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A three generation study with genetically modified Bt corn in rats: Biochemical and histopathological investigation

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Abstract

For the last ten years, in accordance with the increased use of genetically modified (GM) foods for human and livestocks, a large number of feeding studies have been carried out. However, the evidence is still far from proving whether the long-term consumption of GM foods posses a possible danger for human or animal health. Therefore, this study was designed to evaluate the effects of transgenic corn on the rats that were fed through three generations with either GM corn or its conventional counterpart. Tissue samples of stomach, duodenum, liver and kidney were obtained for histopathological examinations. The average diameter of glomeruli, thickness of renal cortex and glomerular volume were calculated and number of affected animals/number of examined animals for liver and kidney histopathology were determined. Amounts of urea, urea nitrogen, creatinine, uric acid, total protein, albumin and globulin were determined; enzyme activities of aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, gamma glutamyltransferase, creatine kinase and amylase were measured in serum samples. No statistically significant differences were found in relative organ weights of rats within groups but there were some minimal histopathological changes in liver and kidney. Changes in creatinine, total protein and globulin levels were also determined in biochemical analysis.
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Keywords: Transgenic Bt corn; Three generation study; Histopathology; Biochemical analysis; Wistar albino rat

1. Introduction

The global area of approved genetically modified (GM) crops such as soybean, corn, cotton, rice, canola, tomato have risen from 1.7 million hectares in 1996 to 102.0 million hectares in 2006 and the number of countries planting GM crops increased to 22. GM corn is the second principal biotech crop occupying 25.2 million hectares, after the GM soybean (James, 2006). The views endorsing the development of GM crops created with recombinant DNA technology, are based on improving the yield and quality of crops, solving the famine that would be a dangerous risk in the next 25 years, founding renewable sources for vaccines, drugs and bioplastics (Coghlan, 1995). Meanwhile, this technology poses scientific, technological, environmental, social, ethical, economical and political issues as well as health risks (Jones, 1999). In order to assess the potential risks of transgenic organisms, International Food Biotechnology Council (IFBC) has initially reported the safety evaluation of GMOs and then Organisation for Economic Cooperation and Development (OECD), Food and Agriculture Organisation of the United Nations (FAO), World Health Organisation (WHO) and International Life Science Institute (ILSI) have established safety assessment guidelines. OECD, developed the concept of substantial equivalence defined as comparison between GM organism and its traditional counterpart and used the data for future safety assessments (Kuiper et al., 2001).

European corn borer (*Ostrinia nubilalis*) and Southwestern corn borer (*Diatraea grandiosella*) have caused significant yield losses in corn (*Zea mays* L.) agriculture

Abbreviations: Bt, *Bacillus thuringiensis*; Cry, crystal protein; GM, genetically modified.

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(MacKenzie et al., 2007; Williams et al., 2005). Therefore, for over 40 years an insecticidal spray that includes a mixture of spores and associated protein crystals belonging to a Gram (–) bacterium *Bacillus thuringiensis* (Bt) has been being worldwide used (Nester et al., 2002). Intracellular crystal inclusions produced during sporulation of Bt are called parasporal crystals (*crystal protein*) which have insecticidal activity on insects midgut epithelium. After applications of recombinant DNA technology, more direct and controlled ways have been employed, namely genetically modified Bt crops, to fight with the aforesaid pests. Bt toxin encoded by bacterial cry gene, transferred to crops and expressed in their specific tissues. During the process of obtaining Bt protein from cultures in order to reach sufficient amounts, unwanted processes may cause toxicity in different organisms (Kuiper et al., 2001). Various nutritional analysis and short term feeding studies have been performed to demonstrate the possible effects of GM Bt corn on human and animal health (Brake and Evenson, 2004; Hammond et al., 2006; El Sanhoty et al., 2004; Netherwood et al., 2004). However, no reports on the possible health effects of GM crops through multigeneration in rats were obtained. Therefore, this three generation study in which rats fed with transgenic Bt corn was designed to clarify and enlighten the safety of long-term Bt corn consumption.

2. Materials and methods

2.1. Animals and housing

Eighteen female and nine male Wistar albino rats, obtained from Experimental Animals Production Center of Başkent University, in Ankara, Türkiye were 10 weeks of age at the beginning of the study. The study conforms the National Research Council guidelines for animal experimentation (National Research Council, 1996). The rats were allowed to acclimate to the housing conditions for 1 week during which they kept on basal diet. All rats were provided with tap water ad libitum through the study and were housed singly in polycarbonate cages with stainless steel cover. Laboratory conditions were maintained 12 h light/dark schedule, at temperature of 23 ± 3 °C and a relative humidity of 47 ± 5. Animals were observed two times daily for general wholesomeness and care.

