been expressed regarding GM food safety. **Aim of the Work:** The aim was to assess the possible effects arising from GM corn on pancreatic acinar cells and β-cells. **Materials and Methods:** Twenty rats were divided equally into two groups. Group I (Control Group) received non-GM corn, Group II (The Experimental Group) fed with GM corn for 3 months. After 3 months, blood samples from all rats were collected for blood glucose estimation, and fresh specimens were taken from the pancreas and processed for light and transmission electron microscopic examination. **Results:** There was a significant increase of blood glucose level in rats fed with GM corn (Group II). Moreover, evident structural changes in the pancreatic acinar cell as evident by interstitial edema, irregular nuclei with dilated perinuclear space, dilated rough endoplasmic reticulum, vacuolation and lysosome-like lamellated structure, decreased electron-dense secretory granules, and formation of apoptotic bodies. Examination of β-cells revealed evident destruction and decreased secretory granules. **Conclusion:** A diet containing significant amounts of GM corn seems to influence zymogen synthesis in pancreatic acinar cells as well as β-cells function

**Key words:** Genetically modified corn, genetically modified organism, pancreatic acinar cell, rats, β-cells

## INTRODUCTION

The exocrine pancreas is an essential organ for food processing. Both functional and structural modifications of pancreatic acinar cells have been described in relation to dietary changes. In addition, endocrine pancreas regulates the blood glucose level (Lee *et al*., 2006; Brannon, 1990).

Genetic modification of foods is an area of biotechnology that is developing very rapidly with many potential

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applications. Genetically modified organisms (GMOs) have widespread applications as they are used in biological and medical research, production of pharmaceutical drugs, experimental medicine, and agriculture (Rodenhiser and Mann, 2006; Trojer and Reinberg, 2006; Jones and Baylin, 2007; Charu *et al*., 2011).

The use of gene technology in food production has become interesting due to increased needs of food as well as its improved quality. Among the different GMOs, in recent years, GM plants (GMPs) have attracted a large amount of media attention. You have been eating GMOs in steadily increasing amounts since 1996. Most genetic modification of foods has primarily focused on cash crops in high demand by farmers such as corn, soybean, cottonseed oil, and sugar cane. GMOs are now present in the majority of processed foods. Commercialized genetically modified (GM) corn which is present in food and feed in the world, has been modified to be tolerant to the broad spectrum herbicide Roundup (glyphosate) and thus contains residues of this formulation, others engineered to synthesize two different Bt toxins used as insecticide and vitamin – enriched corn with increase in β carotene, Vitamin C, and folate (Seralini *et al*., 2007; Mishra *et al*., 2009; Domingo and Gine Bordonaba, 2011; Seralinin *et al*., 2011; Hardisty *et al*., 2013).

As with any new food technology, the safety of the products derived from this technology must be assessed. To date, the studies related to GMOs have been performed on animals, with consistent and documented toxicity, including immune dysregulation (asthma, allergy, and inflammation), accelerated aging, infertility, dysregulation of genes associated with cholesterol synthesis, insulin regulation as well as altered structure and function in the liver, kidney, spleen, and gastrointestinal tract. A very few data are available about the response of exocrine and endocrine pancreas to GM food intake (Celec *et al*., 2005; Sanvido *et al*., 2007; Seralini *et al*., 2012; Snell *et al*., 2012.

With a view to address this lack of information, we have performed this work to investigate the possible effects rising from GM corn on pancreatic acinar cell and β-cells on rats.

## MATERIALS AND METHODS

This study was carried out on twenty adult male albino rats, each of an average weight ranging from 150 to 200 g, and of age about 3 to 4 months. The animals were maintained under standard laboratory conditions of temperature and humidity and 12 h light/dark cycle. Guidelines for care and use of animals, approved by the Animal House Center, Faculty of Medicine, University of Alexandria, were followed.

In the experiment, the animals were randomly divided into two groups:

- Group I (Control Group): Ten rats fed with non-GM corn with water *ad libitum* for 3 months
- Group II (experimental group): Ten rats fed with GM corn for 3 months (Seralini *et al*., 2012; Hardisty *et al*., 2013).

### Biochemical Study

At the end of the experiment blood samples from all rats were collected and blood glucose level was assessed.

