

## THE BIOCHEMICAL AND HISTOLOGICAL CHANGES OF HEPATOCYTES OF ADULT MALE ALBINO RATS FED ON GENETICALLY MODIFIED CORN

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### ABSTRACT

**Introduction:** Genetically engineered foods saturate our diet today. A food safety assessment of genetically modified corn has been performed by different researchers on nutrition, allergenicity and toxicology analyses in agreement to the substance equivalence principle, and only few were supported by histopathological confirmations.

**Aim:** is to study the biochemical and histological changes of hepatocytes of adult male albino rats fed on genetically modified corn.

**Materials and methods:** Twenty adult male albino rats were randomly classified into two main groups: Group I: (control group) that were given standard diet for three months. Group II: (genetically modified corn group) were fed 50% genetically modified corn and 50% standard diet for three months. At the end of experimental period, analysis of aspartate aminotransferase (AST)/ serum glutamic oxaloacetic transaminase (SGOT) and alanine aminotransferase (ALT)/ serum glutamic-pyruvic transaminase (SGPT) were done. Fresh specimens were taken from the right lobe of liver of all rats for light microscopic examination, Gomori's trichrome stain, Best's carmine stain, Aoyama's stain and Electron microscopy.

**Results:** In the current work, the mean of ALT and AST in group (II) was significantly increased compared to that of control group. Light microscopic examination of the histological sections of group II showed, diffuse affection of the hepatic lobules. Gomori's trichrome revealed newly formed collagen fibers in the portal tract. Best's carmine stain showed diffuse increase in glycogen granules in the majority of hepatocytes. Aoyama's stain revealed dilatation and proliferation of bile canaliculi. Electron microscopic examination revealed evident ultra-structural changes.

**KEY WORDS:** Genetically modified corn (GMC), Hepatocytes, Albino Rats.

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### INTRODUCTION

Genetically built foods saturate our diet these days. Plant foods created through gene-splicing, as well as soybean, maize, canola, rice, and potatoes, have already reached the consumer business center. But, the 2 most developed

metric weight unit crops are maize and soybean, that represent the staple constituents of the many foods [1,2]. In several health edges there lies associate evergrowing concern that manufacturing overseas genes into food plants might have associate sudden and negative result on

human health. A recently offered study examined the results of metric weight unit potatoes on the duct in rats. This analysis explicit composition, nutrition, allergenicity and materia medica analyses in agreement to the substance equivalence principle, and solely few were supported by histopathological confirmations[3-5].

**Aim:** The aim of the present work is to study the biochemical and histological changes of hepatocytes of adult male albino rats fed on genetically modified corn.

## MATERIALS AND METHODS

This study was carried out on twenty adult male albino rats, each of average weight ranging from 150-200 g and 6-8 weeks of age. The rats were obtained from animal house of Al - Mouwasat Hospital. The animals were maintained under standard laboratory conditions of temperature and humidity and 12 hours light/dark cycle. The present study was approved by the Ethical guidelines of Alexandria University on laboratory animals and the national institute for the care and use of laboratory animals (NHI Publications No80-23, revised 1978). Further the Alexandria faculty of medicine ethical committee approval was obtained.

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

**Group I:** (control group) that were given standard diet (milk, bread and tap water) for three months.

**Group II:** (genetically modified corn group) were fed 50% genetically modified corn and 50% standard diet (milk & bread) for three months.

### Biochemical study of liver functions tests:

At the end of experimental period (three months), the animals were sacrificed after ether anesthesia. After the sacrifice of the rats, trunk blood samples of all animals were collected in plain tube vacutainers and allowed to stand for 30 minutes to clot. Serum was then separated by centrifugation 300×g for 10 min and frozen at -80 °C until analysis of aspartate aminotransferase (AST)/ serum glutamic oxaloacetic transaminase (SGOT) and alanine aminotransferase (ALT)/ serum glutamic-pyruvic transaminase (SGPT). Enzyme activity was expressed in

International Units per liter (I.U. /l). The analysis was checked for accuracy by concurrent analysis of control sera for ALT and AST [6].

### Histological study

#### Light microscopic study:

11 Fresh specimens were taken from the right lobe of liver of all rats.

**One piece** was fixed in 10% formol saline and processed to get 6 μm thick paraffin sections. These sections were stained with:

**Haematoxylin and Eosin (H&E) stain**[7, 8].

**Gomori's trichrome stain**:[7, 8]

It was used for demonstration of collagen.

