The Comparative Effects of Genetically Modified Maize and Conventional Maize on Rats

Genetik Olarak Değiştirilmiş Mısır ve Konvensiyonel Mısırın Sıçanlardaki Etkilerinin Karşılaştırılması

Genetiği Değiştirilmiş Mısır / Genetically Modified Maize

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Özet

Amaç: Genetik olarak değiştirilmiş ürünlerin dünyanın beslenme problemlerini çözme potansiyelleri vardır. Öte yandan, bu yeni ürünlerin çevre, hayvan ve insan sağlığı üzerine olan etkilerinin test edilmesi ve risk hesabının yapılması gereklidir. Bu çalışmada, sıçan yavrularının kuru gıdaya başlaması ile cinsel olgunluğa ulaşıncaya kadar geçen sürede yedikleri genetiği değiştirilmiş mısırın, sıçanlar üzerindeki pozitif veya olası negatif etkilerinin incelenmesi amaçlanmıştır. Gereç ve Yöntem: Bu çalışmada Wistar türü otuz sıçan kullanıldı. Sıçanlar Bacillus thuringiensis mısır ve konvensiyonel mısırla 40 gün boyunca beslendi. Deneysel periyot sonrasında, sıçanların organ uzunluğu, yüksekliği ve ağırlıkları ile serum kimyası ve kan değerleri ölçüldü. Bulgular: Bacillus thuringiensis mısır grubundaki sıçanların karaciğer, dalak, akciğer ve böbreklerinin uzunluk, yükseklik ve ağırlıklarının konvensiyonel ve kontrol grubuna göre faklılıklar gösterdiği belirlendi. Glikoz, üre, total protein, kolesterol, trigliserid, çok düşük yoğunluklu lipoprotein, düşük yoğunluklu lipoprotein, kalsiyum, fosfor, sodyum, potasyum ve klor gibi serum kimyası ve kan parametreleri incelendiğinde ise Bacillus thuringiensis mısır grubundaki sıçanları ile konvensiyonel ve kontrol grubu arasında önemli farklılıklar bulundu. Sonuç: Bu çalışmadan elde edilen sonuçlara bakıldığında, Bacillus thuringiensis mısırın yalnızca organ uzunluk, yükseklik ve ağırlıkları üzerinde etkisinin olmadığı aynı zamanda serum kimyası ve kan değerleri üzerinde de değişikliklere sebebiyet verebileceği görülmüştür.

Anahtar Kelimeler

Bt Mısır; Genetiği Değiştirilmiş Organizmalar; GDO; Sıçan

Abstract

Aim: Genetically modified crops have a potential to solve many of the world's nutrition problems. On the other hand, the impact of these novel crops on environmental, animal and human health should be tested and their risk assessment is required. In this study, the aim of this study was to investigate the positive or possible negative effects of genetically modified maize on offspring rats which were between the start of dry food feeding and the time interval until they reached puberty. Material and Method: Thirty Wistar albino rats were used in this study. The rats were fed with transgenic Bacillus thuringiensis maize and conventional maize during 40 days. After the experimental period, the length, height and weight of organs and serum chemistry and hematology values were measured. Results: The length, height and weight of liver, spleen, lung and kidneys in Bacillus thuringiensis maize group of rats were different from those in control and conventional groups. When mean values of serum chemistry and hematology parameters, which were glucose, urea, total protein, cholesterol, triglyceride, very low-density lipoprotein, low-density lipoprotein, calcium, phosphorus, sodium, potassium, chlorine were examined, some obvious differences were found between the rats fed with transgenic maize and its conventional counterpart and control groups. Discussion: The results of this study showed that Bacillus thuringiensis maize may not only have an effect on the length, height and weight of organs of the maturing term of rats but also lead to alterations in serum chemistry and hematology values.