#### *Collection of blood samples*

Rat fasting glucose level was estimated after an overnight fast. Blood was collected from the tail vein. Blood samples were then taken after 2 h following the oral administration of glucose solution (3 g/kg b.wt). The rat-tail was immersed in warm water (40°C) for 2 min. The tip of the tail was cut using a new scalpel blade. Blood was collected into heparinized capillary tubes by gently squeezing the tail. Then, the capillary tube was centrifuged in a microcentrifuge for 5 min at 3000 rpm. Blood glucose level was estimated according to the method of (Siest and Schielef, 1981).

### Histological Study

The animals were sacrificed, and the pancreas of all rats was taken and cut into two specimens as follows:

1. The first specimen was fixed in 10% formal saline and processed to get 6 $\mu$m thick paraffin sections. The histological sections of the pancreas were stained with hematoxylin and eosin stain and Gomori's Trichrome stain (Carletons *et al*., 1980)
2. The second specimen was cut into small pieces (1/2–1 $mm^3$) and immediately fixed in 3% glutaraldehyde solution and processed to get ultrathin sections for transmission electron microscopic examination (Bozzola and Russell, 1992).

### Statistical Analysis

The data were analyzed using one-way analysis of variance followed by Least Significant Difference analysis to compare various groups with each other results were expressed as mean ± standard deviation and values of $P > 0.05$ were considered statistically nonsignificantly different, while those of $P < 0.05$ and $P < 0.01$ were statistically significantly and highly significantly different, respectively (PC-STAT, 1995).

## RESULTS

During the experiment, all rats were carefully monitored for behavior, appearance, palpable tumors, and infections. No mortality was detected in all groups during the study period (3 months). There was neither rejection of the

diet with or without GM corn nor any major difference in the body weight.

## Biochemical Results

### *Blood glucose level*

Rats fed with GM corn showed a significant increase in fasting and postprandial blood glucose concentration as compared with the control group [Table 1].

## Histological Results

### *Light microscopic results*

#### Group I

Examination of specimens from the control rat pancreas (fed non-GM corn) showed normal histological structure of the pancreas consisting of the exocrine part formed of closely packed secretory acini with narrow lumina and endocrine part formed of islets of Langerhans which were scattered throughout the exocrine tissue. The pancreatic acini consisted of pyramidal cells with rounded, pale-stained nuclei surrounded by basophilic cytoplasm, and the apices of the cells were packed with acidophilic secretory granules [Figure 1a and b].

#### Group II

Examination of specimens of rat pancreas (fed with GM corn) revealed disturbance of the normal architecture of the pancreas as evident by interstitial edema, congested blood vessels, pyknotic nuclei of the acinar cells, cytoplasmic vacuolation, associated with decrease in number of β-cells in the center of the islets of Langerhans [Figures 2a, b and 3a, b].

As compared with Gomori's Trichrome stained control rat pancreas which revealed delicate collagen fibers around islets of Langerhans, around the pancreatic acini, and blood vessels [Figure 4a], the rat pancreas fed with GM corn revealed increased collagen fibers and congestion of the blood vessels [Figure 4b and c].

**Table 1: Blood glucose concentration values of control and rats fed with genetically modified corn after fasting and after 2 h (postprandial)**

| Groups | Fasting blood glucose | Postprandial blood glucose |
|---|---|---|
| Control group (Group I) | 79.17±16 | 107.25±31 |
| Experimental group (Group II) | 110.15±11 | 160.87±24 |

LSD at 5% level is 13.931, LSD at 1% level is 18.776. Data are expressed (10 rats in each group) as mean±SD. SD - Standard deviation, LSD - Least Significant Difference

### *Ultrastructural results*

#### Group I

Examination of specimens obtained from the control rat pancreas showed the exocrine acinar cells containing rounded nuclei with prominent nucleoli surrounded by numerous cisternae of rough endoplasmic reticulum (rER). Their apical poles were occupied by numerous electron-dense secretory granules of variable sizes. The lumina of the acini were narrow and contained microvilli [Figure 5a and b].

#### Group II

Specimens obtained from rat fed with GM corn revealed acinar cells with large vacuoles that appeared either empty or containing lamellated lysosome-like bodies, damaged mitochondria, and few electron-dense secretory granules [Figure 6a and b]. Other acinar cells showed dilated rER and perinuclear space, destruction of mitochondria [Figure 7a and b], and apoptotic bodies were frequently observed [Figure 7c].

