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Biosafety of genetically modified wheat (*Triticum aestivum*) Hi-line 111

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Abstract

Genetically modified (GM) crops were approved for edible use in several countries but their biosafety for organisms remains to be crucial. The objectives of this work were to compare GM wheat (*Triticum aestivum*) Hi-line 111 (GMW) with native non-GMW wheat (NGMW) to find the differences, if any, in their biosafety. Three groups of albino rats (*Rattus norvegicus*) were used to study the biosafety of GMW for 30 days. Group 1 was fed on a basal diet (control), and group 2 on a control diet with 30 % replacement of starch with NGMW, while group 3 was fed on the control diet with 30 % replacement of starch with GMW. There were no significant signs of adverse impacts noted in the clinical appearance of animals fed on GMW in terms of initial body weight, absolute or relative organ weights and serum profile in comparison with the control group. However, slight histopathological changes were observed in the organs of animals fed on GMW. Though our results demonstrate GMW biosafety regarding its biochemical parameters, however, detailed description of submucosal edema and further studies on allergenic potential with long feeding periods should be performed to conclude its impacts on health.

Introduction

Agricultural biotechnology and genetic engineering have opened an avenue in the development of genetically modified (GM) plants with improved abiotic stress tolerance, herbicide or insect resistance, and nutritional value (Sthrestha *et al.* 2008; Raybould 2012; Elfattah *et al.* 2016; Sun *et al.* 2019). With the international commercialization of GM organisms (GMOs), GM crops were engineered to solve problems such as weed management, disease, improving functional properties and enhancing production efficiency (Shin *et al.* 2013; Klymiuk *et al.* 2018).

Genetic modification introduces new genetic information, new genes, and new compounds in the cells of food-producing organisms. They might alter the cellular metabolism of the food-producing organisms in unanticipated ways. These new proteins might also be toxic or cause allergy (Taylor 1997). In addition, the food-producing organism could fail to produce some nutrients or vitamins. Thus, GM foodstuffs could lack some nutrients that are present in the corresponding non-GM foods. Genetic engineering could cause unintended adverse changes in the food chemical composition or food characteristics, thereby rendering foodstuffs to be unsafe (Elsanhoty

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et al. 2004, 2013; Herman *et al.* 2009). Based on the criteria for safety, some GM crops have been approved for food or feed uses in several countries (Oguchi *et al.* 2010). Consumer concerns regarding GM products related to unexpected health impacts that might arise from GM products consumption (Dona and Arvanitoyannis 2009; Martinez-Povida *et al.* 2009). As recommended by food safety agencies, biosafety and nutritional assessment of GM crops is an important aspect (Chassy 2010). Pressure from public demand and consumers has led some countries to label GMO in foods (Ramadan and Elsanhoty 2012; Elsanhoty *et al.* 2013; Castigliego *et al.* 2015; Turkec *et al.* 2016).

Many countries have imposed biosafety laws and surveillance programs to regulate GMOs uses (De Jong 2010). Within EU countries, regulations and laws on the traceability and labeling of GMOs require mandatory labeling of food and/or feed containing GM ingredients above > 0.9 % (The Commission of the European Communities 2003a, b). EU has adopted a zero-tolerance policy towards the low-level presence of unauthorized GMOs in foods, with a 0.1 % threshold for the permissible presence of unauthorized GMO in the feed (The Commission of the European Communities 2011; Turkec *et al.* 2016).

Many studies were performed to investigate the safety of GM food and feed (Elsanhoty *et al.* 2004, 2006; He *et al.* 2008; Magaña-Gómez and de la Barca 2009; Appenzeller *et al.* 2009a,b; Snell *et al.* 2012). Feeding trials in which rats were fed GM foods for prolonged periods were reported (Brake and Evenson 2004; Brake *et al.* 2004). Some experiments indicated histopathological abnormalities in organs or tissues and clinical impacts (Appenzeller *et al.* 2009a,b; Snell *et al.* 2012; Kaspareit and Rittinghausen 2013; Abdo *et al.* 2014). Some reports assessed the safety of GM plants and reported changes in the serum parameters of animals treated with GM foodstuffs (Elsanhoty *et al.* 2004, 2006; Hammond *et al.* 2006). They noted some differences between animals fed on GM potato Spunta and its control as well as GM corn (MON 810) and its control. However, monitoring of biosafety and nutritional quality for GM food and/or feed require detailed knowledge of the plant composition.

