Histopathological Changes in Some Organs of Male Rats Fed on Genetically Modified Corn (Ajeeb YG)

El-Shamei[1], Z. S., Gab-Alla[1], A.A., Shatta[1], A. A, Moussa[2], E. A. and Rayan[1], A. M.

[1] Food Technology Department, Faculty of Agriculture, [2] Department of Anatomy and Embryology, Faculty of Veterinary Medicine, Suez Canal University, Ismailia, Egypt. ammrayan@yahoo.com

Abstract: Ajeeb YG is a genetically modified (GM) insect resistant corn produced by incorporated the MON 810 (Monsanto) borer resistance trait in the best corn germplasm Ajeeb. The safety of Ajeeb YG corn was assessed by comparison of toxicology response variables in rats consuming diets containing Ajeeb YG with those containing Ajeeb corn grains. Corn grains from Ajeeb YG or Ajeeb were incorborated into rodent diets at 30% concentrations administered to rats (n= 10/group) for 91 days. An additional negative control group of rats (n= 10/group) were fed AIN93G diets. Rats fed on GM corn showed histopathological changes. Liver displayed cytoplasmic vacuolation of centrolobular hepatocytes and fatty degeneration of hepatocytes. Kidneys showed congestion of renal blood vessels and cystic dilatation of renal tubules. Testes revealed necrosis and desquamation of spermatogoneal germ cells lining seminiferous tubules. Spleen showed slight lymphocytic depletion and splenic congestion. Small intestine showed hyperplasia, hyperactivation of mucous secretory glands and necrosis of intestinal villi were detected. Due to these observations, we suggest that the risk of GM crops cannot be ignored and deserves further investigations in order to identify possible long-term effects, if any, of GM food consumption that might help in the post market surveillance.

[El-Shamei, Z. S., Gab-Alla, A.A., Shatta, A. A, Moussa, E. A. and Rayan, A. M. **Histopathological Changes in Some Organs of Male Rats Fed on Genetically Modified Corn (Ajeeb YG).** *J Am Sci* 2012;8(10): 684-696]. (ISSN: 1545-1003). http://www.jofamericanscience.org. 93

Key words: genetically modified corn; histopathology; rats; liver, kidney; small intestine

1. Introduction

Ajeeb YG (YieldGard corn, event MON-00810-6) is a genetically modified insect resistant corn produced by incorporated the MON 810 (Monsanto) borer resistance trait in the best corn germplasm "Ajeeb" (a trade mark of Dekalb). The *Bacillus thuringiensis* (Bt) crystalline protein "Cry1Ab" in YieldGard corn gives it's the protection against European corn porer (*Ostrinia nubilalis*), Pink stem borer (*Sesamia cretica*) and Purple-linked borer (*Chilo Agamemnon*). The protection comes from a naturally occurring soil bacterium called *Bacillus thuringiensis*. In nature, this bacterium produces a protein that is harmful to certain lepidopteran larvae (the stack borers). The gene in the bacterium that produces the protein called the "Bt-gene".

Several animal studies indicate serious health risks associated with GM food, including infertility, immune problems, accelerated aging, insulin regulation, and changes in vital organs and the gastrointestinal system. There are several reasons why GM plants present unique dangers (Verma et al., 2011). First of these, the process of genetic engineering itself creates unpredicted alterations, irrespective of which gene is transferred. This creates mutations in and around the insertion site and elsewhere (Wilson et al., 2006). The biotech industry confidently asserted that gene transfer from GM foods was not possible; the only human feeding study on GM foods later proved that it does take place (Verma et al., 2011). The genetic material in soybeans that make them herbicide tolerant transferred into the DNA of human gut bacteria and continued to function, that means that long after we stop eating a GM crop, its foreign GM proteins may be produced inside our intestines (Netherwood et al., 2004).

Some scientific reports have described structural and molecular modifications in different organs and tissues of GM-fed animals (Ewen and Pustzai 1999; Malatesta et al., 2002a, b, 2003, 2005; Vecchio et al., 2004; Tudisco et al., 2006 and Trabalza-Marinucci et al., 2008). These observations suggest that the risk of genetically modified crops cannot be ignored and deserves further investigations in order to identify possible long-term effects, if any, of GM food consumption that might help in the post market surveillance (Kuiper et al., 2004).

An important problem seems to be related to the safety assessment of new GM foods, which is initially based on the use of the concept of "substantial equivalence." This concept is based on the following principle: "if a new food is found to be substantially equivalent in composition and nutritional characteristics to an existing food, it can be regarded as being as safe as the conventional food" (SOT, 2003). Although application of this concept is not a safety assessment per se, it enables the identification of potential differences between the existing food and the new product, which should then be investigated further

with respect to their toxicological impact. It is a starting point rather than an end point (Kuiper et al., 2002).

Some studies did report adverse changes at a cellular level caused by some GM foods, concluding that "More scientific effort and investigation are needed to ensure that consumption of GM foods is not likely to provoke any form of health problem" (Le Curieux-Belfond et al., 2009). A study published in 2006 found that the testicles of both mice and rats fed roundup ready soybeans showed dramatic changes. In rats, the organs were dark blue instead of pink. In mice, young sperm cells were altered (Oliveri et al., 2006). Abdullah (2008) observed that feeding rats with transgenic wheat flour (T-840) resulting in increasing in the gobbler cells from mucosal layer with thickening in intestinal villi. Key et al. (2008) found clear negative impact on liver and kidney function in rats consuming GM maize varieties for 91 days. Therefore, this study was carried out to provide new information about the negative effects of genetically modified corn and its effect on the tissues of vital organs of male rats.

2. Materials and Methods

2.1. Plant materials

Transgenic corn sample (Ajeeb YG) and its near-isogenic line (Ajeeb) were obtained from the agricultural administration, Hehya, Sharkia governorate, Egypt. The Cairo based company Fine Seed International is partnering with Monsanto Company to distribute the variety in Egypt.

2.2. Diet formulation

Flours from Ajeeb YG and Ajeeb corn grains were formulated into rodent diets at concentration of 30%. These diets were produced in accordance with AIN93G guidelines (Reeves et al., 1993). An additional AIN93G grain-based diet was included as negative control. The composition of all diets is presented in Table 1.

Table 1. Diet formulation (%)

Ingredients	AIN 93 (Standard)	Ajeeb (Control)	Ajeeb YG (GM)
Corn	33.3	30	30
Corn starch	34.4	37.7	37.7
Casein	17.3	17.3	17.3
Grass	5	5	5
Di-calcium phosphorous	2.5	2.5	2.5
Salt	0.5	0.5	0.5
Lime stone	0.6	0.6	0.6
Soy oil	4.8	4.8	4.8
Premix[a]	1	1	1
DL-methionine	0.4	0.4	0.4
Lysine	0.2	0.2	0.2

[a] The premix supplied per kilogram of diet vitamins (vitamin A, 7000 IU; vitamin D_3, 1500 IU; vitamin E, 60 mg; vitamin K, 3 mg; thiamine, 10 mg; riboflavin, 10 mg; pyridoxine, 10 mg; nicotinic acid, 45 mg; D-calcium pantothenate, 20 mg; folic acid, 10 mg; biotin, 0.2 mg; inositol, 400 mg; vitamin B_{12}, 0.05 mg; choline chloride, 1250 mg and vitamin C, 460 mg) and minerals (Cu, 10 mg; Fe, 100 mg; Mn, 75 mg; Zn, 40 mg; Se, 0.2 mg; I, 0.5 mg; NaCl, 3.3 g; Mg, 0.5 g and K 1.2 g).

2.3. Animals and housing

Thirty male apparently healthy rats, approximately three-weeks of age with an average body weight of 45±5 g were obtained from the Research Institute of Ophthalmology (Giza– Egypt). All animals were housed individually with ad libitum access to water and commercially obtained AIN93G feed. Animal rooms were maintained at a temperature 22 ± 2 ºC and 40–70% of relative humidity with a 12 h light/dark cycle. Rats were acclimatized for 5 days with AIN93G control diet and then divided into treatment groups randomly as 10 rats/group with mean body weights across each group not varying more than 10%. Experimental groups were fed diets formulated with 30% (wt/wt) Ajeeb YG; referred as genetically modified diet (Group III). And corresponding control groups were fed diets containing either 30% (wt/wt) Ajeeb corn grains; referred as control group (Group II). Third group of rats were fed AIN93G diet as an additional negative control; referred as standard group (Group I).