**Second piece** was fixed in absolute alcohol, processed to get 6 M thick paraffin sections. These sections were stained with:

**Best's carmine stain**[7, 8]: It was used for demonstration of glycogen in the liver.

**A third piece** was fixed in Aoyama's fluid and processed to get 6 M thick paraffin sections. These sections were stained with Aoyama's stain.

**Aoyama's stain**[7, 8]: It was used to demonstrate the bile canaliculi.

**Electron microscopic study:** The last piece was cut into small pieces (1/2- 1 mm<sup>3</sup>) and immediately fixed in 3% glutaraldehyde solution and processed to get ultrathin sections for transmission electron microscopic examination [8, 9].

The grids were examined and photographed by (Jeol 100 CX, Tokyo, Japan) transmission electron microscope at the Electron Microscopy Unit, Faculty of Science, University of Alexandria.

**Statistical analysis of the data:** Data were fed to the computer using IBM SPSS software package version 20.0. Quantitative data were described using mean and standard deviation (S.D.). For normally distributed data, comparison between more than two populations were analyzed using F-test (ANOVA) and Post Hoc test (Scheffe). Significance of the obtained results was judged at the 5% level [10, 11].

## RESULTS

### Biochemical results:

#### Biochemical study of Liver Functions Tests and statistical analysis:

**Serum ALT:**

**Table 1:** Comparison between the studied groups regarding ALT.

| ALT            | Group I | Group II |
|----------------|---------|----------|
| Range          | 55-85   | 149-540  |
| Mean           | 67.5    | 302.6    |
| S.D.           | 18.9    | 102.6    |
| F              | 85.65   |          |
| P              | 0.001*  |          |
| P <sub>1</sub> | 0.0001* |          |

F = ANOVA test

P is significant if < 0.05

P1 value for Post Hoc test (Scheffe) for comparison between group I and group II

\*: Statistically significant at  $p \leq 0.05$ .

The determination of plasma (ALT) and (AST) in rats fed on genetically modified corn showed a significant increase of ALT&AST in group II after 3 months.

**Serum AST:**

**Table 2:** Comparison between the studied groups regarding AST.

| AST            | Group I | Group II   |
|----------------|---------|------------|
| Range          | 35-62   | 92.5-420.0 |
| Mean           | 52.6    | 265.6      |
| S.D.           | 11.23   | 56.9       |
| F              | 105.6   |            |
| P              | 0.0001* |            |
| P <sub>1</sub> | 0.0001* |            |

F = ANOVA test

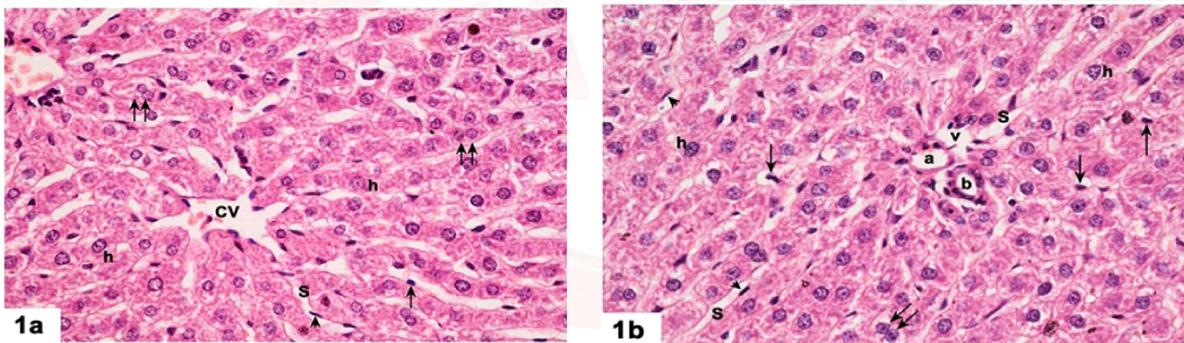
P is significant if < 0.05

P1 value for Post Hoc test (Scheffe) for comparison between group I and group II

\*: Statistically significant at  $p \leq 0.05$ .