Wheat (*Triticum aestivum* L.) is one of the most important cereals that is globally cultivated (Pigolev *et al.* 2018; Sun *et al.* 2019). The GM wheat Hi-Line 111 (GMW) was transformed with plasmid pAB1 harboring the full-length HVA1 cDNA to transform immature GMW Hi-Line 111 cv. Hi-Line embryos (Hong *et al.* 1988; Lanning *et al.* 1992; Sivamani *et al.* 2000). The chemical composition of GMW and non-GMW (NGMW) line as well as the physical and rheological characteristics of the dough were studied (Elfattah *et al.* 2016). The GMW under study was modified by introducing of barley *HVA1* gene, encoding for a member of the group 3 late embryogene sis abundant (LEA) proteins to increase drought tolerance, biomass production and water use efficiency (Sivamani *et al.* 2000; Elfattah *et al.* 2016). Our work is aimed to assess the safety of GMW in comparison with the conventional wheat line or NGMW upon animals feeding study.

Experimental

Materials

GMW Hi-Line 111 and its conventional NGMO control line were cultivated and harvested under the same conditions in the Agricultural Genetic Engineering Research Institute (AGERI, Giza, Egypt). The HVA1 expression cassette, containing the *HVA1* gene under control of the maize ubi promoter was introduced into *T. aestivum* L. cv. Hi-Line genome *via* particle bombardment of immature embryos (Elfattah *et al.* 2016).

Grain samples were freeze-dried prior to mixing to feed in further investigations. The genetic elements in GMW were reported (Elfattah *et al.* 2016). Non-GMW contained 10.7 moisture (%), 15.6 total protein, 1.61 ash (%), 2.13 lipids (%), 2.63 crude fiber (%), and 69.9 total carbohydrates (%). GMW contained 10.1 moisture (%), 15.9 total protein, 1.61 ash (%), 2.73 lipids (%), 2.71 crude fiber (%), and 69.5 total carbohydrates (%).

Experimental conditions

Experimental procedures involving the animals and their care were carried out according to the Guidelines for Care and Use of Laboratory

Table 1. The composition of the basal and experimental diet (g.100 g^{-1}).

Ingredient	CD[a] [g.100 g^{-1} diet]	Experimental diet	
		Group 2	Group 3
Casein[b]	20.0	20.0	20.0
Corn oil	5.0	5.0	5.0
Cellulose	5.0	5.0	5.0
Salt mixture	3.50	3.50	3.50
Vitamin mixture	1.0	1.0	1.0
Starch	55.0	25.0	25.0
Cholin bitartarate	0.2	0.2	0.2
DL methionine	0.3	0.3	0.3
Sucrose	10.0	10.0	10.0
NGMW		30.0	
GMW			30.0

[a] CD means control diet according to Reeves *et al.* (1993).
[b] Casein contained ≥ 80 % protein.

Animals in Biomedical Research as promulgated by the WHO. Fifteen weaning male albino rats (average weight was 65 g) were provided from the Institute of Ophthalmology Research (Giza, Egypt). The animal experiment was ethically approved before collecting the data by the Animal Subjects Committee of the Sadat City University (Egypt). The animals were housed individually in well-aerated cages with screen bottoms and fed on a basal diet as described by Reeves *et al.* (1993). Salt and vitamins mixtures were prepared according to AOAC (2000). Humidity and temperature were maintained at 60 % and 25 °C, respectively. The animals were randomly sorted into three groups each group contains 5 animals. Group 1 was fed on control diet according to Reeves *et al*. (1993), and group 2 was fed on the control diet with a replacement of 30 % from starch by NGMW, while group 3 was fed on the control diet with a replacement of 30 % from starch by GMW. Table 1 presents the diet composition and animal groups. During the experiment (30 days), the diet consumed and the body weight were measured every day. The body weight and the rest of the feed were measured to compare some performance parameters such as food intake, body gain, daily body weight gain, final body weight, and feed efficiency.