2.4. Processing of tissues for histopathology

At the end of each experimental period 45 and 91 days, the animals were fasted overnight, anesthetized with ether and scarified then given a complete gross pathologic examination. A full set of tissues was collected including: liver, kidneys, testes, spleen and small intestine. Following collection, tissues were placed directly into 10% neutral buffered formalin for fixation. The selected tissues representing the major organs from all animals were processed, embedded in paraffin, sectioned (approximately 4 mm), and stained with hematoxylin and eosin using standard histological technique according to Bancroft and Stevens (1996) then, examined by Olympus BX51 light microscope.

3. Results and Discussion

Organs such as liver, kidney, testes, spleen and small intestine were examined by histological approach and the photomicrographs of hematoxalin and eosin stained, specimens were illustrated in Figures (1- 10).

3.1. Liver

Liver represents a suitable model for monitoring the effects of a diet, due to its key role in controlling the whole metabolism. The changes in the liver, as a site responsible for biotransformation and detoxification, suggest alterations in the metabolic processes. Photomicrographs of liver from rats after 45 and 91 days of feeding on different experimental diets were illustrated in Figures (1, 2). For both ages, liver from rats of group I fed on standard diet (Figure 1, A and B) and group II fed on control diet (Figure 2, A and B), displayed normal histopathological structure of hepatic lobules. On the other hand, rats fed on diet containing

GM diet (group III), showed after 45 days histopathological changes as; cytoplasmic vacuolation of centrolobular hepatocytes (Figure 1, C), congestion of hepatic sinuoids and fatty degeneration of hepatocytes (Figure 1, D) and kupffer cells activation and dilatation of hepatic sinuoids (Figure 1, E). After 91 days, rats of group III revealed cytoplasmic vacuolization of hepatocytes, pyknosis of nuclei and fibroplasias in the portal traid (Figure 2, C and D), and some sort of focal hepatic haemorrhage (Figure 2, E).

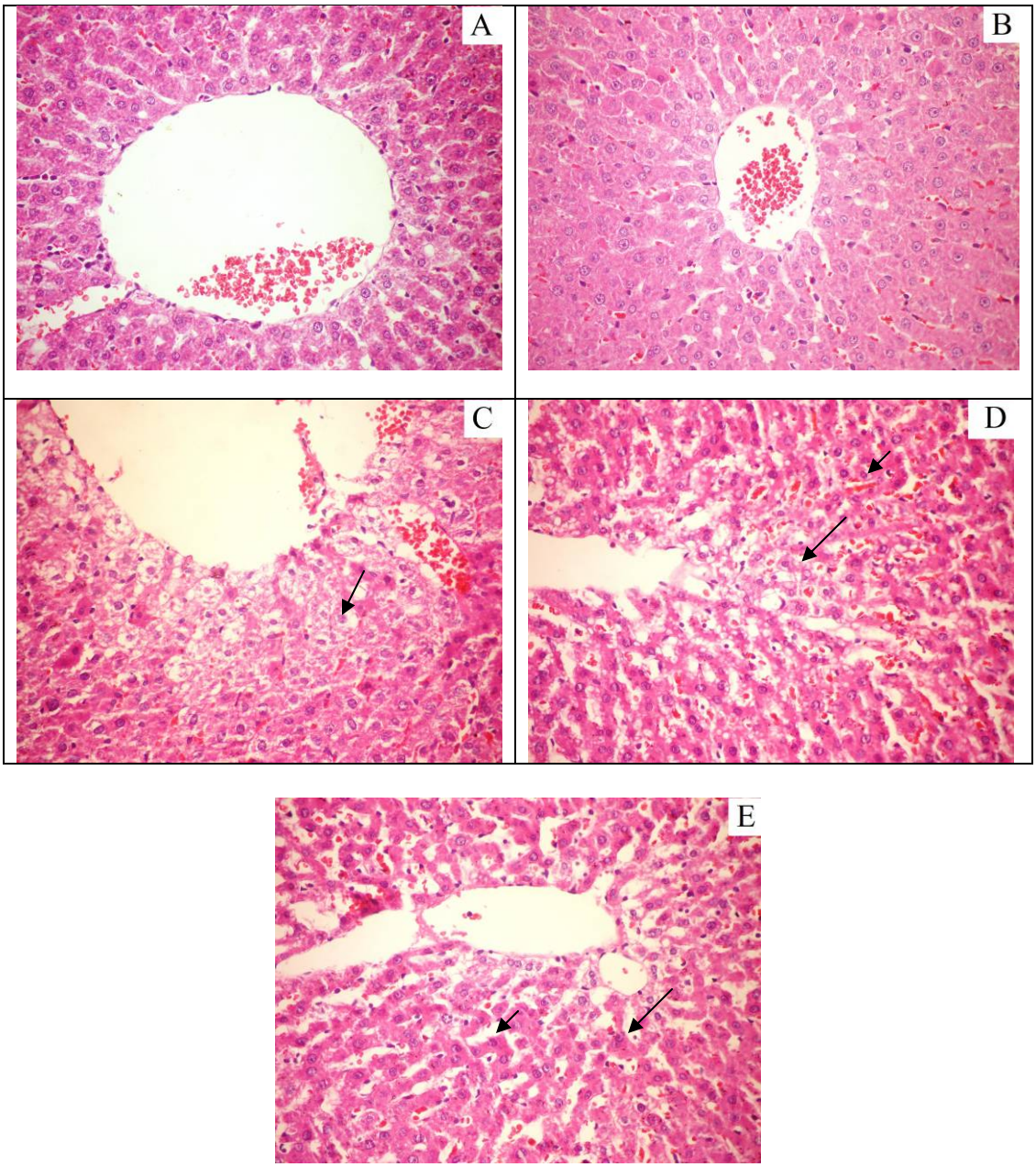


Fig. 1. Photomicrograph of liver from rats after 45 days of feeding on different experimental diets: (A) Standard, showing the normal histopathological structure of hepatic lobules (H&E X 400). (B) Control, showing no histopathological changes (H&E X 400). (C) GM, showing cytoplasmic vacuolation of centrolobular hepatocytes (arrow) (H&E X 400). (D) GM, showing congestion of hepatic sinuoids (small arrow) and fatty degeneration of hepatocytes (large arrow) (H&E X 400). (E) GM, showing kupffer cells activation (small arrow) and dilatation of hepatic sinuoids (large arrow) (H&E X 400).