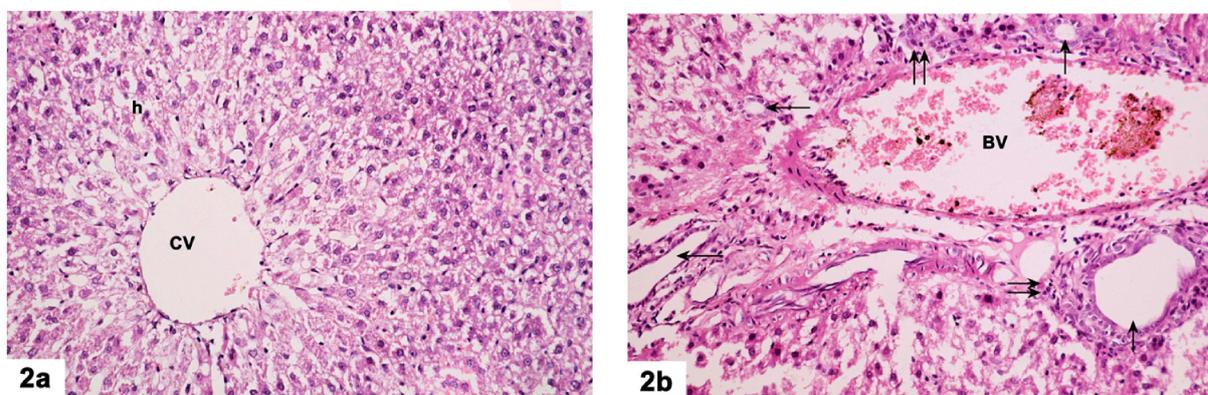
**HISTOLOGICAL RESULTS:**

**Light microscopic results**

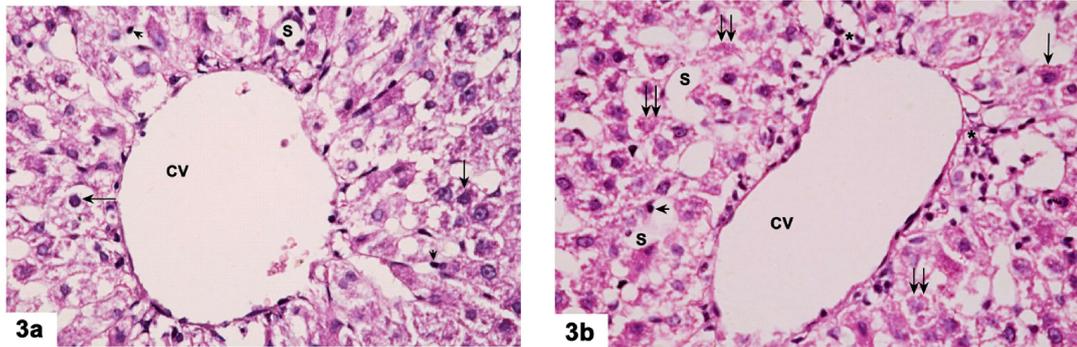


**Fig. 1 a, b:** Photomicrograph of the control rat liver showing, hepatocytes (h) arranged in cords radiating from the central vein (CV). They are polyhedral in shape with slightly vacuolated acidophilic granular cytoplasm and vesicular nuclei. Blood sinusoids (S) separating the hepatic cords are seen lined by endothelial cells (▲) and few Kupffer cells (↑). Note portal tract with its structures, branches of portal vein (v), hepatic artery (a), bile duct (b). Binucleated hepatocytes are also seen (↑↑). (H&Estain, Mic.Mag. x 400)