Keywords

Bt Maize; Genetically Modified Organisms; GMO; Rat

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Introduction

The application of biotechnology in the field of plant agriculture over the last two decades has provided extraordinary opportunities for developers to introduce novel traits into plant crops [1]. So, these plants were named with the term of genetically modified crops (GMC). GMC are plants whose DNA is modified using genetic engineering techniques. In most cases the aim is to introduce a new trait to the plant which does not occur naturally in this species [3]. GMC occupied more than 143 million ha in 23 countries, with soybean, cotton, maize and rapeseed as the dominant crops [4]. One area of interest is the protection of crops from insect pests by the synthesis of insecticidal proteins. A popular strategy for achieving this end has been to transfer genes from the soil bacterium Bacillus thuringiensis (Bt) into plant crops (primarily into corn and potatoes) that synthesize insecticidal protein crystals [1]. To reduce reliance on insecticide sprays, plants have been genetically engineered to make insecticidal proteins derived from the common bacterium Bt. These Bt toxins kill some devastating insect pests [2]. Transgenic maize (Bt maize) has become widely adopted in all over the world. For example, in 2009, Bt maize was planted on more than 22.2 million hectares, constituting 63% of the U.S. crop [5]. GMC may not only have better nutritional and pharmaceutical values but are also resistant to pest and diseases, tolerant to extreme temperatures and herbicides. But they pose many challenges for the governments, scientists, industrialists and policy makers, especially in the areas of safety testing, regulation, international policy and food labelling [6]. So, the GMC food and feed are compared with their non-GMC counterparts in order to identify intended and unintended (unexpected) differences which subsequently are assessed with respect to their potential impact on the environment, safety for humans and animals, and nutritional quality [7]. In this study, the rats were nourished with Bt maize and its conventional counterpart in order to identify safety, similarities and differences between Bt maize and conventional maize.

Material and Method

Our research proposal was submitted to the Atatürk University ethics committee in December 2011. No changes were requested to the protocol, and final approval was received two weeks later.

Thirty Wistar albino rats were used in this study. The rats were 3–4 weeks old (That time was the starting time of rats feeding with standard rat diet) at the initiation of the study. The animals were housed in stainless steel wire cages at 22 ± 1 oC, relative humidity 55 ± 5%, air change 10 times/hour and electric light between 08.00 am and 20.00 pm. In this study, rats were fed with transgenic Bt maize during 40 days. The rats were fed until the day of 60. That time was the age at puberty of rats. In general, rats reach puberty around 50 to 60 days of age [8]. That time interval was particularly chosen in order to see the short time effect of Bt maize on feeding and puberty periods of rats. Organ length, height and weight of the rats were examined, also serum analysis were performed. Study was begun with 3 groups consisting of control. Conventional and Bt and each of them containing 10 male rats. Control group was fed with standard rat pellet. Conventional group was fed with 80% standard rat pellet and 20% conventional maize and Bt group was feed with 80% standard rat pellet and 20% Bt maize. The experiment was completed when the rats became 60 days old. At the end of the experiment, 30 rats were dissected. The values of different parameters such as urea, creatinine, uric acid, total protein etc. and the enzyme activities were measured in serum samples by using otoanalyzer (Roche Diagnostica). The length, height and weight of rats' organs in Bt maize, conventional maize and control groups were determined by measuring devices.

Statistical Analysis

Independent Samples T-Test was used for comparing parameters among the three groups. All data were expressed as mean ± Standard error of means. Differences were considered significant when the P values were less than 0.01.

Results

There were not any histopathologically changes of organs in rats of Bt maize group when compared with those of conventional and control groups. When the length, height and weight of organs which were liver, spleen, stomach, heart, lungs, testicular and kidneys in experimental groups were examined, the length, height and weight of liver, spleen and kidneys, also the length and weight of lungs in Bt maize group rats were seen as significantly different from conventional maize and control's values (Table 1 and Table 2). No significant differences were seen among groups on the length, height and weight, including stomach, heart, testicular and no significant differences were seen between the organs of conventional maize group and the control group parameters. Clinical biochemistry values analyzed were: serum aspartate aminotransferase (AST), serum alanine aminotransferase (ALT), alkaline phosphatase (ALP), total bilirubin, blood urea, Uric acid, blood creatinine, cholesterol, very low-density lipoprotein (VLDL), low-density lipoprotein (LDL), Creatine kinase (CK), Amylase, triglycerides, glucose, total serum