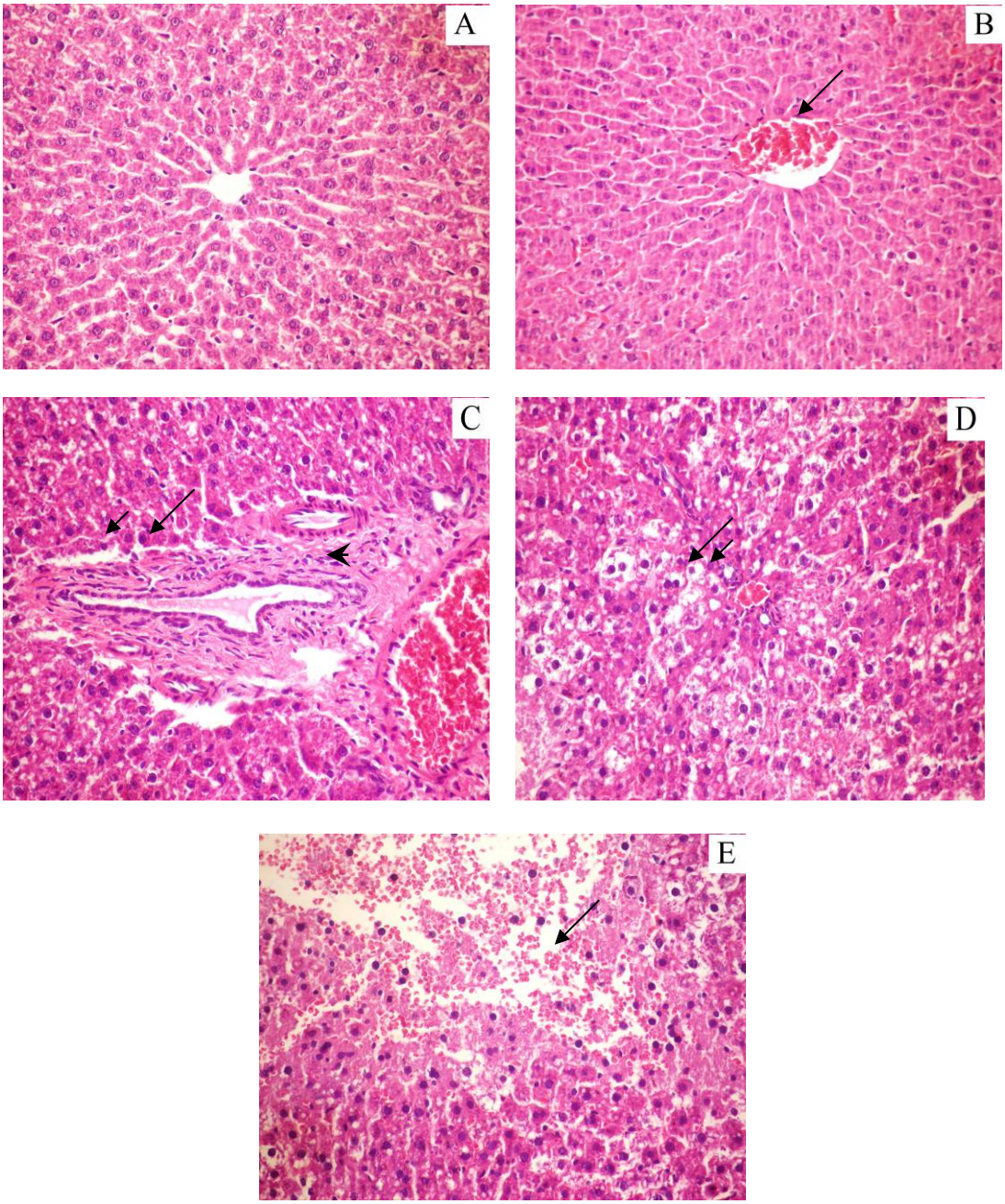


Fig. 2. Photomicrograph of liver from rats after 91 days of feeding on different experimental diets: (A) Standard, showing the normal histopathological structure of hepatic lobules (H&E X 400). (B) Control, showing slight congestion of central vein (arrow) (H&E X 400). (C) GM, showing cytoplasmic vacuolization of hepatocytes (small arrow), pyknosis of nuclei (large arrow) and fibroplasias in the portal traid (arrow head) (H&E X 400). (D) GM, showing cytoplasmic vacuolization of hepatocytes (small arrow) and pyknosis of nuclei and fibroplasias in the portal traid (large arrow) (H&E X 400). (E) GM, showing focal hepatic haemorrhage (H&E X 400).

These findings are in accordance with Schmucker, (1990) who illustrated markedly severity level of granular degeneration was seen in Bt diet containing rat groups but not in control and reference rat groups. Hepatocyte nuclear size change related to both age and food was observed. Therefore diets containing Bt may cause excess fatty supply for animals. Granular degeneration was statistically significant only in male rats in group fed on genetically modified corn. Additionally, nuclear border changes found

statistically significant in female and male rats in the same group. In this aspect, Malatesta et al. (2002) observed irregular shaped hepatocyte nuclei and increase in number of nuclear pore at electron microscopy in offspring's of GM soybean fed pregnant mice. Thirty-five-day feeding study with GM corn in porcine showed the presence of transgene Cry1A(b) in tissues of liver, spleen, kidney and in blood but not in muscle (Mazza et al., 2005). In the same field, morpho-functional characteristics of the liver from 24-month-old mice, fed from weaning on control or GM soybean were investigated by Quaglino et al. (2002), who found several proteins belonging to hepatocyte metabolism, stress response, calcium signaling and mitochondria were differentially expressed in GM-fed mice. This indicates a more marked expression of senescence markers in comparison to controls. Moreover, hepatocytes of GM-fed mice showed mitochondrial and nuclear modifications indicative of reduced metabolic rate. The authors also demonstrate that GM soybean intake can influence some liver features during ageing. Although the mechanisms remain unknown, underlines the importance to investigate the long-term consequences of GM-diets and the potential synergistic effects with ageing, xenobiotics and/or stress conditions. Moreover, Gazzanelli et al. (2002) carried out an ultrastructural morphometrical and immunocytochemical study on hepatocytes from mice fed on GM soybean. The object of this study was to investigate eventual modifications of nuclear components of these cells involved in multiple metabolic pathways related to food processing. The observations demonstrate significant modifications of some nuclear features in GM-fed mice. In particular, GM fed-mice showed irregularly shaped nuclei, which generally represent an index of high metabolic rate, and a higher number of nuclear pores, suggestive of intense molecular trafficking. Recently altrations have also been observed in hepatocyte cells and enzymes (Poulsen et al., 2007 and Peng et al., 2007).

3.2. Kidney

Histopathological examination of kidney sections from group I and group II, Figure (3, 4) after 45 and 91 days feeding showed no histopathological changes and the normal histopathological structure of renal parenchyma. Photomicrograph of Kidney from rats of group III after 45 days indicates some histopathological changes. These include congestion of renal blood vessels and perivascular oedema (Figure 3, C). Vacuolation of endothelial lining glomerular tufts and epithelial lining of renal tubules (Figure 3, D). Atrophy of glomerular tufts and cystic dilatation of renal tubules (Figure 3, E). After 91 days, it showed congestion of glomerular tufts and intertubular blood capillaries, perivascular oedema (Figure 4, C). Congestion of renal blood vessels (Figure 4, D) and cystic dilatation of renal tubules (Figure 4, E).

Such histopathological observations in kidneys were agreed with some studies that reported histopathological changes during feeding the experimental animal with GM corn. Smith (2005) demonstrated that feeding rats with MON 863 Bt corn led to inflammation in kidney and lesions in liver and kidney. Seralini (2005) observed decreases in weight of kidney, tubular changes and inflammation in male rats fed with 33% MON 863 Bt corn in a 90-day study. As well as, Kilic and Akay (2008) observed enlargements in parietal layer of Bowman's capsule and minimal tubular degenerations at different ratios in groups. The decreases in average short and long diameter of glomeruli and glomerular volume in rats fed with standard diet (containing 20% reference corn) and rats fed with standard diet (containing 20% transgenic Bt corn) were statistically different from controls while changes in the thickness of cortex was not significant among groups.

3.3. Testes

For groups I and Group II, there were no histopathological changes and normal seminiferous tubules with normal spermatogoneal cells as well as complete normal spermatogenesis were detected during both period of study (Figures 5, 6). For group III, there were several histopathological changes. Feeding rats with genetically modified corn for 45 days resulted in necrosis and desquamation of spermatogoneal germ cells lining seminiferous tubules as well as atrophy of seminiferous tubules (Figure 5, C), In addition interstitial oedema (Figure 6, D). After 91 days feeding, the changes were desquamation of germ cells in the lumen of seminiferous tubules and interstitial aedema (Figure 6, C). As well as vacuolations and necrosis of spermatogoneal cells lining seminiferous tubules (Figure 6, D).

These histopathological changes in current study were similar to findings of Vecchio et al. (2004), who observed that mice fed on GM Roundup tolerant soy for over 8 months showed nuclear transcription abnormalities in testes during the feeding. Also, they found that the number of perichromatin granules is higher and the nuclear pore densities lower for the GM- fed mice of all ages. Moreover, they detected enlargements in the smooth endoplasmic reticulum in GM-fed mice Sertoli cells. These changes might be due to Roundup herbicide (Monsanto) toxic effects. Similar to those observed on mammalian cells (Richard et al., 2005).