• **Group II (Experimental group)**

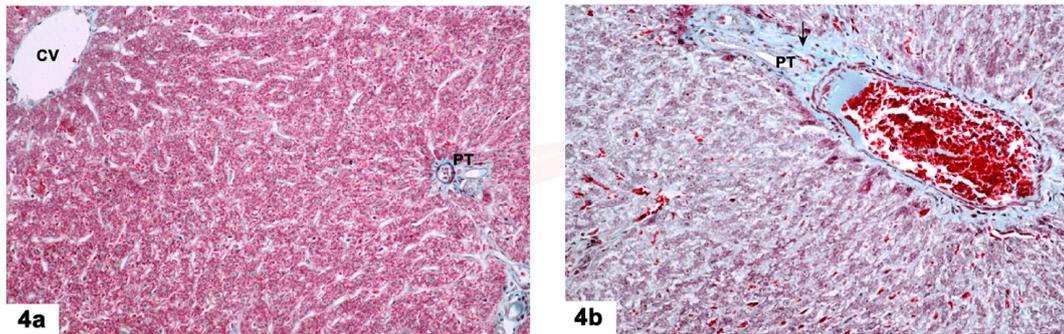


**Fig.2 a, b:** Photomicrograph of rat liver (group II) showing, evident disorganization of hepatic architecture. Hepatocytes (h) appear vacuolated. Notice proliferation of bile duct (↑), congested blood vessels (BV) and some cellular infiltration in the portal tract (↑↑). CV: central vein (H&Estain, Mic.Mag. x100)



**Fig.3 a, b:**Photomicrograph of rat liver (group II) showing, extensive vacuolation of most of hepatocytes. Some cells show dark nuclei (↑), others reveal karyolytic nuclei (↑↑).Note prominent nuclei of kupffer cells (▲), cellular infiltration (\*) and dilated central vein (CV). S: sinusoid (**H&Estain, Mic.Mag. x 400**)

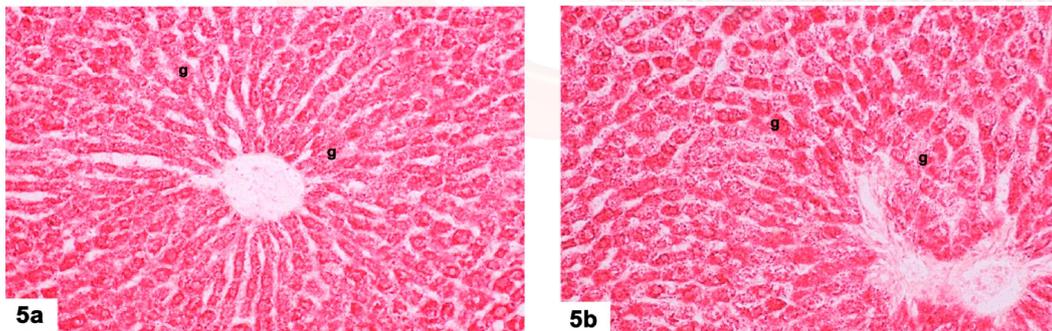
**Gomori's trichrome stain:**



**Fig.4 a:** Photomicrograph of the control rat liver showing normal distribution of collagen fibers in the portal tract (PT) and surround the central vein (CV).

**Fig.4 b:**Photomicrograph of rat liver group II showing newly formed collagen fibers (↑) in the portal tract (PT). (**Gomori's Trichrome stain, Mic.Mag X 100**)

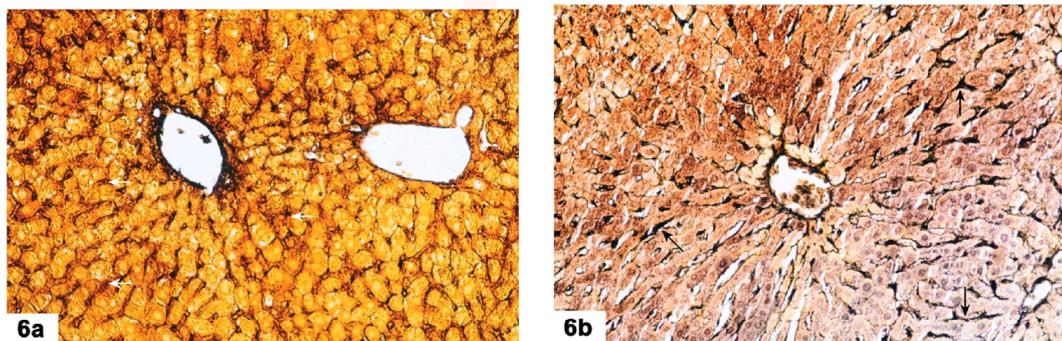
**Best's carmine stain:**



**Fig.5a:**Photomicrograph of the control rat liver showing, normal distribution of glycogen granules (g) within the liver cell.