Blood biochemistry and serum analysis

At the end of the experiment (30 days), blood samples were collected from treated groups from the orbital venous plexuses using a capillary tube. Blood was centrifuged for 15 min at 3,000 rpm. to recover the serum, which kept at -18 °C until analysis. Serum was analyzed according to Schermer (1967). Kidney function parameters (urea, and creatinine) and liver function enzymes (ALT, and AST) were analyzed using kits obtained from Bio-diagnostic (Cairo, Egypt). Blood glucose was measured according to Trinder (1969). Serum total cholesterol (TC) was determined according to Thomas (1992). Glutamate-pyruvate aminotransferase (GPT), and glutamate oxaloacetate aminotransferase (GOT) activates were measured at 540 nm (Reitman and Frankel 1957). Serum urea was determined at 580 nm according to Patton and Crouch (1977). Total lipids (TL) content was measured by the reaction of TL with the sulfophosphovanillic mixture (Coudon and Bouige 1973). Low-density lipoprotein (LDL)-cholesterol was calculated as the difference between TC and the high-density lipoprotein (HDL)-cholesterol content of the supernatant after precipitation of the LDL by the polyvinyl sulphate in the presence of polyethylene glycol monomethyl ether (Demacker *et al.* 1984). Triacylglycerols (TAG) content was analyzed as described by Fossati and Prencipe (1982).

Organ weights, gross necropsy, and histopathology

At the end of the experimental period (30 days), the rats were slaughtered. The organs of each animal (kidney, liver, heart, spleen and testes) were weighed. Kidneys, liver, spleen, and stomach tissues from those organs as well as macroscopically evident lesions were fixed in 4 % buffered formaldehyde for the histological test. Tissues were embedded in paraffin and sections, 4 – 6 lm thick, stained by the standard hematoxylin-eosin (H & E) for light microscopy. The intact small intestines were flushed with a 0.9 % NaCl solution and the length was recorded (Drury and Willington 1980).

Statistical analysis

Results are presented as means ± confidence intervals ($p < 0.05$). Means were considered as significantly different, if their confidence

Table 2. Changes in the body weights of animal groups at the end of the experimental period (30 days)[a].

Parameter	Animal group		
	Control group	NGMW [b] group	GMW[c] group
Initial body weight [g]	65.2 ± 4.64[a]	65.6 ± 4.87[a]	65.8 ± 4.44[a]
Final body weight [g]	108.4 ± 4.74[b]	147 ± 4.64[a]	142.6 ± 7.16[a]
Body weight gain [g]	43.2 ± 4.69[b]	81.4 ± 4.75[a]	76.8 ± 5.8[a]

The same letter in the same row is not significantly different ($p > 0.05$).
[a]mean ± standard error ($n = 5$)
[b]NGMW = non genetically modified wheat
[c]GMW = genetically modified wheat

intervals were not overlapping. Results from the animal studies were presented as mean ± SD where appropriate according to Gomez (1984).

Results and Discussion

Biosafety monitoring research performed on GM wheat based on the application of the principle of substantial equivalence, which adopted by leading international food and regulatory bodies such as WHO (WHO 1995). A new food derived from GM crop was found to be equivalent to its non-modified counterpart and safe as the food or feed from the non-modified plant varieties. Substantial equivalence might be classified into; substantially equivalent, substantial equivalent expects for some defined traits, and lack of equivalence (Momma *et al.* 2000). The present experiment was performed to study the nutritional effects and safety of GMW. A rat performance study was conducted to compare GMW with NGMW.

Clinical observations and body weight

During the experimental period, no treatment-related signs of adverse impacts in the clinical appearance of rats were noted. The body weights of animals were comparable at the beginning of the experiment, while throughout the study period there was an increase in body weight of NGMW and GMW groups (Table 2). This increase may be due to the high nutrient value of wheat that replaced starch in the feeding formula. Differences between groups were tested for statistical significance ($p < 0.05$). The results revealed no statistically significant differences between groups in the initial body weight, while at the end of the experiment, there were statistically significant differences between control and other groups. The results agreed with previous reports (Elsanhoty *et al.* 2004, 2006; Abdo *et al.* 2014), where no statistically significant differences between groups observed in the initial body weight of rats during feeding of GM potato Spunta, and Bt-corn (MON810: Ajeeb YG) during feeding study. However, the results disagreed with some studies (Zhu *et al.* 2004; Séralini *et al.* 2007; Zhu *et al.* 2015), where some GMOs (Roundup tolerant and MON863) increased the body weight. Zhu *et al.* (2015) found significant differences in organ weights among dietary groups fed on GM rice (expressing Cry1Ab/1Ac protein) and non-GM rice. Séralini *et al.* (2007) reported significant

Table 3. Changes in the relative organ weight (%) of animal groups at the end of the experimental period (30 days) [a].