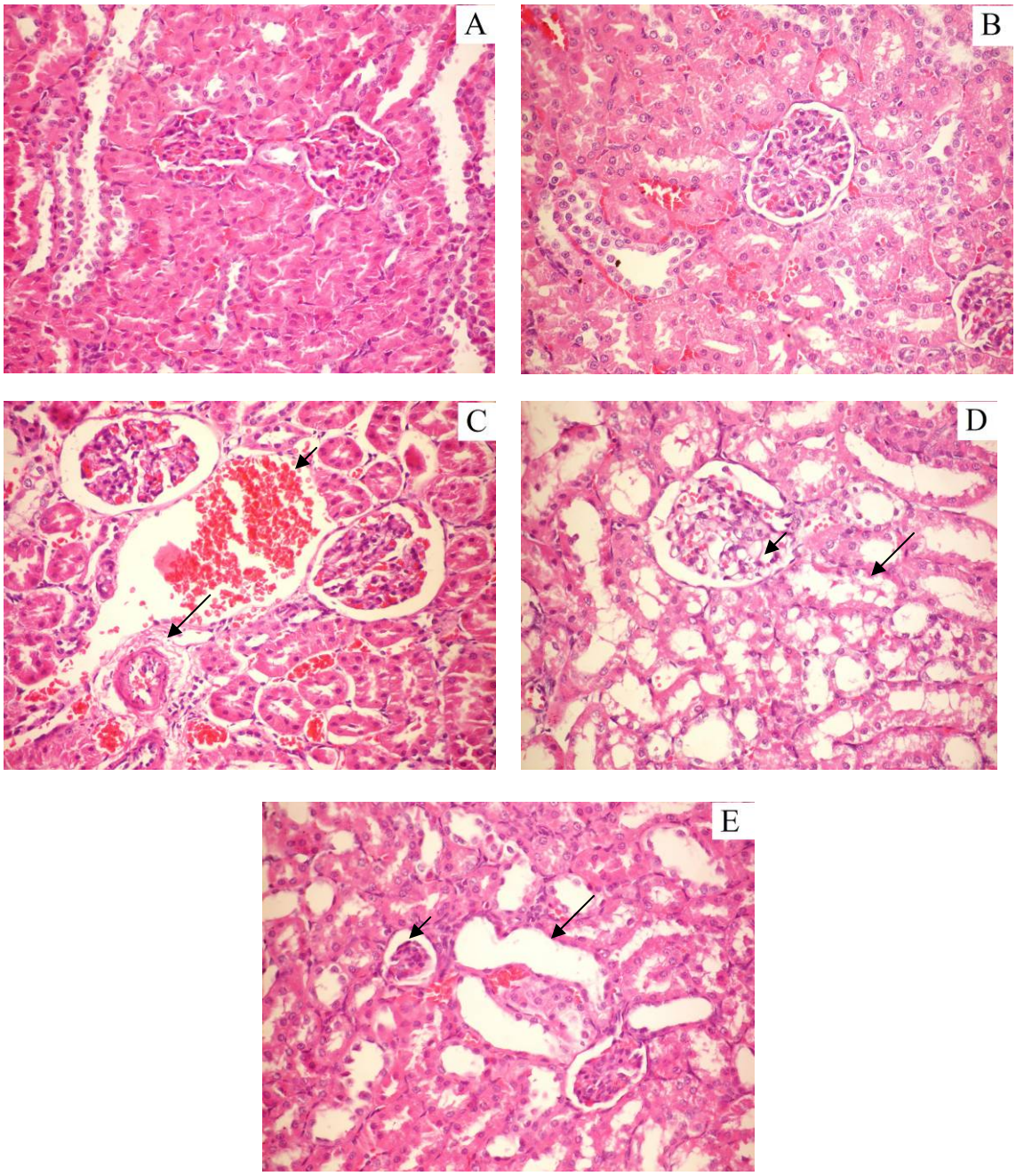


Fig. 3. Photomicrograph of kidney from rat after 45 days of feeding on different experimental diets: (A) Standard, showing the normal histopathological structure of renal parenchyma (H&E X 400). (B) Control, showing no histopathological changes (H&E X 400). (C) GM, showing congestion of renal blood vessels (small arrow) and perivascular aedema (large arrow) (H&E X 400). (D) GM, showing vacuolation of endothelial lining glomerular tufts (small arrow) and epithelial lining renal tubules (large arrow) (H&E X 400). (E) GM, showing atrophy of glmerular tufts (small arrow) and cystic dilatation of renal tubules (large arrow) (H&E X 400).

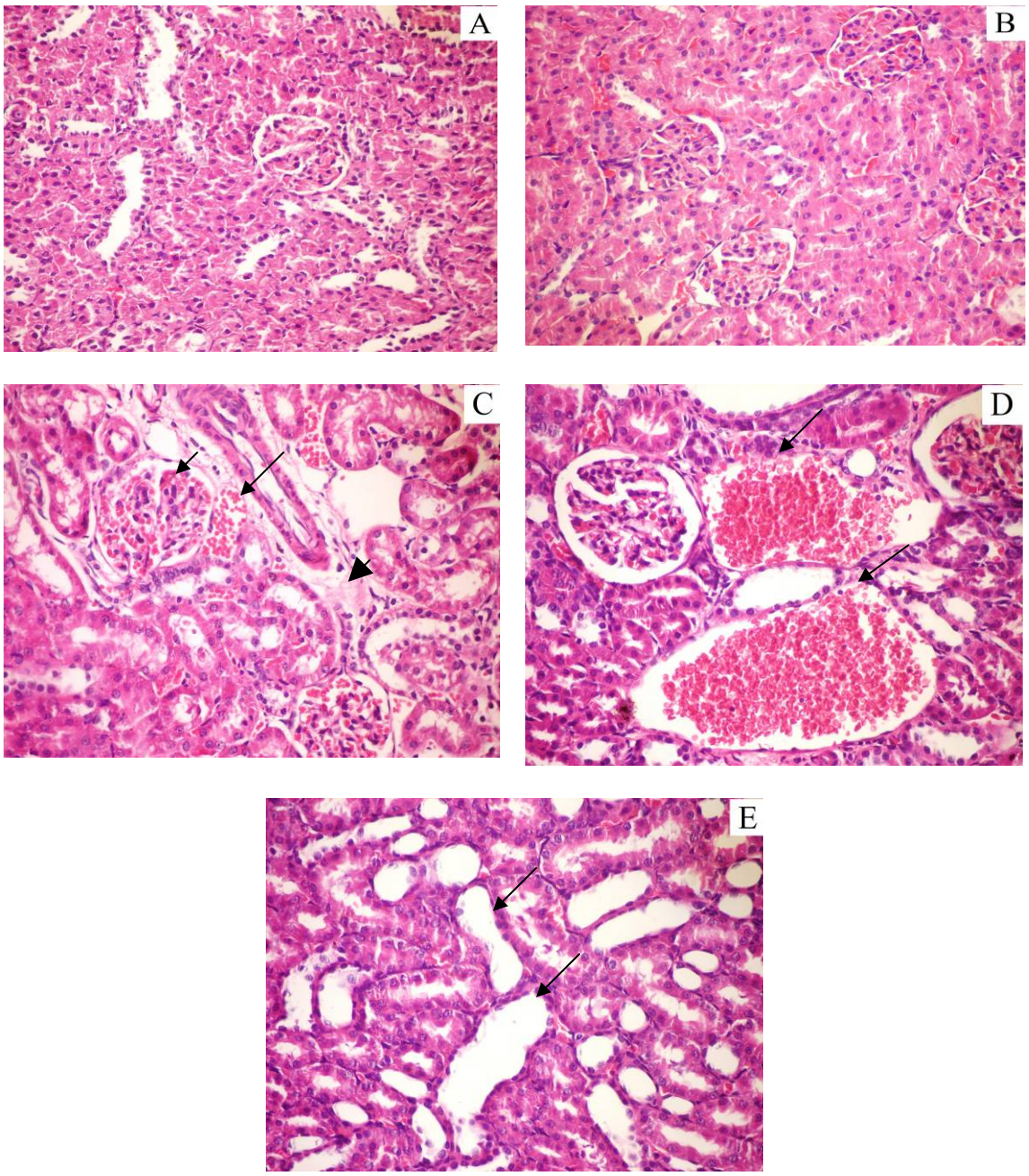


Fig. 4. Photomicrograph of kidney from rats after 91 days of feeding on different experimental diets: (A) Standard, showing the normal histopathological structure of renal parenchyma (H&E X 400).(B) Control, showing no histopathological changes (H&E X 400). (C) GM, showing congestion of glomerular tufts (small arrow) and intertubules blood capillaries (large arrow) as well as perivascular aedema (arrow head) (H&E X 400). (D) GM, showing dilatation and congestion of renal blood vessels (arrows) (H&E X 400). (E) GM, showing cystic dilatation of renal tubules (arrows) (H&E X 400).