2.2. Experimental diets

Bt corn that has insect resistance trait for the most invasive corn borers and its reference (*same genetic and breeding background but lack of the Bt transgene*) were obtained by the agency of Turkish Ministry of Agriculture and Rural Affairs. Besides, standard rat diet were purchased from Dokuz Tuğ Yem A.Ş., Ankara, Türkiye. Composition list of experimental diets are given at Table 1. Rats in Group I were fed only with standard diet, those in Group II with standard diet containing 20% reference corn and the ones in Group III with standard diet containing 20% transgenic Bt corn. These percentages were chosen in order to maintain a balanced rodent diet in this long-term feeding study. Corn grains and standard food were ground weekly to have a homogenous-mixed diet. Experimental diets were kept at 4 °C to preserve their protein content. Mixing ratios of standard and experimental diets are given at Table 2. The amount of dam's diet was 25 g/rat/day during gestation and lactation on the other hand offspring's diet was 20 g/rat/day from the beginning of 1.5 months after the birth.

Table 1
Composition of experimental diets for rats (%)

Ingredients	Bt corn	Non-Bt corn
Water	11.00	11.50
Dry nutrient	89.00	88.50
Crude protein	9.28	9.37
Crude fat	3.39	3.10
Starch	60.70	54.20
Sugar	2.33	2.10

2.3. Experimental design and treatment

Animals were randomly assigned to three groups, depending on their body weight means. Eighteen female Wistar albino rats (6 rats/each group) were mated with 9 male rats (one male for two female rats) overnight. Vaginal lavages were examined on light microscopy and the day that sperm was detected was considered to be the first day of pregnancy. Then, pregnant rats (F_0) were started to feed with either the diet containing 20% transgenic corn or 20% reference corn or standard rat diet depending on their groups. Dams and their offsprings were fed with the diets during the periods of mating, gestation, lactation, offspring care and pubescence. The offsprings of different dams in a group of each generation were mated among themselves throughout three generations. F_1, F_2 and F_3 generations were acquired by the same procedures described above. F_3 rats were also fed with either standard diet or experimental diets until they reached to 3.5 months age. The male rats that were used in mating were out of study. Male and female gender ratio of F_1, F_2, F_3 rats were compared.

2.4. Processing of tissues for histopathology

All F_3 rats were weighed and sacrificed by cervical dislocation at the end of the treatment. Their tissues of stomach (*corpus*), small intestine (*duodenum*), liver and kidney were removed, weighed and immediately fixed in Bouin's fixative for 8 h or 10% formaldehyde for 10 h. After the routine procedure, the fixed tissues were embedded in paraffin and 5 μm thick tissue sections were stained with routine haemotoxylin and eosin (H&E) or periodic acid schiff (PAS) in order to examine under light microscopy. All tissue sections were observed; only liver and kidney tissues were photographed. For histopathological changes "affected number of animals/examined number of animals" of liver and kidney, and their percentages were calculated. Forty glomeruli for each kidney specimen were selected and the maximum diameter and the thickness of cortex of selected glomeruli in serial sections was measured by Bs200prop program in Olympus BX51 system light microscope. The diameters were calculated as the mean of the longest and shortest diameters (Yamashita et al., 2002). The glomerular volume was calculated from the mean glomerular diameter, $d(G)$, using the formula: $4\pi(d(G)/2)3/3$ (Sugimoto et al., 1998).

2.5. Biochemical analysis

Blood samples were taken from heart of F_3 rats under ether anaesthesia at the end of the study. After centrifugation at 3000 rpm for 15 min, serum was separated. Serum samples were analysed for determination of the amounts of urea, urea nitrogen (BUN), creatinine, uric acid, total protein, albumin and globulin and for the measurement of enzyme activities of aspartate aminotransferase (AST), alanine aminotransferase

Table 2
Ratios of standard and experimental diets (%)

Groups	Diets (%)		
	Transgenic corn	Reference corn	Standard diet
Group I	0	0	100
Group II	0	20	80
Group III	20	0	80

(ALT), alkaline phosphatase (ALP), gamma-glutamyltransferase (GGT), creatine kinase (CK) and amylase by closed system of Olympus Au autoanalyser in Ölçüm Tıp Laboratory in Ankara, Türkiye.