## β-cells

Pancreatic islets of Langerhans of the control rats are characterized by a predominant proportion of insulin – producing β cells in the core of the cluster and scarce alpha, delta, and PP (gamma) cells in the periphery. The cytoplasm of β-cells contains numerous electron-dense secretory granules surrounded by wide lucent halo [Figure 8]. β-cells of rat fed with GM corn showed evident decrease of the characteristic secretory granules, vacuolation, and dilated rER [Figure 9a and b] on the other hand, the alpha cell showed many specific secretory granules [Figure 10a and b].

# DISCUSSION

Livestock and poultry demand for feeds is expected to grow in the next 50 years as food requirements increase parallel with the doubling of human population.

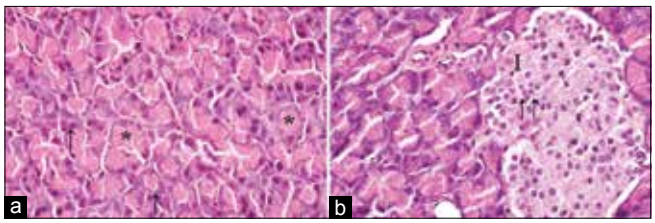


**Figure 1:** (a and b) Photomicrograph of the control rat pancreas (Group I) showing, pancreatic acini lined by pyramidal acinar cells with classical pattern of apical acidophilia (*) and basal basophilia (↑). Note pale stained islets of Langerhans (I) with numerous centrally located β-cells (↑↑) (H and E; Mic., ×400)

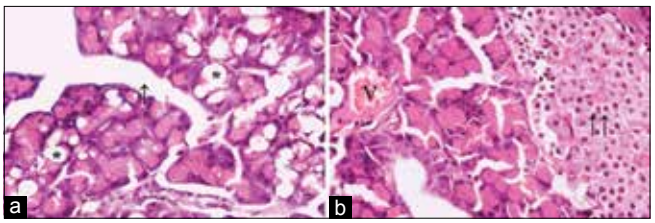


**Figure 2:** (a and b) Photomicrograph of rat pancreas fed with genetically modified corn for 3 months (Group II) showing, widely separated acini due to edema (↑), vacuolation (*), congested blood vessels (V) and few pyknotic nuclei of β-cells in the center of the islets of Langerhans (↑↑) (H and E; Mic., ×400)

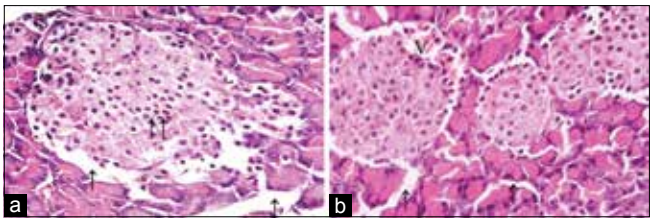


**Figure 3:** (a and b) Photomicrograph of rat pancreas (Group II) showing, evident destruction and loss of architecture of islets of Langerhans associated with decrease number and pyknotic nuclei of β-cells (↑↑) and congested blood vessels. Note the presence of edema (↑) between the acini and loss of normal architecture (H and E; Mic., ×400). V - Vessels

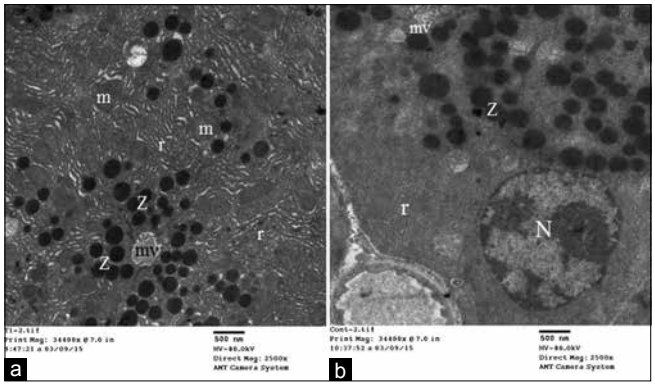


**Figure 5:** (a and b) Electron micrograph of the control rat pancreas (Group I) showing, pancreatic acinar cell with few apical microvilli projecting into the lumen, nucleus with prominent nucleolus, (N), rough endoplasmic reticulum, mitochondria, and electron-dense zymogen granules. mv - Microvilli, N - Nucleolus, r - Rough endoplasmic reticulum, z - Zymogen granules, m - Mitochondria