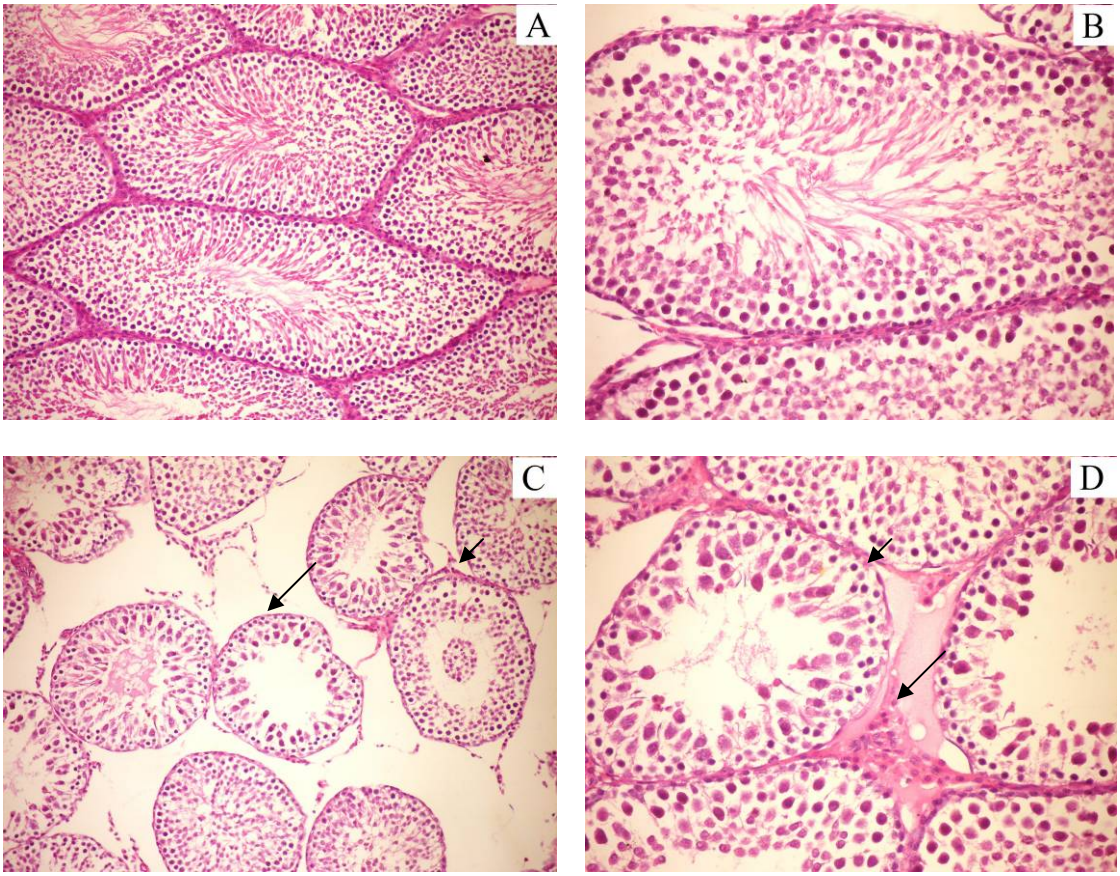
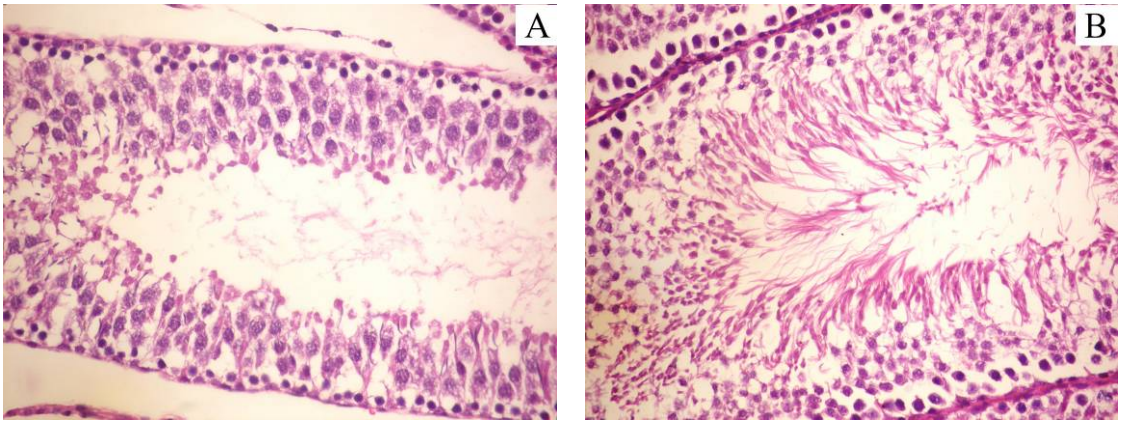


Fig. 5. Photomicrograph of testis from rats after 45 days of feeding on different experimental diets: (A) Standard, showing normal seminiferous tubules with normal spermatogoneal cells and complete spermatogenesis (H&E X 200). (B) Control, showing normal semineferous tubules (H&E X 400). (C) GM, showing necrosis and desquamation of germ cells lining seminiferous tubules (small arrow) as well as atrophy of seminiferous tubules (large arrow) (H&E X 200). (D) GM, showing necrosis of spermatogoneal cells lining seminiferous (small arrow) and interstitial aedema (large arrow) (H&E 400).



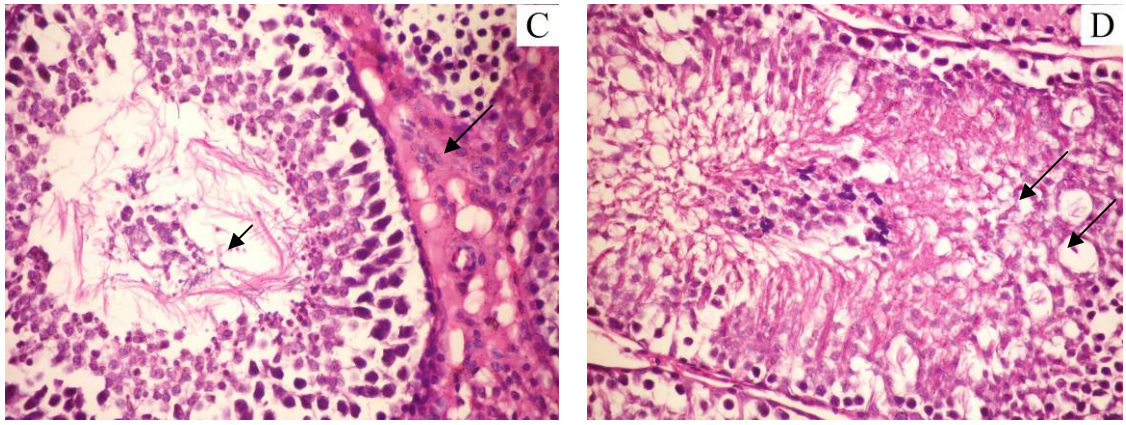


Fig. 6. Photomicrograph of testis from rats after 91 days of feeding on different experimental diets: (A) Standard, showing normal seminiferous tubule (H&E X 400). (B) Control, showing no histopathological changes (H&E X 400). (C) GM, showing desquamation of germ cells in the lumen of seminiferous tubules (small arrow) and interstitial aedema (large arrow) (H&E X 400). (D) GM, showing vacuolations and necrosis of spermatogoneal cells lining seminiferous (arrows) (H&E 400).

3.4. Spleen

No histopathological changes in spleen were observed after 45 days of feeding for all examined rat groups (Figure 7; A, B and C). After 91 days, rats from group I and II showed normal lymphoid follicles and no histopathological changes (Figure 8, A and B). On the other hand, rats fed on diet containing genetically modified corn showed slight lymphocytic depletion and splenic congestion (Figure 8, C and D).