Table 1. Organ length and height mean values ± SD (%) in male rats following 30 days of exposure to different food groups

Organ	The length of Organ (mm) (X ± SD)			Height of Organ (mm) (X ± SD)		
	Control (n=10)	GM (n=10)	Conventional (n=10)	Control (n=10)	GM (n=10)	Conventional (n=10)
Liver	44.65 ± 5.30	77.88±4.89*	47.41 ± 3.05	49.62 ± 3.15	67.72± 3.87*	56.06 ± 3.25
Spleen	7.79 ± 1.18	10.18±0.86*	8.40 ± 1.58	33.63 ±3.39	40.95±3.75*	34.82 ± 3.68
Stomach	20.60 ±6.44	18.03± 1.61	17.31 ± 1.22	32.86 ±4.46	31.87± 2.85	29.93 ± 4.26
Lung	21.52 ±2.78	25.57±2.15*	21.12 ± 2.44	26.95 ±2.91	28.80± 3.02	26.72 ± 1.82
Heart	10.49 ±1.40	11.36±1.80	12.09 ± 1.28	14.29 ±2.13	15.29±1.64	15.05 ± 1.06
Testicular 1	10.08 ±0.48	10.39±0.93	10.40 ± 0.23	18.58± 1.30	18.68±1.16	19.17 ± 0.54
Testicular 2	10.32± 0.48	10.47±0.85	10.48 ± 0.38	18.54± 0.74	18.26±0.95	19.08 ± 0.55
Kidney 1	14.23 ±0.56	11.49±1.11*	14.44 ± 0.99	9.09 ± 0.70	9.45 ± 2.26*	15.01 ± 0.56
Kidney 2	15.02 ±0.79	9.79±0.57*	15.00 ± 0.68	9.06 ± 0.58	14.85±2.70*	9.22 ± 0.97

The number of animals was 10 rats; asterisks (*) in the same line are statistically different, data is presented as group mean values ± SD *p< 0.01
Abbreviations; GM: Genetically Modified Maize

Table 2. Organ weight mean values ± SD (%) in male rats following 30 days of exposure to different food groups

Organ	Weight of Organ (mg) (X ± SD)		
	Control (n=10)	GM (n=10)	Conventional (n=10)
Liver	6514.60± 1051.62	8325.07±1396.21*	6728.95±1550.28
Spleen	777.06 ± 212.42	1514.05±231.04*	775.74±217.73
Stomach	1002.54±118.14	1014.17 ± 116.77	970.0±8116.21
Lung	0.975.25 ± 134	1217.88± 249.9*	1103.9± 141.7
Heart	556.01±110.44	612.57 ± 118.85	582.48 ± 91.88
Testicular 1	986.90±109.19	972.92 ± 171.43	1003.61±53.38
Testicular 2	1074.25±103.94	967.72 ± 165.14	1016.52±71.13
Kidney 1	507.82 ± 24.86	682.05 ± 106.41*	511.10 ± 29.63
Kidney 2	513.49 ± 26.25	695.73 ± 81.44*	519.29 ± 31.19

The number of animals was 10 rats; asterisks (*) in the same line are statistically different, data is presented as group mean values ± SD *p< 0.01
Abbreviations; GM: Genetically Modified Maize

protein, Unsaturated Iron Binding Capacity (UIBC), Total Iron Binding Capacity (TIBC) albumin, calcium, inorganic phosphorus, Magnesium, sodium, potassium, iron and chlorine. According to serum chemistry and hematology analyses of the rats in Bt maize, conventional maize and control groups, significant changes in glucose, urea, total protein, cholesterol, triglyceride, VLDL, CK, LDL, calcium, phosphorus, sodium, potassium, chlorine levels were determined. While there were no differences among the other parameters of groups (Table 3). The results of serum chemistry and hematology analyses and the length, height and weight of organs show that rats fed with genetically modified Bt maize throughout maturing period of rats have notable changes on organs (Table 3).