Nutrition enhancement in crops targets manipulation of the levels of proteins and amino acids, fats and oils, vitamins and minerals, carbohydrates, and fiber quality as well as decreasing the levels of undesirable components in major feed crops (Flachowsky *et al*., 2007; Brake *et al*., 2003).

Biotechnology offers a variety of potential benefits and risks. Once GM plants are released into the environment, they cannot be controlled or recalled. Genetic pollution is irreversible living pollution that self-replicates. Contamination of other plants is a major problem because the genes from any crop can move, through seed and pollen flow (Snell *et al*., 2012; FAO, 2009).

Plants are now engineered for insect resistance, fungal resistance, viral resistance, herbicide resistance, changed nutritional content, improved taste, and improved storage. Corn is GM for developing insect resistant plants. It is called Bt-corn because the insect-killing gene in the plant comes from the bacteria *Bacillus thuringiensis*. GM corn could pollute aquatic ecosystems. Pollen and other plant parts containing toxins from GM insect-resistant Bt-corn are washing into streams near cornfields and laboratory traits show that consumption of Bt-corn byproducts produced increased mortality and reduced growth in

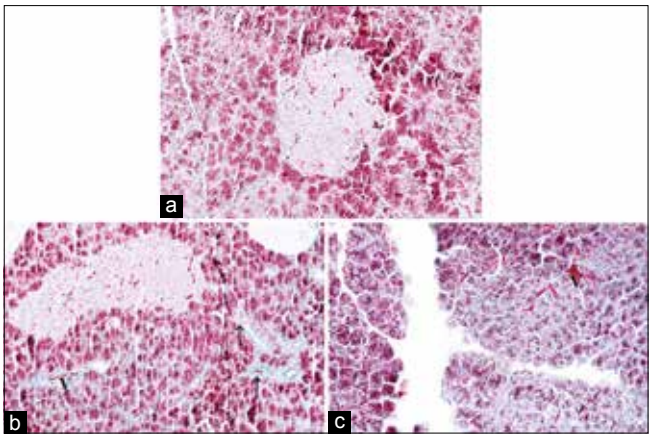


**Figure 4:** (a-c) Photomicrograph of rat pancreas (a) Control Group (Group I) revealing normal distribution of collagen fibers between the acini and in the islets of Langerhans. (b and c) (Group II) revealing increase collagen fibers (↑) and mild congestion of the blood vessels (V) (Gomori's Trichrome, ×200)

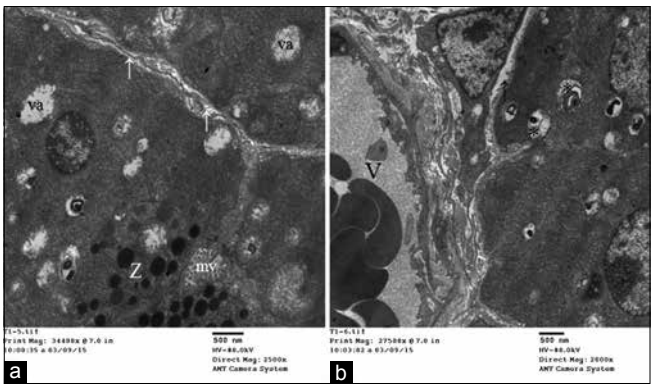


**Figure 6:** (a and b) Electron micrograph of rat pancreas (Group II) showing increase intercellular space (↑), microvilli projecting into the lumen (mv), cytoplasmic vacuolation, lamellated lysosome-like bodies (*) and few electron-dense zymogen granules. Note congested blood vessels. va - Vacuolation, mv - Microvilli, z - Zymogen granules, V - Vessels

caddisflies and aquatic insects that are related to the pests targeted by the toxin in Bt-corn (König *et al*., 2004).