2.6. Statistical analysis

Statistical analysis were performed using a SPSS 11.5 program for Windows. Data were expressed as mean ± standard error (SE) and statistical significance was assigned at the $p \leqslant 0.05$ level. The homogeneity of variance and normal distribution between groups was evaluated by General Linear Model procedure and Kolmogorov–Smirnov *nonparametric* test. Serum parameters were analysed by two-way ANOVA in male and female rats separately. To identify the sources of significant main effect, *post hoc* comparisons (Games–Howell, Tukey) were used. Body and relative organ weights were examined by one-way ANOVA and Games–Howell *post hoc* test (Sokal and Rohlf, 1995). Besides, histopathological findings were compared by using Fisher's exact test as described by Gad and Weil (1989).

3. Results

3.1. Clinical observations, number of offspring, body and relative organ weights

No signs of adverse effects were seen in clinical appearance of new borns in all three generation. The dams gave fertile progeny and successfully continued their strips. Number of offsprings in F_1, F_2, F_3 generations are shown in Table 3.

The final body weights, relative kidney and liver weights of female and male F_3 rats are given at Table 4. There were no significant differences in final body weights of rats in all groups whereas relative liver weights of female rats in Groups II and III and also relative kidney weights in Group II showed decreases. A statistically significant decrease was determined in the relative kidney weight of male rats in Group II.

3.2. Histopathology

3.2.1. Stomach and duodenum

No histopathological finding was observed in the stomach and duodenum of Bt rats in Group III. Gastric glands, surface epithelium preserved their structure in stomach. Villi and microvilli were continuous and there were no deformations in lacteals and goblet cells in duodenum.

3.2.2. Liver

Different levels of minimal granular degeneration were seen in all groups. The degrees and percentages of granular degeneration among groups are shown in Fig. 1. Granular degeneration level in 10% of examined sections was maximum (level 4) in Group III while no degeneration was observed at level 4 in Groups I and II. Additionally, degeneration level 3 was seen in 6.6% of Group II. Besides, focal mononuclear cell infiltration, congestion and nuclear border changes were determined locally in some ratios among groups and some of them were statistically significant (Table 5; Fig. 2B and C).

Table 3
Number of female and male offsprings in Groups I–III for three generation

Generations										
Groups	F0	F1			F2			F3		
	♀	♀	♂	Total	♀	♂	Total	♀	♂	Total
Group I	6	10	16	26	8	14	22	5	14	19
Group II	6	12	10	22	15	22	37	10	22	32
Group III	6	18	6	24	18	16	34	14	16	30

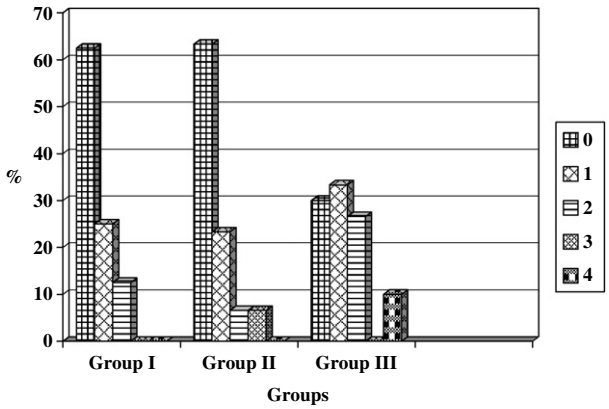
Table 4
Final body weights, relative liver and kidney weights of F_3 rats

	Groups		
	Group I	Group II	Group III
n	5	10	14
Females			
Body weight (g)	204.30 ± 4.35	246.15 ± 1.69	251.33 ± 6.43
Liver ($g \times 10^{-3}$)	33.46 ± 0.65	6.70 ± 0.78[a]	25.11 ± 0.34[a]
Kidney ($g \times 10^{-3}$)	3.36 ± 0.075	2.68 ± 0.053[a]	2.84 ± 0.002
n	14	22	16
Males			
Body weight (g)	294.20 ± 6.22	289.12 ± 6.30	299.89 ± 4.76
Liver ($g \times 10^{-3}$)	28.43 ± 0.25	30.10 ± 0.42	29.13 ± 0.53
Kidney ($g \times 10^{-3}$)	3.04 ± 0.04	2.97 ± 0.06[a]	2.99 ± 0.10

Each value is mean ± SE.
n: number of rats.
[a] Significantly different from control group, $P \leqslant 0.05$.

3.2.3. Kidney

Enlargements in parietal layer of Bowman's capsule and minimal tubular degenerations were observed at different ratios in groups (Table 5; Fig. 2E and F). The decreases in average short and long diameter of glomeruli and glomerular volume in Groups II and III were statistically different from controls while changes in the thickness of cortex was not significant among groups (Table 6).