**Fig.5b:**Photomicrograph of rat liver group II showing diffuse increase in glycogen granules (g) within the liver cells. (**Best's carmine stain, Mic.Mag. X 100**)

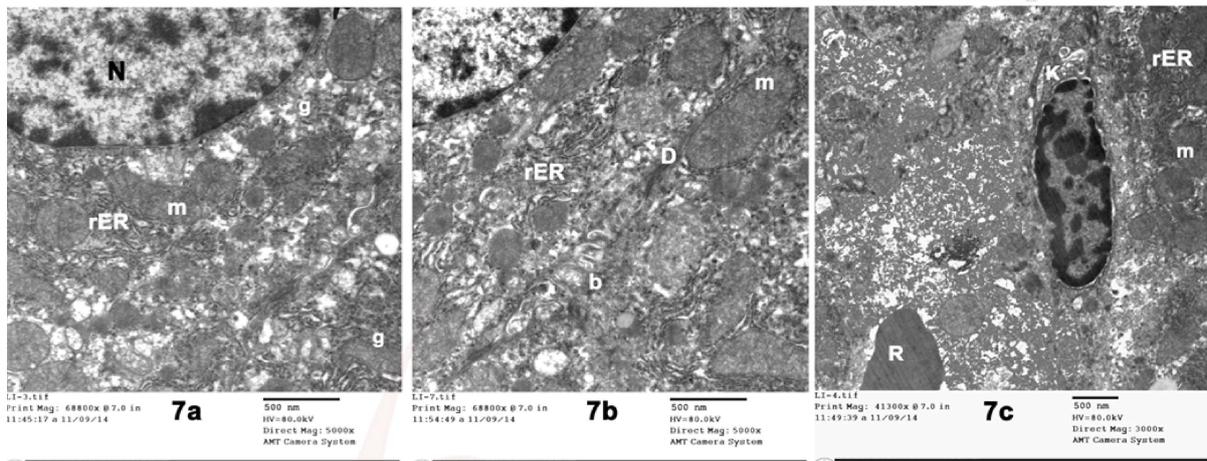
**Aoyama's method for bile canaliculi:**



**Fig.6 a:**Photomicrograph of the control rat liver showing, bile canaliculi ( ↑) in the form of brown narrow spaces in between hepatic cords.

**Fig.6 b:**Photomicrograph of rat liver group II showing, dilatation and proliferation of bile canaliculi (↑). (**Aoyama's method for bile canaliculi x 100**)

## ELECTRON MICROSCOPIC RESULTS: GROUP I (Control Group)

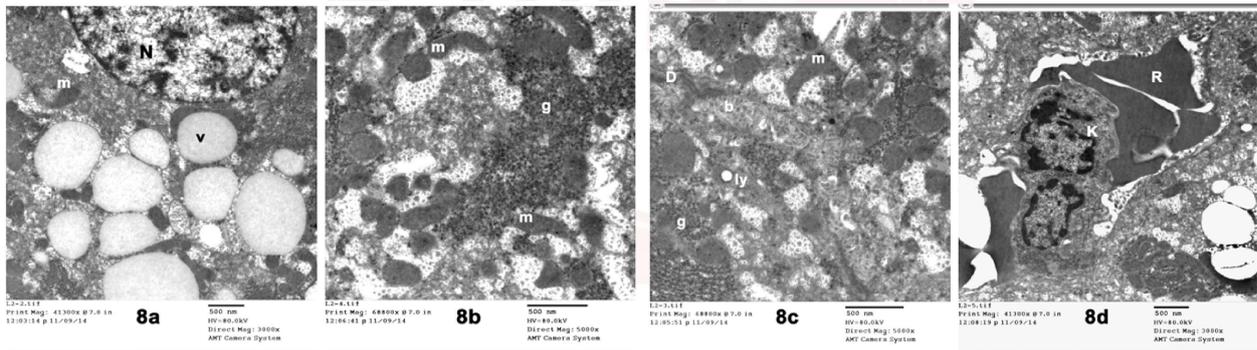


**Fig.7a:**Electron micrograph of the control rat liver showing, normal appearance of hepatocytes with euchromatic nucleus (N) of regular outline. The cytoplasm shows numerous mitochondria (m), rough endoplasmic reticulum (rER) and glycogen granules (g).