Parameter	Animal group		
	Control group	NGMW [b] group	GMW[c] group
Final body weight [g]	108.4 ± 4.740[b]	147 ± 4.640[a]	142.6 ± 7.160[a]
Liver [%]	3.916 ± 0.068[a]	3.597 ± 0.241[a]	3.438 ± 0.093[a]
Spleen [%]	0.459 ± 0.032[a]	0.489 ± 0.045[a]	0.432 ± 0.051[a]
Kidney [%]	0.718 ± 0.054[a]	0.737 ± 0.044[a]	0.759 ± 0.017[a]
Stomach [%]	0.757 ± 0.024[a]	0.652 ± 0.044[b]	0.632 ± 0.026[b]

The same letter in the same row is not significantly different ($p > 0.05$).
[a]mean ± standard error ($n = 5$)
[b]NGMW = non genetically modified wheat
[c]GMW = genetically modified wheat

Table 4. Changes in the serum biochemical values of animal groups at the end of the experimental period (30 days) [a].

Parameter	Animal group		
	Control group	NGMW [b] group	GMW[c] group
GOT [U.L^{-1}]	10.6 ± 0.39[a]	11.4 ± 0.81[a]	10.8 ± 0.58[a]
GPT [U.L^{-1}]	11.2 ± 1.53[a]	10.6 ± 0.51[a]	10 ± 1.76[a]
Cholesterol [mg.dL^{-1}]	71.2 ± 10.0[a]	63.2 ± 10.9[a]	69.2 ± 17.7[a]
HDL cholesterol [mg.dL^{-1}]	64.6 ± 22.7[a]	43.8 ± 8.87[a]	57 ± 13.0[a]
Triglyceride [mg.dL^{-1}]	73.8 ± 5.46[a]	62.6 ± 18.6[a]	54.6 ± 7.45[a]
Total lipids [mg.dL^{-1}]	517.4 ± 26.0[a]	379.6 ± 89.6[ab]	257 ± 72.8[b]
Creatinine [mg.dL^{-1}]	0.396 ± 0.02[a]	0.396 ± 0.02[a]	0.506 ± 0.05[a]
Urea [mg.dL^{-1}]	106.2 ± 10.6[a]	85.8 ± 10.1[a]	79.2 ± 10.5[a]

The same letter in the same row is not significantly different ($p > 0.05$).
[a] mean ± standard error ($n = 5$)
[b] NGMW = non genetically modified wheat
[c] GMW = genetically modified wheat

variation in the weights of rats fed on MON 863 compared to the control animals. The findings of the present study showed no significant differences in the absolute body weights of animals fed on GMW in comparison with NGMW and control groups.

Relative organ weight

The impact of feeding diets containing 30 % GMW and NGMW on the relative weights of some organs (kidney, liver, spleen, and stomach) to the animal body weight is presented in Table 3. The relative ratio of liver to the body weight recorded a value of 3.91 %, 3.59 %, and 3.43 % for control, NGMW group, and GMW group, respectively. The relative ratios of spleen weight for control and NGMW group (0.43 – 0.48 %) were in lower levels as the value resulting from feeding on GMW. In addition, comparable findings were noted for the relative weights of the kidney (Table 3). From these findings, it is clear that there is no significant statistical differences between the studied groups. However, NGMW group, and GMW group showed a significantly lower range of relative ratio for stomach weight (-14 %) and (-16.5 %), respectively. These findings agree with Elsanhoty *et al.* (2004) and Poulsen *et al.* (2007) who found an increase in the relative weight of the small intestine (+10 %) in rats fed GM rice, andan increase in the absolute and relative weight of the adrenals. On the other hand, Poulsen *et al.* (2007) found statistically significantly reduce in the absolute weight of the adrenals (-15 %) in male rats fed on GM rice. Similar results were reported in the previous study on GM potato Spunta (Elsanhoty *et al.* 2004), wherein no significant differences in the spleen, kidney and heart weights were observed after 1.5 months of feeding. Kilic and Akay (2008) reported that the liver weights were increased in females' rats, but there were no significant differences in males. Séralini *et al.* (2011) reported that *Bt*-corn or GM soybean caused significant differences in organs' weights, especially liver and kidney because of these organs responsible for the detoxification. The obtained results disagree with Abdo *et al.* (2014) who reported significant differences in all organs weights among different groups after 45 days of feeding study.