Cumulative severity score on a 4-point scale: 0, no lesions; 1, slight severity; 2, minimal severity; 3, moderate severity; 4, marked severity.

Fig. 1. Levels and percentages of granular degenerations in liver of rats in control, reference and Bt group.

Table 5
Incidence of histopathological changes observed in liver and kidney of rats in control and experimental groups

Tissue	Finding	Groups					
		Female			Male		
		Group I	Group II	Group III	Group I	Group II	Group III
Liver	Focal infiltration	1/5	2/10	7/14	2/16	3/20	8/16[b]
	Congestion	0/5	2/10	10/14[a,b]	2/16	4/20	7/16
	Granular degeneration	1/5	2/10	9/14	3/16	6/20	13/16[a,b]
	Nuclear border change	0/5	3/10	10/14[a]	3/16	6/20	13/16[a,b]
Kidney	Enlargement in parietal layer of Bowman's capsule	0/5	1/10	6/14	2/14	1/15	5/11[a,b]
	Tubular degeneration	0/5	3/10	13/14[a,b]	2/14	4/15	9/11[a,b]

Data are expressed as number of affected/number of examined animals. Each value is mean ± SE.
[a] Significantly different from Group I (control) group, $P \leqslant 0.05$ (Fisher's exact test).
[b] Significantly different from Group II (reference) group, $P \leqslant 0.05$ (Fisher's exact test).