GM corn is also utilized herbicide tolerance (glyphosate-tolerant genes) was isolated from a strain of *Agrobacterium*. These were inserted into the genome of the plant through a multistep process resulting in a plant that can withstand the direct application of the herbicide. The herbicide-tolerant plants absorb the poisons and become toxic to humans and domestic animals eat them. It was shown in the previous articles that there has been a huge increase in the amount of glyphosates applied to corn and soy crops. Glyphosate disrupts the endocrine system and the balance of gut bacteria; it damages DNA and is a driver of mutations that lead to cancer. Endocrine disruptors can lead to failure in all systems in the body that are controlled by hormones. Imbalances and malfunctions of the endocrine system can lead to diabetes, hypertension, and obesity (Seralini *et al*., 2007; Spiroux de vendomois *et al*., 2009; Spiroux de vendomois *et al*., 2010; Vandenberg *et al*., 2012).

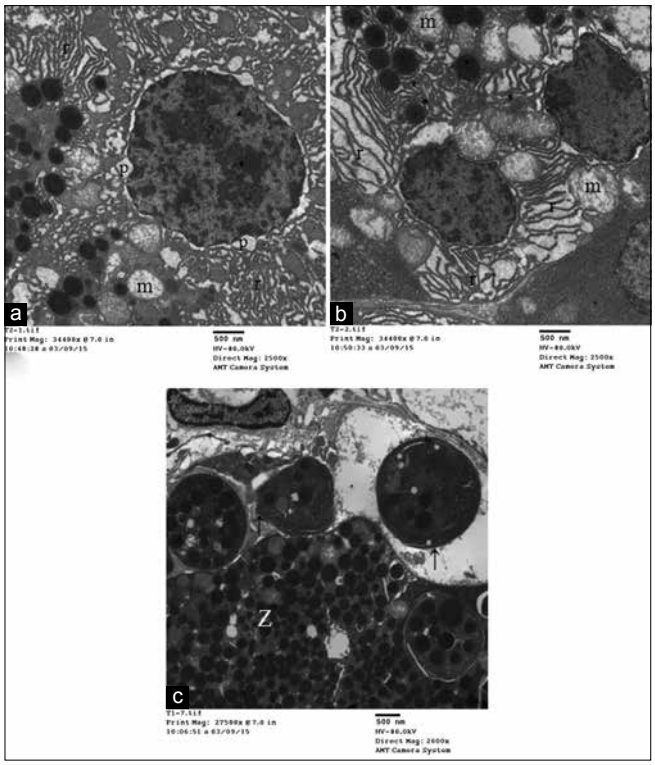


**Figure 7:** (a-c) Electron micrograph of rat pancreas (Group II) showing, (a and b) irregular nuclei with dilated perinuclear space, evident dilatation, degranulation, and retained secretion in rough endoplasmic reticulum associated with destructed mitochondria. (c) Many electron-dense zymogen granule and formation of several apoptotic bodies (↑). *P* - Perinuclear space, r - Rough endoplasmic reticulum, m - Mitochondria, Z - Zymogen granule

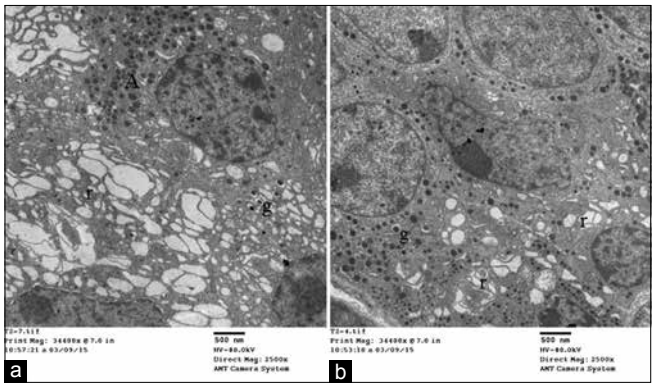


**Figure 9:** (a and b) Electron micrograph of rat pancreas showing β-cells in the islets of Langerhans with evident dilatation and degranulation of rough endoplasmic reticulum, marked decrease in specific secretory granules, and alpha cell with many specific granules. r - Rough endoplasmic reticulum, g - Specific secretory granules, A - Alpha cell with many specific granules

The data for corn and soy crops have been plotted against the incidence rates of cancers of the thyroid and liver (Chakraborty *et al*., 2007; Balley *et al*., 1976).