Effect of GMW on serum parameters

The effects of feeding NGMW, and GMW on serum biochemical parameters that reflecting kidney and liver functions are given in Table 4. The results showed no significant differences among the studied parameters in all groups. The health conditions of all groups coincided with normal values of GPT, GOT, cholesterol, HDL-cholesterol, TAG, TL, creatinine, and urea. The results indicated that the kidney and liver functions were in a good state without suffering from acute infections. The total cholesterol was also in the normal physiology level. The results revealed that the general health and metabolic process of the animals were not affected by feeding on GMW, wherein there were no significant differences ($p < 0.05$) between groups. The exception was in the values of total lipids,

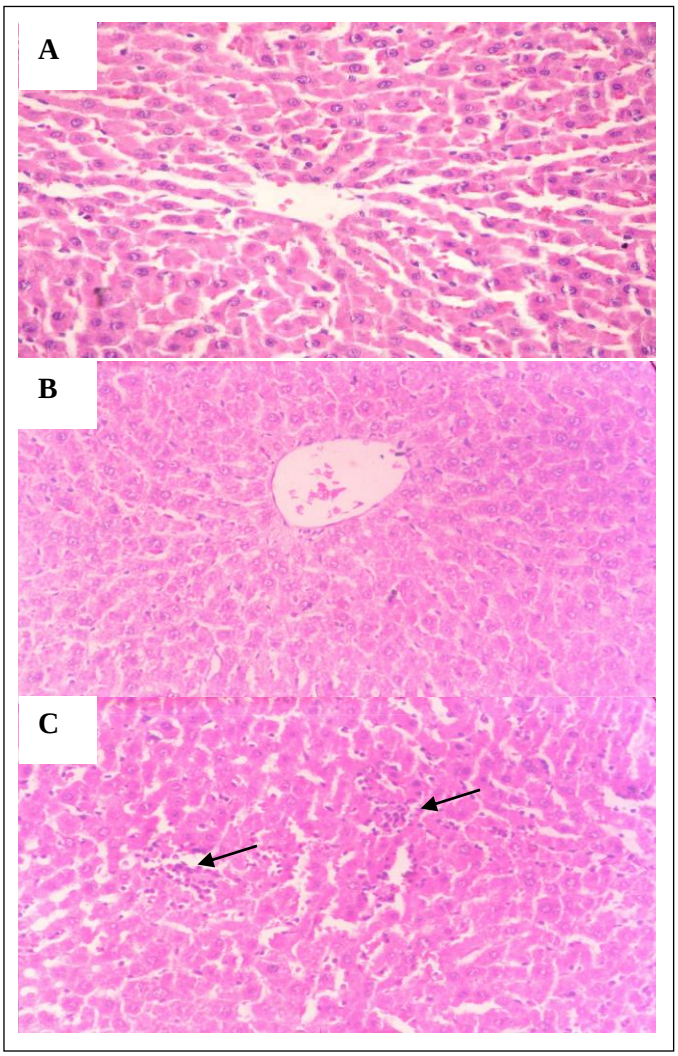

Fig. 1. Liver sections of control (**A**), NGMW group (**B**) and GMW group (**C**). Control group (**A**) and NGMW group (**B**) showing the normal histological structure of hepatic lobule, while GMW group (**C**) showing leucocytes in the hepatic sinusoids (arrows) (H&E X 200).