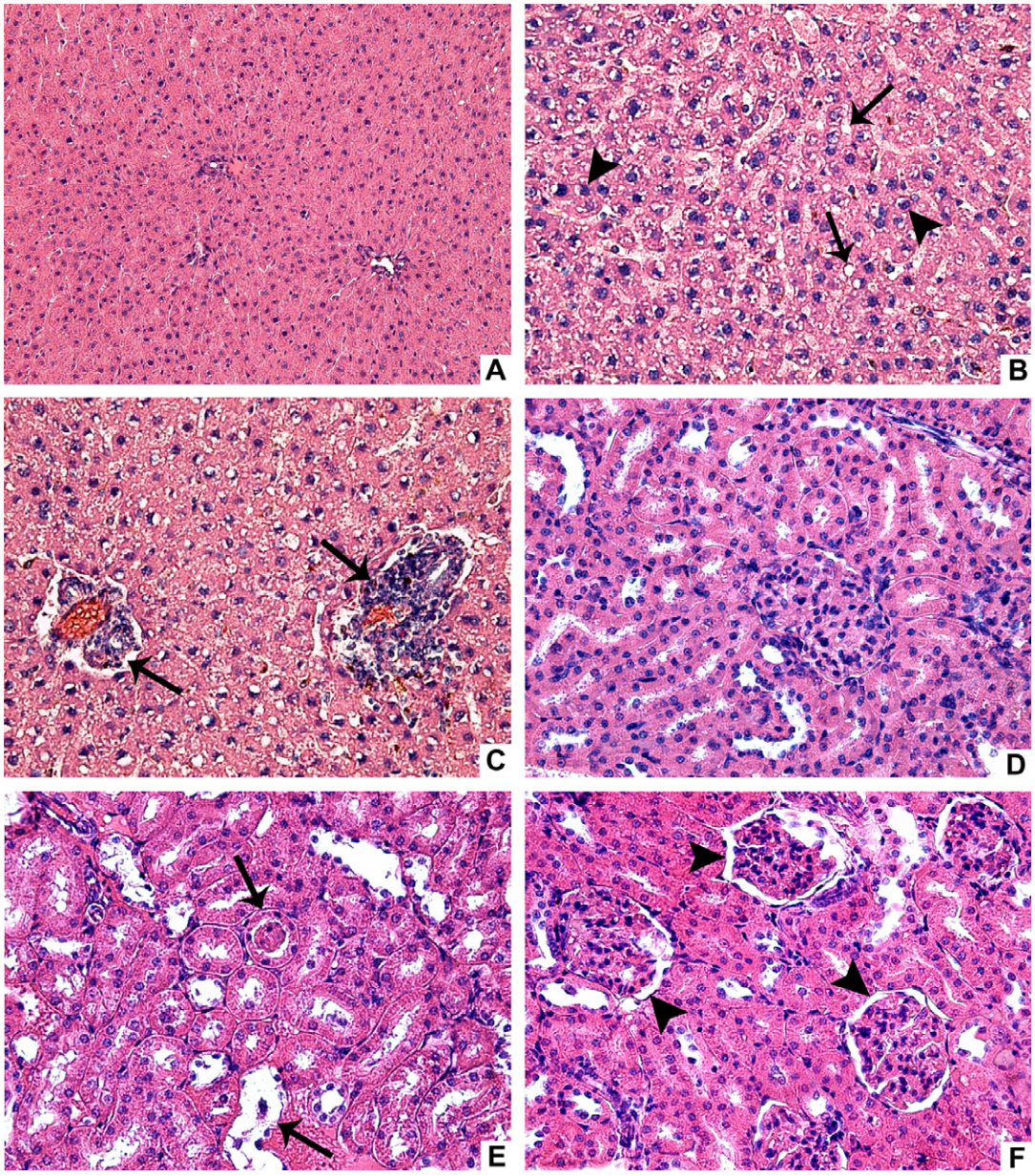


Fig. 2. Photomicrographs of liver (A–C) and kidney (D–F) tissues of rats stained with H&E. A and D are of control groups; B, C, E and F are of Bt corn groups. In liver, minor granular degeneration (arrows), nuclear border changes (B) (arrow heads) and (C) focal mononuclear cell infiltration (arrows) are shown. In kidney, (E) minimal tubular degeneration (arrows), (F) enlargements in parietal layers of Bowman's capsule (arrow heads) (magnification: A, 100×; B–F, 200×).

Table 6
Measurements of glomerular diameter, glomerular volume and thickness of cortex of rats in control and experimental groups

Groups	Diameter of glomerulus		Glomerular volume (10^4 μm^3)	Thickness of cortex (μm)
	x-axis	*y*-axis		
Group I	193.73 ± 38.88	187.96 ± 24.96	360	389.53 ± 27.50
Group II	172.89 ± 27.67[a]	184.89 ± 30.04	299	370.89 ± 41.04
Group III	168.19 ± 29.15[a]	164.73 ± 30.65 [a,b]	241	370.38 ± 44.70

Each value is mean ± SE.
[a] Significantly different from Group I (control) group, $P \leqslant 0.05$.
[b] Significantly different from Group II (reference) group, $P \leqslant 0.05$.

Table 7
Serum analysis values of female rats in control and experimental groups

Parameter	Groups		
	Group I	Group II	Group III
Urea (mg/dl)	38.50 ± 4.50	36.00 ± 3.00	36.25 ± 1.31
Urea nitrogen (mg/dl)	18.00 ± 2.10	16.73 ± 1.43	16.95 ± 0.61
Creatinine (mg/dl)	0.47 ± 0.005	0.54 ± 0.003[a]	0.50 ± 0.01
Uric acid (mg/dl)	1.69 ± 0.60	1.75 ± 0.14	1.63 ± 0.10
Total protein (g/dl)	6.07 ± 0.07	6.86 ± 0.15[a]	6.25 ± 0.13[b]
Albumin (g/dl)	3.15 ± 0.07	3.31 ± 0.008	3.42 ± 0.14
Globulin (g/dl)	2.92 ± 0.14	3.55 ± 0.16[a]	2.98 ± 0.14[b]
AST (U/L)	250.70 ± 53.20	152.50 ± 12.71	285 ± 22.98
ALT (U/L)	80.55 ± 17.55	50.06 ± 3.88	61.00 ± 5.54
ALP (U/L)	105.80 ± 12.62	63.98 ± 9.00	86.88 ± 14.75
GGT (U/L)	2.65 ± 0.15	1.76 ± 0.27	4.35 ± 0.91
Creatine kinase (U/L)	1368.00 ± 144.0	833.33 ± 90.87	1560.00 ± 100.51
Amylase (U/L)	443.5 ± 23.50	355.0 ± 7.09	369.2 ± 5.64

Each value is mean ± SE and each group consists of five rats.
[a] Significantly different from Group I (control) group, $P \leqslant 0.05$.
[b] Significantly different from Group II (reference) group, $P \leqslant 0.05$