In the present study, it appeared that GM corn affects animal physiology with no gross modifications of indicators, such as body and organ weight. We investigated the role of GM corn intake in the induction of morphofunctional modifications in the pancreatic acinar cells and β-cells of albino rats.

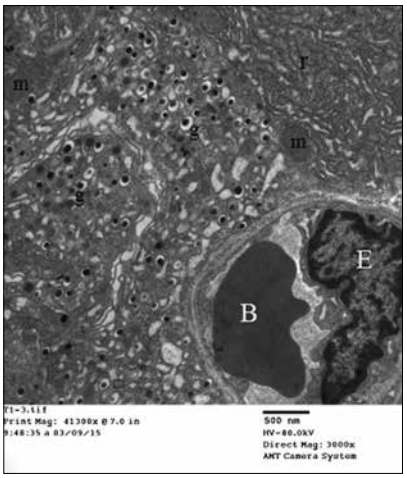


**Figure 8:** Electron micrograph of the control rat islets of Langerhans revealing β-cells with rough endoplasmic reticulum, mitochondria, specific secretory granules, and blood capillary lined with endothelial cell and contain red blood cell. r - Rough endoplasmic reticulum, m - Mitochondria, g - Specific secretory granules, E - Endothelial cell, B - Blood cell

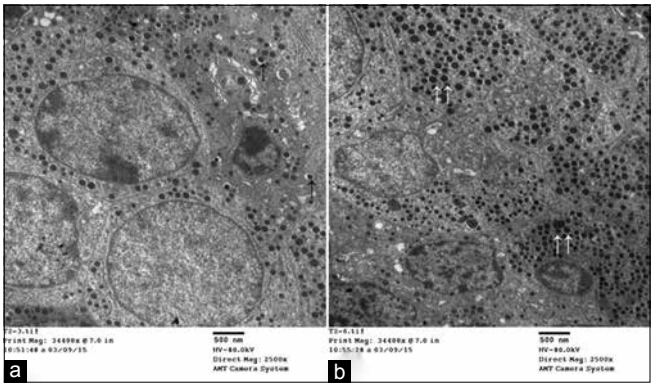


**Figure 10:** (a and b) Electron micrograph of rat pancreas (Group II) showing, alpha cell In the islets of Langerhans with many specific granules (↑↑) and β-cells with very few granules (↑)

In the present study, we described both nuclear and cytoplasmic changes in the pancreatic acinar cells of rat fed with GM corn. We demonstrated alteration in general organization and the fine morphology of pancreatic acinar cells as evident by irregular nuclei with dilated perinuclear space and large prominent nucleolus. The nucleolus is a very dynamic structure able to rapidly change its architecture in response to various stimuli including diet (Brannon, 1990, Schwarzacher and Wachtler, 1993, Lopez-Navarro *et al*., 1996, and Malatesta *et al*., 2005).

Large nucleoli are generally found in cells with a higher metabolic rate, which is also an index of increased rRNA synthetic rate (Schwarzacher and Wachtler, 1993), these modifications similar to those observed in animals fed with GM soybean. Interestingly, most of the effects of the diet observed in pancreatic acinar cells are comparable to those previously reported for hepatocytes submitted to the same experimental treatment, indicating that GM soybean can exert a similar influence mainly on

both RNA and protein synthesis/processing on different tissues. It is likely that the exocrine pancreas and the liver are especially sensitive to change in the diet due to their central role in the digestion and processing of food (Gasnier *et al*., 2010).

At the present study, no conclusive evidence exists as to the factor(s) which can induce the modifications observed. GM corn used in our study has been treated in the field with the herbicide Roundup (glyphosate – tolerance); it is worth considering the possible presence in the crops of traces of Roundup or its metabolites. Interestingly, Roundup has been demonstrated to interfere with nuclear functions as well as to alter many metabolic pathways (Gasnier *et al*., 2009; Mesnage *et al*., 2010).