**Fig. 7b:**Electron micrograph of the control rat liver and its bile canaliculi. Higher magnification revealed two adjacent hepatocytes enclosing a bile canaliculus (b) with microvilli protruding into the lumen and bounded by desmosomes (D), numerous mitochondria (m) and (r ER).

**Fig.7c:**Electron micrograph of the control rat liver showing, red blood cell (R) and a kupffer cell (K) lining the sinusoid. Part of hepatocyte is seen, its cytoplasm shows mitochondria (m) and r ER.

## Group II (Experimental Group)



**Fig.8a:**Electron micrograph of rat liver group II revealing markedly affected hepatocytes. The cytoplasm of the cells reveals many vacuoles (v) and mitochondria with dense matrix (m).N: nucleus

**Fig. 8b:**Electron micrograph of rat liver of group II showing, evident accumulation of glycogen granules (g) and pleomorphic mitochondria with dense matrix (m).

**Fig. 8c:**Electron micrograph of rat liver of group II showing, proliferated bile canaliculi between two adjacent hepatocytes (b) bounded with desmosomes (D), marked accumulation of glycogen granules (g), pleomorphic mitochondria with dense matrix (m) and numerous lysosomes (ly) are seen near the bile canaliculus.

**Fig.8d:**Electron micrograph of rat liver group II revealing congested blood sinusoids (R) with evident kupffer cells (k).

## DISCUSSION

The worldwide populace is consistently developing, while the measure of arable land is consistently diminishing [12].

Advances in genetics and molecular biology have empowered the development and commercial release of genetically modified organisms (GMOs) with attributes that rise above the species boundaries[13].

As the predominance of genetically modified

organisms (GMOs) keeps on ascending, there has been an increasing public interest for the wellbeing of these products. How the GMO may influence the environment or may affect the consumer are the major public concerns. The advantages resulting from the utilization of GMO result in increased benefit for producers and provide therapeutic products. The adverse influence of GMOs on human health could result from differences in dietary substance, allergic response, or undesired side effects such

as toxicity, organ damage, or gene transfer. There have been over hundred research studies comparing the effects of traditional food to genetically modified food, the results of which have been reviewed in various journals [14, 15]. The effects of GMO to purchasers' health and life are the potential for synthesis in their cells and tissues of poisonous products or products activating neoplastic processes. Genetically modified food is responsible for food allergies and resistance to antibiotics. The presence of dangers related with a broad utilization of GMO provides the basis for criticism from the side of biotechnology opponents [16, 17].

Novel pesticide residues contained in GM corn will be available in food. Subsequently, the potential consequences on physiological parameters, due either to the perceived mutagenic impacts of the GM change process or the above mentioned novel pesticides present within these plants can be assessed in animal feeding studies [18].

The present study aimed to explore subchronic toxicological impacts of these GM cereals in mammals, as it utilized in vivo tests performed on mammals consuming these GMOs. The animals were checked for blood and organ parameters. In the current study, the liver was chosen since it is the largest metabolic parenchymatous organ in the human body, and it is highly susceptible to toxic injury due to its essential role in the metabolism of foreign substances [19]. The liver is a good indicator of nutritional pathology because of its capacity in processing items originating from the digestive tract [20].

Animal experiments are important and give important data about the safe use of a GM plant by human [15].

In the present study, the blood sera were separated to evaluate ALT and AST enzymes activity. According to Oraby et al., [13] biochemical analysis of AST and ALT activity revealed significant differences between the control animals and the animals fed on the GM diet.

Evident increase in the levels of serum AST and ALT was observed in rats fed the GM diet for 3 months. Increased levels of these two transferases are known to be an indicator of liver

damage which was induced in the current work by the GM diet. This biochemical results were in perfect agreement with work done by some researchers. Limdi and Hyde [21] and Pratt and Kaplan [22] reported significantly higher plasma activities of ALT in female rats fed GM rice, while Poulsen et al., [23] revealed insignificant effects. Walsh et al., [24] revealed slightly increased ALT and AST in cows fed on GM maize during gestation.

Malatesta et al., [25] found slight modification in ALT and AST activity in mice fed on GM soybean compared to a non-GM diet. Chemistry measurements including ALT activity and creatinine levels done by Séralini et al., [26] revealed signs of hepatorenal toxicity in rats fed on GM maize.