Table 3. Serum chemistry and Haematology mean values ± SD in male rats following 30 days of exposure to different food groups

Parameters	Control (n=10) (X ± SD)	GM (n=10) (X ± SD)	Conventional (n=10) (X ± SD)
Glucose (mg/dl)	134.90 ± 21.97a	175.10 ± 27.94b*	138.70 ± 9.04a
Urea (mg/dl)	20.00 ± 4.59a	64.60 ± 6.83b*	19.30 ± 5.48a
Creatinine (mg/dl)	0.37 ± 0.07a	0.35 ± 0.05a	0.32 ± 0.04a
Uric acid (mg/dl)	1.11 ± 0.25a	1.19 ± 0.36a	1.23 ± 0.64a
AST (U/L)	128.10 ± 18.16a	113.50 ± 13.18a	117.30 ± 10.17a
ALT(U/L)	76.30 ± 12.78a	67.80 ± 11.42a	69.30 ± 4.90a
Alkaline Phosphatase (U/L)	322.00 ± 53.01a	329.10 ± 64.16a	286.40 ± 44.93a
Total Protein (g/dl)	6.28 ± 0.24a	5.82 ± 0.37b*	5.85 ± 0.37c*
Albumin (g/dl)	3.71 ± 0.12a	3.68 ± 0.21a	3.69 ± 0.11a
Cholesterol (mg/dl)	87.70 ± 12.35a	108.10 ± 10.30b*	88.10 ± 11.29a
Triglyceride (mg/dl)	56.90 ± 16.17a	82.20 ± 17.79b*	61.10 ± 15.83a
VLDL (mg/dl)	12.00 ± 3.83a	16.50 ± 3.63b*	12.90 ± 4.31ac
CK (U/L)	183.50 ± 42.67a	178.36 ± 50.38a	188.08 ± 30.71a
LDL (mg/dl)	103.30 ± 33.02a	169.60 ± 77.13b*	98.50 ± 48.44a
Amylase (U/L)	2836.20 ± 496.23a	2958.00 ± 464.32abc	2936.60 ± 335.75abc
Calcium (mg/dl)	10.58 ± 0.46a	5.15 ± 0.65b*	11.00 ± 0.47a
Phosphorus (mg/dl)	6.18 ± 0.93a	9.06 ± 0.86b*	6.23 ± 0.67a
Magnesium (mg/dl)	1.96 ± 0.24a	2.20 ± 0.34a	2.16 ± 0.30a
Iron (µg/dl)	163.80 ± 44.05a	206.10 ± 62.99a	216.40 ± 50.57a
UIBC (µg/dl)	397.70 ± 66.03a	353.20 ± 60.25a	365.10 ± 19.87a
TIBC (µg/dl)	597.70 ± 37.08a	596.30 ± 21.07abc	582.00 ± 39.09a
Sodium(mmol/L)	134.00 ± 1.56a	139.90 ± 1.45b*	135.10 ± 1.52a
Potassium (mmol/L)	6.07 ± 0.48a	8.64 ± 1.00b*	6.09 ± 0.20a
Chlorine (mmol/L)	96.15 ± 0.47a	101.26 ± 1.73b*	97.40 ± 1.23a

The number of animals was 10 rats; different letters in the same line are statistically different, data is presented as group mean values ± SD. *p< 0.01
Abbreviations; Aspartate aminotransferase (AST), Alanine aminotransferase (ALT), Alkaline phosphatase (ALP), Very low-density lipoprotein (VLDL), Low-density lipoprotein (LDL), Creatine kinase (CK), Unsaturated Iron Binding Capacity (UIBC), Total Iron Binding Capacity (TIBC).