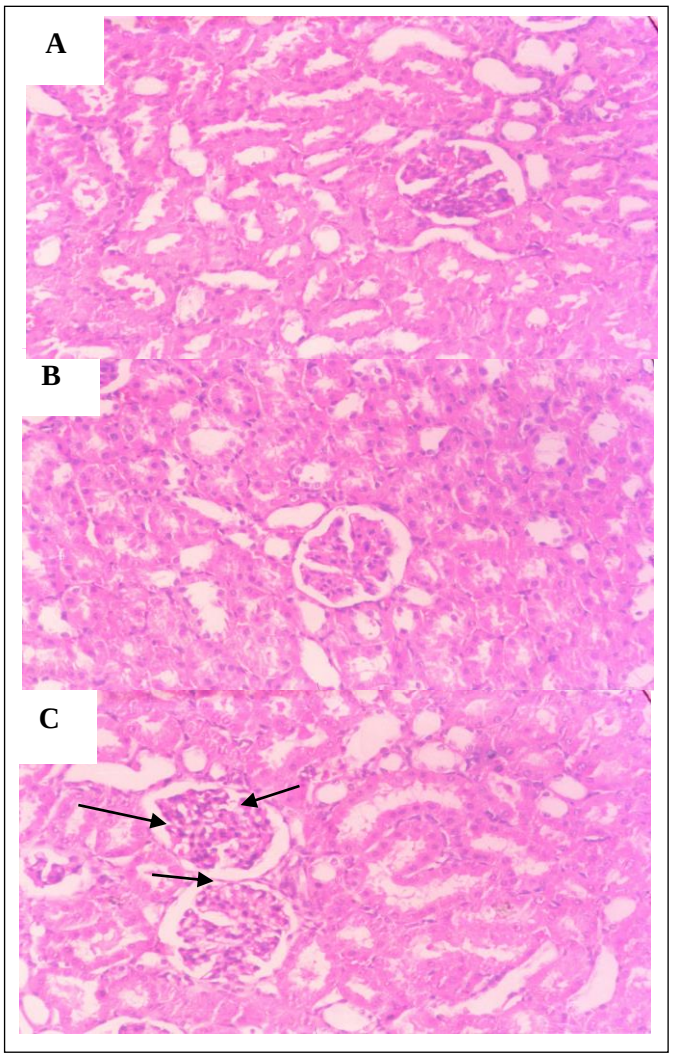

Fig. 2. Kidney sections of control (**A**), NGMW group (**B**) and GMW group (**C**). The control group (**A**) and NGMW group (**B**) showing normal histological structure, while GMW group (**C**) showing slight vacuolations of endothelial liming glomerular tufts (arrows) (H&E X 200).

wherein there were significant differences ($p < 0.05$) between the control group and GMW group. These results agree with Elsanhoty *et al.* (2004), who reported no significant difference in serum biochemical values between groups fed GM potato Spunta and non-GM potato. Also, the results agreed with Poulsen *et al.* (2007) who reported that male rats fed on GM rice had a lower plasma concentration of potassium as well as the levels of protein, and albumin. Abdo *et al.* (2014) reported from subchronic feeding study on rats fed on GM *Bt*-corn that there were many alternations in the organs weights, hematology, and serum biochemical parameters in *Bt*-corn group after 45 days but changes were increased after 90 days. The obtained results of lipid parameters disagreed with Gab-Alla *et al.* (2012) who reported an increase in all lipid parameters (except for HDL-cholesterol) of rats' blood fed on MON810-Ajeeb YG after 91 days of starting the experiment.

Histopathological profile

Microscopically examination of liver in the control group exhibited the normal histological structure of hepatic lobule (Fig. 1A). The liver of the rat from the NGMW group showed no histopathological changes (Fig. 1B). However, the liver of rat from group 3 showed the presence of leucocytes in the hepatic sinusoids (Fig. 1C). Concerning kidney, examined sections from control, and non-GMW group revealed