3.5. Small intestine

As shown in Figure (9, 10) the rats fed on standard and control diets for 45 and 91 days, respectively demonstrated normal histopathological layer (mucosa, submucosa and musculosa) and no histopathological changes were observed. After 45 days of feeding on diet containing genetically modified corn, hyperplasia and hyperactivation of mucous secretory glands and necrosis of intestinal villi were detected (Figure 9, C and D). Furthermore, after 91 days feeding on genetically modified corn, shortening of intestinal villi and leucocytic cells infiltration in lamina propria were developed (Figure 10, C and D).

Our gained results are in the same line with Abdullah (2008) who observed that feeding rat with transgenic wheat flour (T-840) resulting in increasing in the gobbler cells from mucosal layer with thickening in intestinal villi. In this respect, feeding rats with genetically modified potatoes resulting in potentially precancerous cell growth in their stomach and intestines (Stanley et al., 1999). Moreover, mice fed Bt potatoes engineered to produce the insecticide called Bt-toxin also had proliferative cell growth in their small intestine, as well as abnormal and damaged cells (Fares and El-Sayed, 1998). GM potatoes expressing *Galanthus nivalis* lectin (gna) induced proliferative growth in the small-large intestines (Ewen and Pusztai, 1999) and GM soybean type Roundup Ready® caused moderate inflammation in the distal intestine of salmons (Bakke-McKellep et al., 2007).

Conclusion

In conclusion, the present work demonstrate that GM corn intake influenced on the histopathological features of liver, kidney, testis, spleen and small intestine during the physiological process of ageing and, although the mechanisms responsible for such alterations are still unknown and several animal studies indicate serious health risks associated with GM food. Therefore, we recommend that more scientific efforts and investigations are needed to ensure that consumption of GM foods likely to provoke any form of health problem. Moreover, because of the importance that the consumption of GM foods has acquired, as well as its enormous potential in the near future, the performance of a complete case-by-case study seems would be advisable and long-term studies are clearly necessary.

Acknowledgement

This study is a part of Ph.D. Thesis of Ahmed Rayan submitted to Suez Canal University.

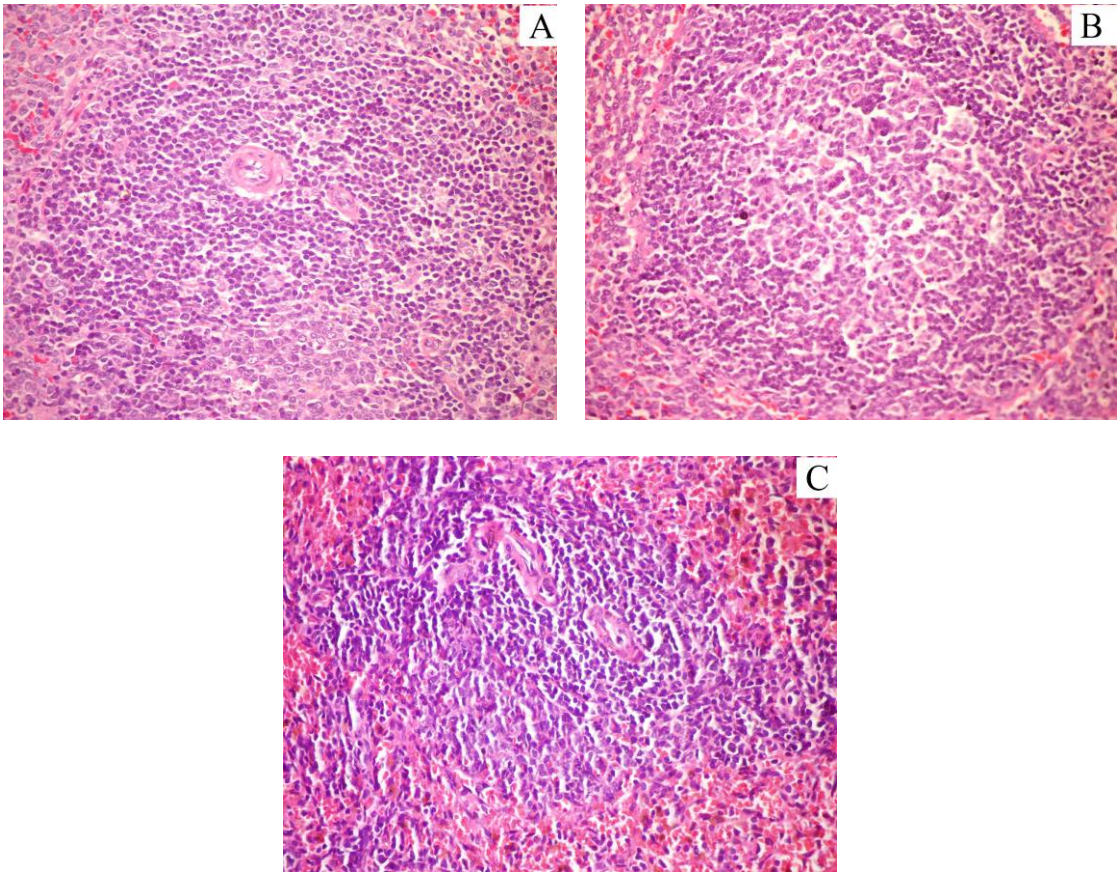
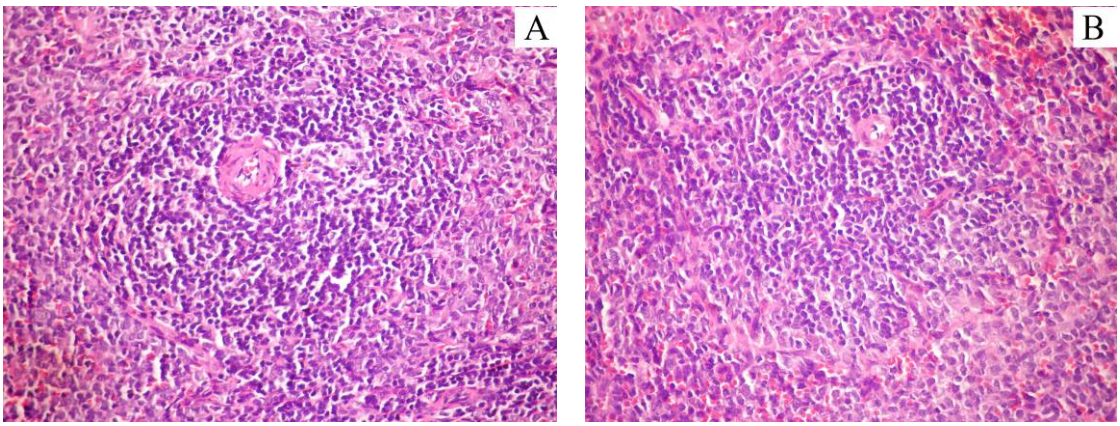


Fig. 7. Photomicrograph of spleen from rats after 45 days of feeding on different experimental diets: (A) Standard, showing normal lymphoid follicle (H&E X 400). (B) Control, showing no histopathological changes (H&E X 400). (C) GM, showing no histopathological changes (H&E X 400).



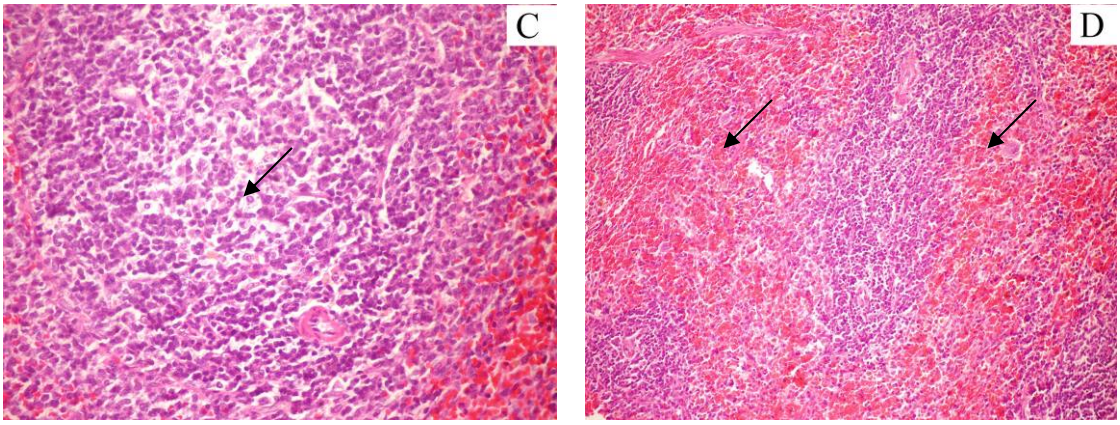


Fig. 8. Photomicrograph of spleen from rats after 91 days of feeding on different experimental diets: (A) Standard, showing normal lymphoid follicle (H&E X 400). (B) Control, showing no histopathological changes (H&E X 400). (C) GM, showing slight lymphocyte depletion (arrow) (H&E X 400). (D) GM, showing splenic congestion (arrow) (H&E X 400).