Discussion

The GMC currently on the market are mainly aimed at an increased level of crop protection through the introduction of resistance against plant diseases caused by insects or viruses, or through increased tolerance towards herbicides. On the other hand, large scale cultivation of genetically engineered plants in some regions of the world have revealed a broad range of adverse impacts on the future of sustainable agriculture, such as increased weed resistance [9], increasing use of pesticides [10], pest resistance [11] and pest replacement [12]. Therefore, for the public acceptance of these GMC materials an important problem seems to be related to the safety assessment of new GMC foods. Taking into account that different GMC include different genes inserted in different ways, the World Health Organization indicates that individual foods and their safety should be assessed in a case-by-case, the present database regarding the toxicological, nutritional and environmental health hazards of GMC are inadequate [13]. So the aim of this was to evaluate the possible risk assessment and to assure safety of Bt maize requires thorough testing and risk assessment. So, rats were fed Bt maize and conventional maize. During the period tested, rats in each group which were Bt, conventional, and control, grew well and there were not any differences among groups' in appearance, food intake or body weight. At the end of the experiment indicated neither pathologic symptoms in all rats tested nor histopathological abnormalities in organs. On the other hand significant differences were found in height, length weights of liver, spleen and kidneys, also the length and weight of lungs. Those differences could be thought that Bt maize has a notable growing affect on internal organs of rats. As a matter of fact that, in a feeding study in rats with MON 863 Bt corn demonstrated inflammation in kidney and lesions in liver and kidney [14]. Also, Séralini et al.[15]. And Li et al. [16] showed that body weight might be significantly altered with the consumption of Mon 863 corn. On the other hand, in another study, Seralini [17] observed decreases in weight of kidney, tubular and in blood composition, among the rats after feeding BT maize. In briefly, administration Bt maize to rats at up to 20% of their diet for 40 days produced several changes for the height, length weights of organs that were not correlated histopathologically. These changes likely represent a nutritional effect as the result of dietary displacement with BT maize, and may not be considered as toxicological in nature. On the other hand, when serum chemistry and hematology analyses of the rats in Bt maize, conventional maize and control groups were examine, significant differences were seen. Especially high cholesterol, triglyc-

eride, VLDL, LDL, levels in Bt maize group were determined (Table 3). The marked hyperlipidaemia in blood characterises the diabetic state. As a matter of fact the glucose levels of in Bt maize group were determined as higher than the glucose levels of control and conventional groups. The diabetic state may therefore be regarded as a consequence of the uninhibited actions of lipolytic hormones on the fat depots [18]. Excess of fatty acids in plasma promoted by Bt maize promotes the liver conversion of some fatty acids to phospholipids and cholesterol. These two substances, along with excess of triglyceride formed in the liver, may be discharged into lipoproteins in the blood [19]. In addition these results, urea, calcium, inorganic phosphorus, sodium, potassium and chlorine reflecting renal function, levels of Bt maize were significantly different from levels of control and conventional groups (Table 3). As a matter of fact, feeding study in rats with GM-maize, significant increases in blood urea nitrogen and higher spleen weight were found. In another 90-day study, higher concentration of urea and reduction in concentration of protein was reported in male rats fed with Bt rice [20]. Those results are in accordance with our biochemical results except for total protein levels (Table 3). Although, there were differences among biochemical parameters of Bt maize, control and conventional maize, interestingly there were not any pathological defect in tissues. Moreover, we did not encounter any abnormal situation for animals in all three groups during the study. This situation could be explained as signs of toxicity of Bt maize rather than proofs of toxicity. On the other hand, in a study that included different developmental stages (larvae, pupa and adult), it was found that generally young larvae are more sensitive to Bt maize than mature larvae or adults [21]. The findings of that study are parallel to our findings. So, acute and medium-term effects can be observed for other species collaboration with new improving technologies. When the other parameters such as AST, ALT, ALP reflecting liver function and creatinine, uric acid, Albumin, CK, Amylase, Magnesium, Iron, Unsaturated Iron Binding Capacity, Total Iron Binding Capacity were examined, there were no significant differences among rats fed conventional maize, Bt maize and control groups parameters. Results obtained from this research show that group of rats consuming Bt maize from weaning age to adulthood had significant organ weight higher than that of conventional maize consuming group and the control, in spite of the fact that Bt maize and conventional maize and control-consuming groups had similar histopathological results. Additionally, biochemical analysis demonstrated that there are significant differences among Bt maize, conventional maize and the control group. On the other hand, the groups of rats consuming conventional maize had similar results to those of the control. Because of the importance that the consumption of GMC have increased, as well as their enormous potential in the near future, taking into account the results of this study, conflicting interests and concerns against GMC. GM crops should be performed on other species collaboration with new improving technologies also further studied in acute, medium-term and longer-term experiments must be implemented in order to assure their safety.

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