The experimental subgroup showed various degrees of structural changes in the liver of animals receiving GMO food. These histological changes were marked and drastic in liver specimens taken from animals that received GM corn for 3 months (group II) as compared to the control group. The most common changes observed in the liver were: hepatocytes vacuolation, liver fatty degeneration, changes in metabolic activity as detected by liver function tests, changes in liver parenchyma and necrosis.

The results of the current study can be clarified on basis of when liver is utilized as organ of choice in histopathology, challenges can happen since the liver is very sensitive to any source of contamination. Cells exposed to numerous damaging substances causing similar cellular swelling and vacuolar appearance within the cytoplasm of cells and this was called vacuolar degeneration or Non-alcoholic fatty liver disease (NAFLD). The pathogenesis of fat aggregation in the liver stays to be clarified. Many hypotheses have been suggested recently. The first hypothesis (the "two hit") hypothesis, ("the first hit") prompts increase free fatty acid (FFA) motion to the liver. Hepato-steatosis creates if FFAs are not oxidized or secreted. This hepatic steatosis predisposes the liver to "second hits" such as mitochondrial dysfunction, cytokines, adipokines, endoplasmic reticulum (ER) stress and bacterial endotoxins. Lipotoxicity make the liver vulnerable to injury

by “various equal hits” oxidative damage, activation of fibrogenic pathways, activation of hepatic stellate cells, altered expression of adipokines which end to steatosis and fibrosis[27].

The present work demonstrated that liver histology is inevitable in nutrition studies. They also showed an increase in glycogen deposition in hepatocytes of rats fed with corn. These findings were in agreement with Poleksiæ et al.,[28]Raškoviæ et al., [29]who revealed increase in glycogen in sea bass, fed the pea meal diets.

In the current work, ultra-structural examination of liver specimens of group II confirmed the light microscopic results. Most liver cells of animals of group II showed markedly affected hepatocytes with irregular electron dense nuclei. The cytoplasm showed marked rarefaction compressing the organelles around the nucleus or towards the periphery of the cell membrane. It revealed many vacuoles, pleomorphic mitochondria with dense matrix and partial degranulation and dilatation of rough endoplasmic reticulum. Many lysosomes in some liver cells were seen especially close to dilated bile canaliculi.

The cytoplasmic rarefaction observed in the studied group represents the sign of cell swelling. As cell increases in volume, the cytoplasm is diluted without a related increase in cytoplasmic organelles. Electron lucent territories of cytoplasmic matrix separate the organelles and inclusions with an apparent loss of free ribosomes, endoplasmic reticulum and glycogen[30].

In the current work, the experimental group revealed affection of the nuclei of hepatocytes by light and electron microscopic examination. The cells showed irregular dense eccentric nuclei. Karyolytic nuclei were observed in some cells. The fading and the dense eccentric nuclei indicated irreversible damage and death of liver cells.

These findings were parallel with Malatesta et al., [25] who observed that a GM herbicide-tolerant soybean available on the market was used to feed mice, it caused the presence of irregular hepatocyte nuclei, a lot of nuclear pores,

various little fibrillar centers, and plentiful dense fibrillar indicating increased metabolic rates. The herbicide deposits could be the cause for that because this specific GM plant can absorb these chemicals. Such chemicals may be the cause of the previously mentioned pathological features. This became even clearer when similar features in rat hepatic cells were provoked by Roundup residues directly in vitro[31, 32]. The reversibility observed in some instances for these parameters in vivo [33] might be clarified by the heterogeneity of these chemicals in the feed. Anyway, these are specific features of ultra-structural dysfunction, and the relevance is clear[34].

Histological changes in the kidneys and the liver of rats were confirmed in some studies with maize after GM feed consumption. Such changes involve congestion, cell nucleus border changes, and severe granular degeneration within the liver [35]. The results exhibit potential adverse impacts in hepatic metabolism. The pesticide created by GM plants may additionally induce liver reactions, like several different pesticides[36].