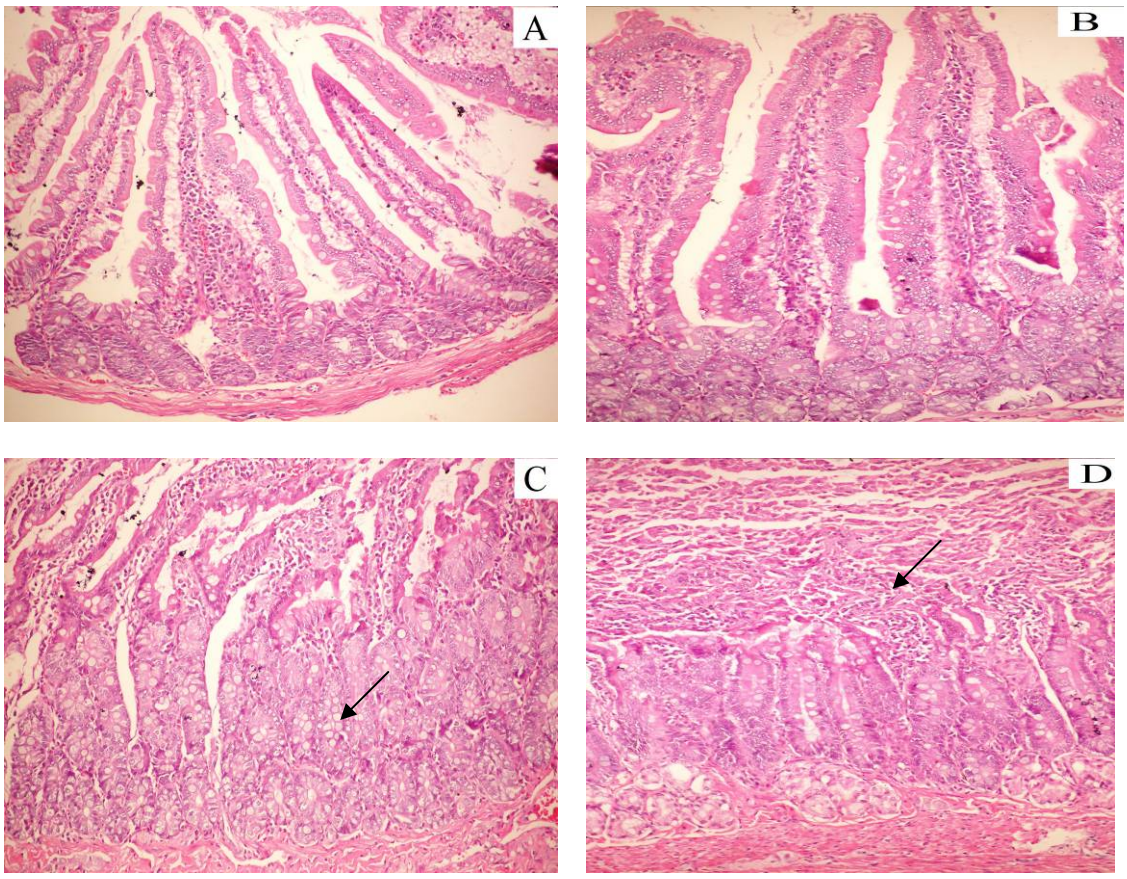


Fig. 9. Photomicrograph of intestine from rats after 45 days of feeding on different experimental diets: (A) Standard, showing normal histopathological layer (mucosa, submucosa and musculosa) (H&E X 200). (B) Control, showing no histopathological changes (H&E X 200). (C) GM, showing hyperplasia and hyperactivation of mucos secretory glands (arrow) (H&E X 200). (D) GM, showing necrosis of intestinal villi (arrow) (H&E 200).

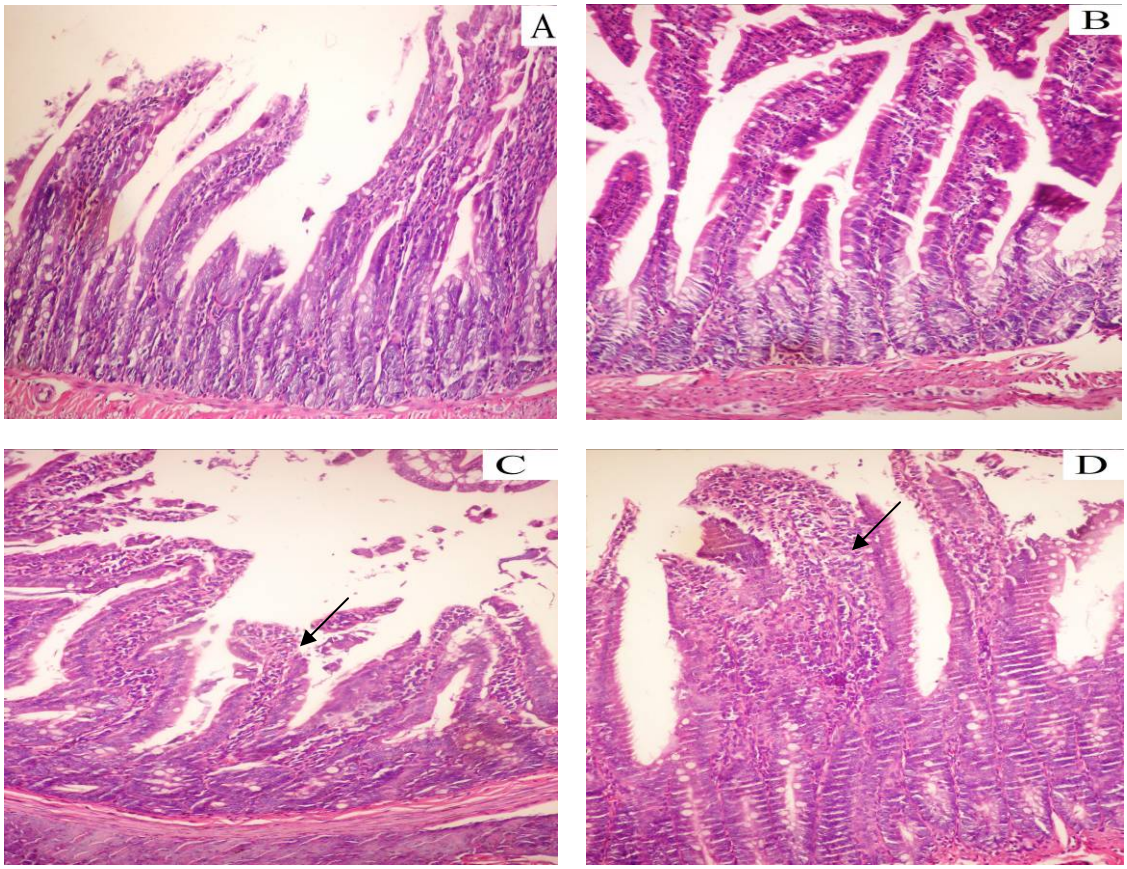


Fig. 10. Photomicrograph of intestine from rats after 91 days of feeding on different experimental diets: (A) Standard, showing no histopathological changes (H&E X 200). (B) Control, showing no histopathological changes (H&E X 200). (C) GM, showing shortening of intestinal villi (H&E X 200). (D) GM, showing leucocytic cells infiltration in lamia propria (arrow) (H&E 200).

4. References

1. Abdullah, A. B. (2008). Safety parameters and quality of some genetically modified and organically produced foods. Ph.D thesis, Faculty of Agriculture, Ain Shams University, Egypt.