Table 8
Serum analysis values of male rats in control and experimental groups

Parameter	Groups		
	Group I	Group II	Group III
Urea (mg/dl)	40.80 ± 2.63	39.00 ± 1.63	34.33 ± 2.18
Urea nitrogen (mg/dl)	19.06 ± 1.23	18.22 ± 0.75	16.03 ± 1.02
Creatinine (mg/dl)	0.47 ± 0.005	0.49 ± 0.017	0.41 ± 0.006[a]
Uric acid (mg/dl)	1.76 ± 0.14	2.05 ± 0.34	1.33 ± 0.05
Total protein (g/dl)	6.09 ± 0.19	6.62 ± 0.18[a]	6.37 ± 0.05[b]
Albumin (g/dl)	3.10 ± 0.09	3.19 ± 0.04	3.27 ± 0.07
Globulin (g/dl)	2.98 ± 0.10	3.42 ± 0.14[a]	3.10 ± 0.12[b]
AST (U/L)	299.12 ± 63.20	240.35 ± 23.02	547.03 ± 35.43
ALT (U/L)	81.52 ± 9.28	76.80 ± 8.32	122.10 ± 35.20
ALP (U/L)	115.21 ± 15.71	99.53 ± 16.02	120.99 ± 7.47
GGT (U/L)	3.56 ± 0.65	2.65 ± 0.32	2.00 ± 0.05
Creatine kinase (U/L)	1296.20 ± 200.51	1329.50 ± 167.7	823.33 ± 89.09
Amylase (U/L)	521.60 ± 31.46	559 ± 34.84	632.33 ± 84.33

Each value is mean ± SE and each group consists of five rats.
[a] Significantly different from Group I (control) group, $P \leqslant 0.05$.
[b] Significantly different from Group II (reference) group, $P \leqslant 0.05$.

3.3. Biochemical analysis

Results of biochemical analysis for female and male rats are presented in Tables 7 and 8. Alterations in the amounts of creatinine, globulin and total protein were statistically significant in treatment groups. Creatinine level differed depending on the group and also on gender. There were increases in the amount of creatinine in Group II females, on the other hand decreases in Group III males. Amounts of globulin and total protein were statistically different from controls in Group II but not in Group III. No statistically significant differences were noted for other parameters.

4. Discussion

GM technology have the advantage of improvement in productivity and quality of crops which express 0.01–0.1% protein of host's total protein (first generation-transgenic foods) and also prevention of various disease such as diabetes, hypertension, hypercholesterolemia and corpulence with novel crop (second generation-transgenic foods) having more than 1–10% expressing level (Hashimoto et al., 1999). Maize is one of the most widely used crop producing high-fructose corn syrup, glycose, dextrose, starch, oil, flour and meal. By means of transferring insecticidal trait to maize (Bt corn) it is possible to combat with demolishing pests that cause 7% loss of maize products (Kuiper et al., 2001).

The results of sub-chronic feeding studies on rats showed no histopathological, and biochemical effects but caused some minor changes observed suggesting long-term studies (Akay et al., 2003; Seralini, 2005). The reports on long-term feeding studies and comprehensive analysis with transgenic Bt corn are rare so this current study was planned in which rats fed with 20% Bt or reference corn (below the safety margin 33%) containing diets in order to restrain from one way and unbalanced feeding.

In our study, final body weights were not considered to be significant and decreases in relative weights of liver and kidney appeared randomly among all groups and sex, so differences were diet independent. Likewise no differences were observed in body weights and weights of kidney and liver in a recently published 90-day feeding study with Bt (Cry1Ab protein) corn in rats (Schrøder et al., 2007).

Results of a 13 week feeding study in rats with 11% or 33% Roundup Ready corn containing diets showed few increases in weight gain of males (Hammond et al., 2004) and no statistically significant differences were found in body or organ weights in a 90-day feeding study in rats with 11% or 33% MON 810 corn containing diets (Hammond et al., 2006). Similar body weights and increase in relative weight of small intestine and adrenal were found in another 90-day safety study with *Galanthus nivalis* expressing GM rice in rats (Poulsen et al., 2007).

Throughout our study, no adverse behavioural or clinical effects on F_1, F_2, F_3 generation animals were observed. Besides, birthrate and survival of the offsprings did not change among groups demonstrating successful reproduction. Polat (2005), reported no apparent differences were found in histopathological examinations in male and female rat reproductive system that were fed with transgenic Bt corn throughout two generation. Conversely, high level of mortality (55.6%) and decreases in weights of offsprings were reported in GM soybean feeding study in which female rats were fed before mating, during mating and pregnancy (Ermakova, 2005).

Our histopathological examinations in stomach and duodenum pointed out that Bt toxin did not cause deformations in gastrointestinal system. A 105-day feeding study supporting our findings was with Brown Norway rats and mice in which they fed with GM soybean and no histopathological abnormalities in mucosa of small intestine were detected (Teshima et al., 2000). In the only reported study on humans, seven volunteers fed with meal containing GM soybean and low levels of transgene survival were detected in small intestine only in three ileostomists with using molecular biology techniques (Netherwood et al., 2004). On the other hand, increase in hyperplastic cell was observed in the ileum of mice fed with *Bacillus thuringiensis* var. *kurstaki* delta-endoxin treated potatoes through 14 days (Fares and El Sayed, 1998).