In the present study, histological examination of liver sections showed nuclear changes in hepatocytes within the variety of karyorrhexis in the hepatocytes of rats fed the GM diet for 3 months. This was in accordance with Malatesta et al.,[37] Vecchio et al., [38] who observed disturbed liver tissue further as abnormally formed liver cell nuclei and nucleoli in mice fed on GM soy, that shows increased metabolism and probably altered patterns of gene expression. Malatesta et al.,[25] detailed that crucial alteration of some nuclear characteristics and activities in the hepatocytes of GM-fed mice. Malatesta et al., [32] reported that GM soybean feeding may impact some liver options during aging. They verified that senescence pathways were considerably activated in GM-fed mice. El-Shamei et al.,[39]stated that rats fed on GM corn for 90 days histological changes in the testis, kidney and liver were determined. Kiliç and Akay[35] mentioned that rats fed GM Bt maize over 3 generations suffered minor injury to the liver and kidneys and minor changes in blood biochemistry. What is more, De Vendomois et al [18] reported that the changed

maize varieties had toxic impacts on the liver and kidney in mammals. The changes in the liver, an organ accountable for biotransformation and detoxification, suggest modifications within the metabolic procedures, as reported by Malatesta et al.,[40]. Many proteins belonging to hepatocyte metabolism and stress response were differentially expressed in GM fed mice[32]. Within the live of oxidative stress in liver tissue, Del Rio et al., [41] determined that malondialdehyde (MDA) concentration was considerably higher in all animals fed the GM diet for 30, 60, and 90 days. Guet al.,[42] suggested that Bt maize will raise oxidative cellular stress in immunized salmon, Trabalza-Marinucci et al.,[43] declared that female sheep fed GMBt maize more than 3 generations demonstrated disturbances within the digestive system function, whereas their lambs had cellular changes within the liver and pancreas.

In the current work, biliary duct proliferation and bile canalicular dilatation within liver lobules were prominent in light and electron microscopic findings. Similar injuries were accounted with different toxins causing oxidative stress in the liver which is due to increased mitotic activity in the bile ductules and smaller bile ducts with the corresponding increase of their number [44]. The bile ductule enters in the injured peripheral parts of the lobule to build up the pathway for the bile drainage interrupted by the death of the peripheral cells and disintegration of their bile canaliculi. This clarification was accounted by Fawcett,[45] as a steady finding in all periportal hepatic insults. These findings were also in accordance with Choi et al.,[46] who demonstrated that nanoparticles larger than 5.5 nm cannot be excreted from the rat body through kidneys; instead they passed away from the body via the bile. Dragoni et al.,[47] clarified this finding on the basis that uptake of AuNPs by the hepatocyte happens through the endosomal pathway up to dense lysosomes that grouped around the dilated biliary canaliculi.

Cholestatic liver injury is one of the significant reasons of liver fibrosis and cirrhosis in patients with acute or chronic liver disease. Mouse models had been used to analyse the pathophysiological processes prompting cholestatic liver injury and often focus on its causes [48].

In the current study, light and electron microscopic examination of the experimental group revealed the presence of apoptotic cells and membrane bounded apoptotic bodies between the severely affected hepatocytes. The presence of apoptotic bodies might be due to the potential role of ROS and free radicals as mediators for apoptotic cell death. Pan et al.,[49] Kroemer et al.,[50] described that oxidative stress was related to protein and lipid oxidation, causing a significant modification in mitochondrial function that was responsible of cell death.

The present study revealed affection of the mitochondria in the form of dense matrix with pleomorphism in shapes and sizes. These findings were in parallel with Robinson et al., [51] who studied over a long-term (24-month) period the impact of GM soy fed by mice. They demonstrated more acute signs of ageing in the liver in comparison to the control group in the form of changes in the expression of proteins relating to hepatocyte (liver cell) metabolism, stress reaction, and calcium signaling.

In the present study, congestion of central hepatic veins, portal vessels and hepatic sinusoids was also seen in the present light and ultra-structural study and this could be attributed to the reactive oxygen species (ROS) induced by GM foods.

Overall, the present findings suggested that GM corn provoked drastic histological and biochemical changes in the rat hepatocytes in comparison to the control group.

## CONCLUSION

Genetically modified corn induced evident damage in hepatocytes of adult male albino rats.

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**Ethical approval** The present study was approved by the Ethical guidelines of Alexandria University on laboratory animals and the national institute for the care and use of laboratory animals (NHI Publications No80-23, revised 1978). Further the Alexandria Faculty of Medicine ethical committee approval was obtained.

**Conflict of Interest:** The authors proclaim that there is no conflict of interests regarding the publication of this paper.

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