Glyphosate is the primary active constituent of the commercial pesticide Roundup. It induces oxidative stress and activates multiple stress-response pathways. The pesticide increased intracellular $Ca^{2+}$ leading to $Ca^{2+}$ overload within the cells, which set off oxidative stress and necrotic cell death. In addition, stress response of the endoplasmic reticulum and/or depleted antioxidant defenses could contribute to oxidative stress and disruption of normal function (Walsh *et al*., 2000, Duke *et al*., 2003, Hammond *et al*., 2004, Ramudo and Manso, 2010, and Green *et al*., 2011), these findings in agreement with our findings which revealed dilation, degranulation, and retained secretion in the rER and associated with destruction of mitochondria and decrease zymogen granules.

Previous studies on mice fed on a GM soybean showed that changes in zymogen synthesis and processing as well as in the cell nuclear activity take place. Reduction in zymogen area was comparable to the results of (Malatesta *et al*., 2005) for mice chronically fed with a GM soybean diet.

This study hypothesized that the intake of GM corn induces pancreatic stress or injury by analyzing the cellular structure of pancreatic acinar cells as evident by edema between the acini, congested blood vessels, and loss of normal architecture of the pancreatic acinar cells. In line with these findings, the present study showed ultrastructural lesions of acinar cells in specimens of rat fed on GM corn such as dilation of rER, swollen, and damaged mitochondria as well as large vacuoles and lysosome-like lamellated bodies. The same findings were previously recorded by other investigators and were described as acinar cell necrosis. The formation of enlarged secretory vacuoles containing lysosomal and digestive enzymes is paralleled by the activation of lysosomes and degradation of cellular organelles in autophagosomes. This process represents the initial stage for acinar cell destruction and the development of pancreatitis (El-Bakary and Mousa, 2008; Gukovsky and Gukovasky, 2010; Gukovskaya and Gukovsky, 2011; Gukovsky *et al*., 2012).

As regards zymogen granules, the present study revealed variation of zymogen granules. Some acini showed decreased number and others retained their granular content associated with large vacuoles, these findings were also recorded by other investigators who stated that the acinar cells adjacent to fat necrosis (De Lisle, 2005).

They suggested that one of the basic defects in pancreatitis is the uncontrolled release of enzymes into the interstitial space which, in turn presumably by the action of lipase, leads to autodigestive fat necrosis. Moreover, vascular damage and widening of the interstitial tissue were also frequent observations. In this regard, it was reported that chemical mediators such as free radicals which are produced by vascular damage may accelerate the activation of zymogen protease in acinar cells leading to pancreatitis (Degirmenol *et al*., 2005; Yildiz and Hamaloglu, 2010).

The present study revealed a significant increase in fasting and postprandial blood glucose level compared with the control group and structural changes in β-cells confirmed these results as evident by obvious dilation of rER and decreased number of specific granules. Degirmenol *et al*. (2005) reported a decrease in secretory granules of β-cells, vacuolation, and swelling of mitochondria in alloxan-induced damage of β-cells, and demonstrated that mouse β-cells showed signs of both necrotic and apoptotic cell death. Bogolepov, (1983) stated that vacuolation was one of the structural indications of permeability disorders of the membranes, which results in an enhanced transport of water and electrolytes into the cell. The permeability disorder could be attributed to many cellular membrane insults caused by reactive oxygen species-mediated formation of lipid peroxides, which generate self-sustaining lipid peroxidation. It has been found that oxidative stress is associated with the molecular mechanism of the decreased insulin biosynthesis and secretion, which is the main etiology of glucose toxicity. Indeed, it was suggested that the pancreas may be more susceptible to oxidative stress than other tissues and organs because pancreatic islets cells show extremely weak manifestation of antioxidative enzymes (Evans *et al*., 2003).

It is evident that oxidative stress plays a key role in causing insulin resistance and β-cells dysfunction by their ability to activate stress-sensitive signaling pathways (Robertson 2006).

## CONCLUSION

A diet containing significant amounts of GM corn seems to influence zymogen synthesis in pancreatic acinar

cells as well as β-cells function. The reasons leading to these modifications remain not well known, and further investigations are needed. Our data presented here strongly recommend that additional long-term (up to 2 years) animal feeding studies be performed, preferably also multigenerational, to provide true scientifically valid data on the acute and chronic toxic effects of GM crops, feed, and foods.

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Nil.

### Conflicts of Interest
There are no conflicts of interest.

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