2. Bakke-McKellep, A. M., Koppang E. O., Gunnes, G., Senden, M., Hemre, G. I., Landsverk, T. and Krogdahl, A. (2007). Histological, digestive, metabolic, hormonal and some immune factor responses in Atlantic salmon, *Salmo salar* L., fed genetically modified soybeans. J. Fish Dis. 30: 65–79.

3. Bancroft, J.D. and Stevens, A. (1996). Theory and Practice of Histological Techniques, 4th Edn. Churchill Livingstone, London.

4. Ewen, S.W. and Pustzai, A. (1999). Effects of diets containing genetically modified potatoes expressing Galanthus nivalis lecitin on rat small intestine. Lancet. 354: 1353–1354.

5. Fares, N.H. and El Sayed, A.K. (1998). Fine structural changes in the ileum of mice fed on delta-endotoxin-treated potatoes and transgenic potatoes. Nat. Toxins. 6: 219–233.

6. Gazzanelli, G., Tiberi, C., Serafini, S., Rocchi, M. B., Gavaudan, S., Caporaloni, C. and Malatesta, M. (2002). Ultrastructural morphometrical and immunocytochemical analyses of hepatocyte nuclei from mice fed on genetically modified soybean. Cell Struct Funct. 27, 399.

7. Key, S., Ma, J.K. and Drake, P.M. (2008). Genetically modified plants and human health. J. R. Soc. Med. 101: 290-298.

8. Kilic, A. and Akay, T. (2008). A three generation study with genetically modified Bt corn in rats: Biochemical and histopathological investigation. Food Chem. Toxicol. 46: 1164–1170.

9. Kuiper, H. A., Kleter, G. A., Noteborn, H. P. and Kok, E. J. (2002). Substantial equivalence–an appropriate paradigm for the safety assessment of genetically modified foods. Toxicol. 181–182:427–431.

10. Kuiper, H.A., König, A., Kleter, G.A., Hammes, W.P. and Knudsen, I. (2004). Safety assessment, detection and traceability, and societal aspects of genetically modified foods. European network on safety assessment of genetically modified food crops (ENTRANSFOOD). Food Chem. Toxicol. 42: 1195–1202.

11. Le Curieux-Belfond, O., Vandelac, L., Caron, J. and Seralini, G. E. (2009). Factors to consider before production and commercialization of aquatic genetically modified organisms: the case of transgenic salmon. Environ. Sci. Policy. 12: 170-89.

12. Malatesta, M., Caporaloni, C., Gavaudan, S., Rocchi, M.B., Serafini, S., Tiber, C. and Gazzanelli, G. (2002[a]). Ultrastructural morphometrical and immunocytochemical analysis of hepatocyte nuclei from mice fed on genetically modified soybean. Cell Struct. Funct. 27: 173–180.

13. Malatesta, M., Caporaloni, C., Rossi, L., Battistelli, S., Rocchi, M.B.L., Tonucci, F. and Gazzanelli G. (2002[b]). Ultrastructural analysis of pancreatic acinar cells from mice fed on genetically modiWed soybean. J. Anat. 201: 409–416.

14. Malatesta, M., Biggiogera, M. and Manuali, E. (2003). Fine structural analyses of pancreatic acinar cells nuclei from mice fed on GM soybean. Eur. J. Histochem. 47:385-388.

15. Malatesta, M., Tiberi, C., Baldelli, B., Battistelli, S., Manuali, E., Biggiogera, B**. (**2005). Reversibility of hepatocyte nuclear modifcations in mice fed on genetically modifed soybean. Eur. J. Histochem. 49: 237–242.

16. Mazza, R., Soave, M., Morlacchini, M., Piva, G. and Marocco, A. (2005). Assessing the transfer of genetically modified DNA from feed to animal tissues. Trans. Res. Oct. 14: 775–784.

17. Netherwood, T., Martín-Orúe, S.M. and O'Donnell, A. G. (2004). Assessing the survival of transgenic plant DNA in the human gastrointestinal tract. Nat. Biotechnol. 22: 204-209.

18. Oliveri et al. (2006). Temporary deression of transcription in mouse preimplantation embryos from mice fed on genetically modified soybean, 48th Symposium of the Society for histochemistry, Lake Magggiore (Italy), September 7-10, 2006.

19. Peng, D., Chen, S., Ruan, L., Li, L.,Yu, Z. and Sun, M. (2007). Safety assessment of transgenic Bacillus thuringiensis with VIP insecticidal protein gene by feeding studies. Food Chem. Toxicol. 45, 1179–1185.

20. Poulsen, M., Kroghsbo, S., Schrøder, M., Wilcks, A., Jacobsen, H., Miller, A., Frenzel, T., Danier, J., Rychlik, M., Shu, Q., Emami, K., Sudhakar, D., Gatehouse, A., Engel, K.H. and Knudsen, I. (2007). A 90-day safety study in Wistar rats fed genetically modified rice expressing snowdrop lectin Galanthus nivalis (GNA). Food Chem. Toxicol. 45, 350–363.

21. Quaglino, D., Biggiogera, M., Battistelli, S., Baldelli, B., Annovi, G., Boraldi, F. and Malatesta, D. (2002). A long-term study on female mice fed on a genetically modified soybean: effects on liver ageing. Cell Struct. Funct. 4: 173-180.

22. Reeves, P.G., Nielsen, F.H. and Fahey, G.C. (1993). AIN-93 Purified diets for laboratory rodents: final report of the American Institute of Nutrition Ad Hoc Writing Committee on the reformulation of the AIN-76A rodent diet. J. Nutr. 123: 1939– 1951.

23. Richard, S., Moslemi, S., Sipahutar, H., Benachour, N. and Seralini, G.E. (2005). Differential effects of glyphosate and roundup on human placental cells and aromatase. Environ. Health Perspect. 113: 716–720.

24. Schmucker, D.L. (1990). Hepatocyte fine structure during maturation and senescence. J. Electron Microsc. Tech. 14: 106–125.

25. Seralini, G.E. (2005). Report on MON 863 GM maize produced by Monsanto Company. Controversial Effects on Health Reported after subchronic toxicity test: a confidential rat 90 day feeding study, June.

26. Smith, J.M. (2005). Most offspring died when mother rats ate genetically engineered soy spilling. The Beans Newsletter (October), 1–4.

27. SOT (Society of Toxicology). (2003). The safety of genetically modified foods produced through biotechnology. Toxicol. Sci. 71, 2–8.

28. Stanley, W., Ewen, B. and Pusztai, P. (1999). Effect of diets containing genetically modified potatoes expressing Galanthus nivalis lectin on rat small intestine, Lancet, 1999 Oct 16; 354 (9187):1353-1354.

29. Trabalza-Marinucci, M., Brandi, G., Rondini, C., Avellini, L., Giammarini, C., Costarelli, S., Acuti, G., Orlandi, C., Filippini, G., Chiaradia, E., Malatesta, M., Crotti, S., Antonini, C., Amagliani, G., Manuali, E., Mastrogiacomo, A.R., Moscati, L., Haouet, M.N., Gaiti , A. and Magnani, M. (2008). A three year longitudinal study on the effects of a diet containing genetically modiWed Bt176 maize on the health status and performance on sheep. Livestock Sci. 113: 178–190**.**

30. Tudisco, R., Lombardi, P., Bovera, F., d'Angelo, D., Cutrignelli, M.I., Mastellone, V., Terzi, V., Avallone, L. and Infascelli, F**.** (2006). Genetically modifed soya bean in rabbit feeding: detection of DNA fragments and evaluation of metabolic effects by enzymatic analysis. Anim. Sci. 82: 193–199.

31. Vecchio, L., Cisterna, B., Malatesta, M., Martin, T.E. and Biggiogera, M. (2004). Ultrastructural analysis of tests from mice fed on genetically modified soybean. Eur. J. Histochem. 48, 449-54.

32. Verma, C., Nanda, S., Singh, R.K., Singh, R.B. and Mishra, S. (2011). A Review on Impacts of Genetically Modified Food on Human Health. Open Nutraceut. J. 4, 3-11

33. Wilson, A., Latham, J.R. and Steinbrecher, R.A. (2006). Transformation – induced mutations in transgenic plants: Analysis and biosafety implications. Biotechnol. Genet. Eng. 23, 105-109.

9/28/2012