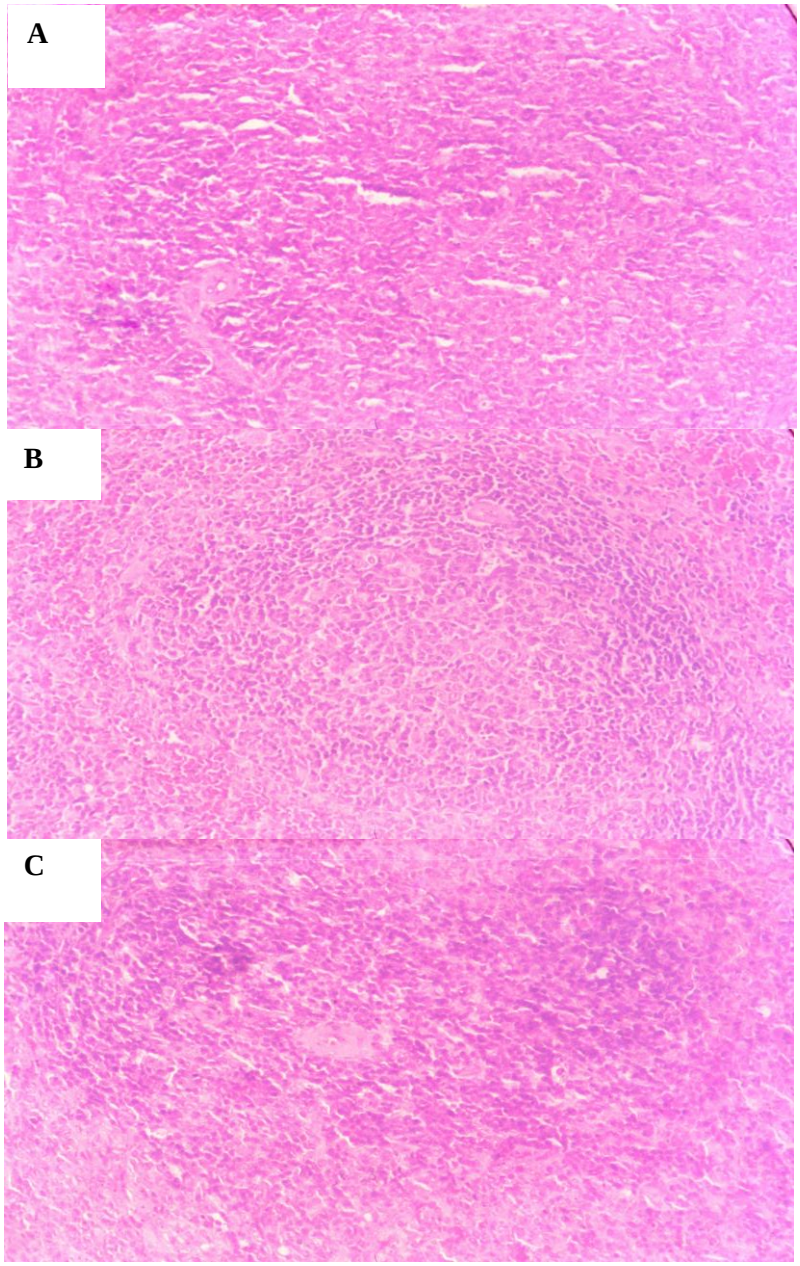

Fig. 3. Spleen sections of control (**A**), NGMW group (**B**) and GMW group (**C**). Control group (**A**), NGMW group (**B**) and GMW group (**C**) showing normal histological structure (H&E X 200).

no histopathological changes (Fig. 2A and B). Meanwhile, kidneys of rat from GMW group showed no changes except slight vacuolations of endothelial lining glomerular tufts (Fig. 2C). Regarding spleen, examined sections from the control group, non-GMW group and GMW group revealed no histopathological changes (Fig. 3A, B and C).

Macroscopically examination of the stomach from control revealed the normal histological structure of gastric layers (Fig. 4A), and examined sections from the NGMW group showed no histopathological changes (Fig. 4B). Moreover, the stomach of the rat from GMW group showed submucosal edema associated with few leucocytic cells infiltration (Fig. 4C). These results indicated that the submucosal edema could be caused by GMW. To explain the nontoxicity of GMW, a detailed description of submucosal edema is needed (Fig. 4C). These results agree with Kilic and Akay (2008) and Gab-Alla *et al.* (2012) who reported minimal histopathological changes in kidney and liver in rats fed on GM corn. The results of histopathological examination agree with the results obtained by Ewen and Pusztai (1999) and Trabalza-Marinucci *et al.* (2008) who observed no histological differences in pancreas, duodenum, liver, spleen, mesenteric lymph nodes,