The changes in the liver, as a site responsible for biotransformation and detoxification, suggest alterations in the metabolic processes. Markedly severity level of granular degeneration was seen in Bt diet containing groups in our study but not in control and reference groups. Hepatocyte nuclear size change related to both age and food (Schmucker, 1990). Therefore diets containing Bt may cause excess fatty supply for animals. But, we also observed granular degeneration at lower levels in rats of control and reference groups not showing health problems. Granular degeneration was statistically significant only in male rats in Group III. Additionally, nuclear border changes found statistically significant in female and male rats in Group III. 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).

One of the most important processes in kidneys is excretion of toxic metabolic waste products by glomerular and tubular filtration so we examined parietal layer of Bowman's capsule and tubular changes. These findings were statistically different in males and females in Group III from control and reference groups in our study. Glomerular diameters and volume reflecting renal functions decreased in experimental groups. Decreases in short glomerular diameter in Group II also short and long diameter in Group III were statistically significant. These alterations were minor changes and parallel to the enlargements in parietal layers. The thickness of renal cortex did not changed significantly among groups. Besides, thickness in Bowman's capsule, basal membrane and glomerular mesangium were not seen at PAS stained sections. In a short term safety assessment in rats fed with GM potato showed neither pathological nor histopathological finding in liver and kidney (Hashimoto et al., 1999). Another feeding study in rats with MON 863 Bt corn demonstrated inflammation in kidney and lesions in liver and kidney (Smith, 2005). 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.

According to the results of biochemical analysis, sex-dependent creatinine levels were detected. Significant lower plasma level of creatinine in Group III may refer to anomaly in working of muscles but we did not encounter any abnormal situation during the study (Vural et al., 1986). Creatinine levels of female serum samples in Group II significantly increased from Groups I and III, depending on individual alterations and diets. Significant differences were observed in amounts of globulin and total protein in reference groups besides, findings in Group III were statistically significant from Group II. Other parameters like AST, ALT, ALP reflecting liver function and like urea, urea nitrogen, uric acid reflecting renal function did not change. Histopathological changes in liver and kidney were in accordance with our biochemical results, showing damages were minor but not critical on animal health. Parallel to our findings, Poulsen et al. (2007) pointed out lower creatinine levels but increased in plasma activity of ALT in female rats fed on GM rice. Slight reduction of albumin/globulin ratio was observed in male rats fed with 33% MON 810 GM corn through 90 day but individually albumin and globulin were not different from control groups (Hammond et al., 2006). In another 90-day study, higher concentration of urea and reduction in concentration of protein was reported in male rats fed with Bt rice (Schrøder et al., 2007).

In conclusion, although the results obtained from this study showed minor histopathological and biochemical effects in rats fed with Bt corn, long-term consumption of transgenic Bt corn throughout three generation did not cause severe health concerns on rats. Therefore, long-term feeding studies with GM crops should be performed on other species collaboration with new improving technologies in order to assure their safety.

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References

Akay, M.T., Koçkaya, E.A., Selmanoğlu, G., Erdoğan,. Y., Çabuk, S., 2003, Transgenik Bt mısırın Sıçanlar Üzerine Etkilerinin Araştırılması, Proje No: F.Bİ. P90. Ankara, s:22–25.

Brake, D.G., Evenson, D.P., 2004. A generational study of glyphosate-tolerant soybeans on mouse fetal, postnatal, pubertal and adult testicular development. Food Chem. Toxicol. 42, 29–36.

Coghlan, A., 1995. Grow your own vaccines. New Scientist (Jan), 21–23.

El Sanhoty, R., Rahman, A.A.A., Bögl, K.W., 2004. Quality and safety evaluation of genetically modified potatoes Spunta with Cry V gene: Compositional analysis, determination of some toxins, antinutrients compounds and feeding study in rats. Nahrung/Food 48 (1), 13–18.

Ermakova, I.V., 2005. Influence of genetically modified soya on the birth-weight and survival of rat pups: preliminary study. Institute of Higher Nervous Activity and Neurophysicology Russian Academy of Science.

Fares, N.H., 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.

Gad, S.C., Weil, C.S., 1989. Statistics for toxicologists. In: Hayes, A.W. (Ed.), Principles and Methods of Toxicology, second ed. Raven Press, New York, pp. 435–483.

Hammond, B., Dudek, R., Lemen, J., Nemeth, M., 2004. Result of a 13 week safety assurance study with rats fed grain from glyphosate tolerant corn. Food Chem. Toxicol. 42, 1003–1014.

Hammond, B.G., Dudek, R., Lemen, J.K., Nemeth, M.A., 2006. Results of a 90-day safety assurance study with rats fed grain from corn borer-protected corn. Food Chem. Toxicol. 44, 1092–1099.

Hashimoto, W., Momma, K., Katsube, T., Ohkawa, Y., Ishige, T., Kito, M., Utsumi, S., Murata, K., 1999. Safety assessment of genetically engineered potatoes with designed soybean glycinin: compositional analyses of potato tubers and digestibility of the newly expressed protein in transgenic potatoes. J. Sci. Food Agric. 79, 1607–1612.

James, C., 2006. Executive Summary Global Status of Commercialized Biotech/GM Crops: 2006 ISAAA Brief 35.

Jones, L., 1999. Science, medicine, and the future, genetically modified foods. BMJ 581–584 (February).

Kuiper, H.A., Kleter, G.A., Noteborn, H.P.J.M., Kok, E.J., 2001. Assessment of food safety issues related to genetically modified foods. Plant J. 27 (6), 503–528.

MacKenzie, S.A., Lamb, I., Schmidt, J., Deege, L., Morrisey, M.J., Harper, M., Layton, R.J., Prochaska, L.M., Sanders, C., Locke, M., Mattsson, J.L., Fuentes, A., Delaney, B., 2007. Thirteen week feeding study with transgenic maize grain containing event DAS-Ø15Ø7-1 in Sprague–Dawley rats. Food Chem. Toxicol. 45 (4), 551–562.

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

Mazza, R., Soave, M., Morlacchini, M., Piva, G., Marocco, A., 2005. Assessing the transfer of genetically modified DNA from feed to animal tissues. Trans. Res. Oct. 14 (5), 775–784.

National Research Council, 1996. Guide for the Care and Use of Laboratory Animals. National Academy, Washington, DC.

Nester, E.W., Thomashow, L.S., Metz, M., Gordon, M., 2002. 100 Years of Bt A Critical Scientific Assessment. American Academy of Microbiology.

Netherwood, T., Martín-Orúe, M.S., O'Donnell, G.A., Gockling, S., Graham, J., Mathers, J.C., Gilbert, H.J., 2004. Assessing the survival of transgenic plant DNA in the human gastrointestinal tract. Nat. Biotechnol. 22 (2), 204–209.

Polat, S., 2005. Transgenik Bt mısırın Sıçanların Prenatal ve Postnatal Gelişimi Üzerine Etkileri Yüksek Lisans Tezi (The Effects of Transgenic Bt Corn on Prenatal and Postnatal Development of The Rats: Master Thesis) Hacettepe Üniversitesi, pp. 46–75.

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., 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.

Schrøder, M., Poulsen, M., Wilcks, A., Kroghsbo, S., Miller, A., Frenzel, T., Danier, J., Rychlik, M., Emami, K., Gatehouse, A., Shu, Q., Engel, K.H., Altosaar, I., Knudsen, I., 2007. A 90-day safety study of genetically modified rice expressing Cry1Ab protein (*Bacillus thuringiensis* toxin) in Wistar rats. Food Chem. Toxicol. 45, 339–349.

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

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.

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

Sokal, R., Rohlf, F.J., 1995. Biometry, third ed. W.H. Freeman and Company, NY (Section 9).

Sugimoto, H., Shikata, K., Matsuda, M., Makino, H., 1998. Increased expression of endothelial cell nitric oxide synthase (ecNOS) in afferent and glomerular endothelial cells is involved in glomerular hyperfiltration of diabetic nephropathy. Diabetologia 41, 1426–1434.

Teshima, R., Akiyama, H., Okunuki, H., Sakushima, H., Takahashi, M., Matsuda, T., Fujimura, T., 2000. Effect of GM and non-GM soybeans on immune system of BN rats and B10 mice. J. Food Hyg. Soc. Jpn. 41, 188–193.

Vural, S., Çetin, E.T., Tuzlacı, U., 1986. Klinik teşhiste laboratuvar. İstanbul. s.75.

Williams, W.P., Windham, G.L., Buckley, P.M., Perkins, J.M., 2005. Southwestern corn borer damage and aflatoxin accumulation in conventional and transgenic corn hybrids. Field Crops Res. 91 (2-3), 329–336.

Yamashita, T., Shikata, K., Matsuda, M., Okada, S., Ogawa, D., Sugimoto, H., Wada, J., Makino, H., 2002. Beraprost sodium, prostacyclin analogue, attenuates glomerular hyperfiltration and glomerular macrophage infiltration by modulating ecNOS expression in diabetic rats. Diab. Res. Clin. Practice 57, 149–161.