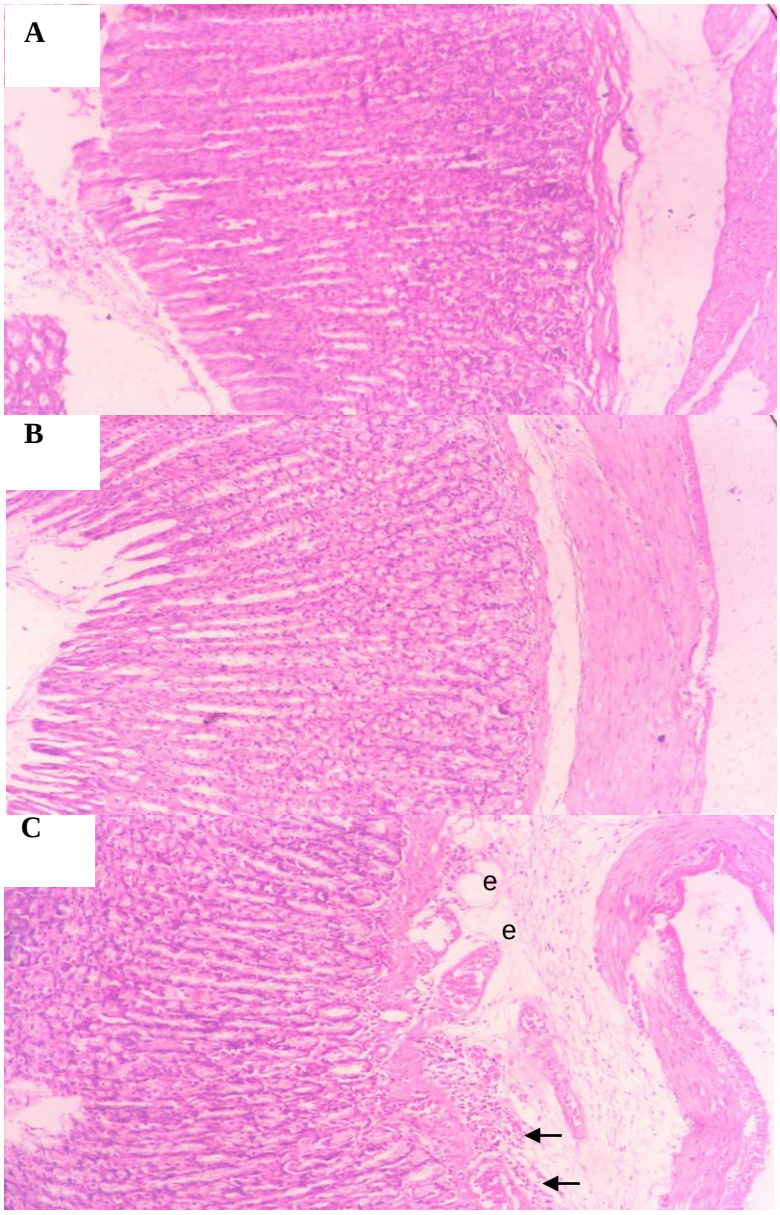

Fig. 4. Stomach sections of control (**A**), NGMW group (**B**) and GMW group (**C**). The control group (**A**) and NGMW group (**B**) showing normal histological structure, while GMW group (**C**) showing submucosal edema (**e**) associated with few leucocytic cells infiltration (arrows) (H&E X 200).

cecal appendix, rumen, and abomasum sections between control group and GM maize-fed groups in sheep and lambs. The differences between the groups (individuals) can fall under individual variability or due to the particular location of the individual histological cuts.

Conclusions

This research aimed to study the biosafety of GMW Hi-line 111. No statistically differences in the biochemical parameters were noted between animals fed on GMW and rats fed on NGMW. When it comes to GMW, which contains a gene from barley, with both the plants belonging to the same genus, we perhaps cannot expect a significant impact on parameters in the serum of the experimental animals compared to commercial wheat of the same strain. Based on the obtained data, it could be concluded that the GMW has no adverse health or toxic effects. However, a detailed description of submucosal edema and further studies on allergenic potential with long feeding periods might be needed.

Conflict of Interest

The authors declare that they have no conflict of interest.

Statements on Compliance with Ethical Standards

The procedures followed were in accordance with the institutional and national ethical standards of the responsible committee on animal/human experimentation.

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