Biosafety
ISSN: 2167-0331

Rahman, et al., Biosafety 2015, 4:2
DOI: 10.4172/2167-0331.1000122

Research Article Open Access

Mammalian Food Safety Risk Assessment of Transgenic Cotton Containing Cry1Ac Gene Conducted Independently in Pakistan

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Rec date: Jun 13, 2015; **Acc date:** Sep 23, 2015; **Pub date:** Sep 28, 2015

Abstract

Impact of transgenic cotton containing Cry 1Ac (Bt cotton) has been witnessed in term of reduced insecticide use and enhanced cotton production, are compelling factors for its rapid adoption worldwide. Though Bt cotton has been released for cultivation based on the biosafety data generated mostly by the developer, and the information on its safe use are yet meager. Hence additional studies are needed to support the food safety issues by developing different cases with independent Bt-cotton genotypes. In the present study, seed and leaves of IR-NIBGE-901 (containing Bt gene) were fed to rabbits over a period of 90 days as to know 1) non-target mammalian food-safety and 2) primary effect as feed to domestic animals. During the course of study, all rabbits both in treated and control groups grew well without any marked differences in appearance, food/water intake or gain in body weight. Similarly, no differences were observed in complete blood composition, liver enzymes, random blood sugar or cholesterol. Necropsy, at the conclusion of the study revealed neither pathological symptoms in any of the rabbits tested nor histopathological abnormalities in liver and kidney. Potential genotoxicity to liver and kidney cells at the DNA level was measured first time by comet assay. Tail like structures following electrophoresis of extracted DNA in agarose gels (indicative of genetic damage) was not observed among the treated or control groups. This study suggests that Bt cotton in the diet has no adverse effect on growth and development of rabbits as one of examples for mammals.

Keywords: Bt cotton; Cry1Ac; Rabbits; Comet assay; Food safety; Dietary risk assessment

Introduction

Globally cotton is an important source of feed and edible oil, and is grown in more than 80 countries [1,2]. crop is vulnerable to bollworm larvae (*lepidopteran* pests). Earlier, spores of *Bacillus thuringiensis* (Bt) and aerial treatment of synthetic pesticides were exploited as a valuable pest management tool by conventional and organic farmers. However, the opinion on safe use of bacterial spores has not been set because of killing of non-target *lepidopteran* [3] initially caused by bacterial spore or other microbial contamination [4]. On the other hand, integrated pest management system has been proposed, and practices have been conducted to avoid the potential risk of non-target damage.

introduction of genetically cotton, expressing toxin genes derived from the bacterium *Bacillus thuringiensis*, (Bt) which has a reduced requirement for the application of insecticides to control insect pests [5,6] has reduced production cost and improved yields in many practices at various countries [7-12]. Bt gene introduced into cotton (Cry 1Ac) confers a high degree of resistance to major *lepidopteran* insect pests in Indo-Pakistan subcontinent. yield advantage of insect-resistant cotton in USA and China, for instance are less than 10% on average [13,14]. However, in India, the yield advantages are much higher [15], and in Pakistan ~30% yield advantage was observed by arranging trials for three consecutive normal cotton growing seasons [6,16].

So far a lot of environmental risk assessments on Bt technology have been reported in many crop species such on maize, potato, soybean, brinjal and cotton [17-24]. is also an intensive research review made on Bt cotton with various case studies on environmental biosafety [25]. However, compared with them environmental information, the non-target feeding safety and base food safety report are rather nonsystematic [26] and meager [27], and also not yet disclosed enough on Bt technology in general.

Earlier, Noteborn and Kuiper [28] studied the of Bt tomato in rodents by supplementing the semi-synthetic rodent diet with 10% (w/w) of lyophilized genetically or control tomato powder, and fed during 91 days. average daily intake was approximately 200 g tomato day-1 per rat. No clinical, toxicological or histopathological abnormalities were observed. Noteborn et al. [29] found non-toxic impact by exposing rats for 90 days on transgenic tomato containing Cry1Ab endotoxin by studying feed consumption rate, body weight, organs weight, blood chemistry and histopathology. In another study, sheep were fed on diets of GM corn (containing Cry 1A) and conventional corn. No change in body weight gain and feeding values were found [30]. Similarly, no in body weight change, feed conversion, biochemical and hematological values were reported for chicken fed on GM corn containing Cry9c gene [31]. Such commonalties were also reported for studies conducted on dairy cattle fed on corn containing Cry1Ab gene [32]. Another experimental model animal 'rat' was exposed to

conventional and GM rice containing Cry1Ab gene, and no substantial were found in animal behavior, weight gain, hematological and biochemical parameters, macroscopic and histopathological examinations of organs among the rats of two groups [33].

possibility of toxicity of Bt pesticidal protein to mammals, and invertebrates was examined by Xu-Chongren et al. [34]. Mice, zebra and eelworms were chosen as experimental model animals, respectively, to evaluate the dietary biosafety of Bt protein expressed in Bt transgenic cotton plants. Many toxicity tests such as acute tests, chronic tests, gene toxicity tests were included. results showed no acute toxicity, chronic toxicity or gene toxicity to each kind of animals mentioned above. However, these are the only few examples on cotton, and extensive information is needed to support the food safety issues by developing cases with independent cotton genotypes with Bt genes.

Pakistan is one the major cotton producing countries, and well know Bt gene (Cry 1Ac Mon 531) was used to introduce in local elite cotton cultivars by various public and private organizations (since there is no patent for this gene exits in Pakistan). More than 14 cotton varieties containing Bt gene were recommended for cultivation in Pakistan [35]. Information on food safety aspects is yet meager on Bt cotton and a series of tests should be done as earlier studies are not explicit in two ways. food safety studies are limited to commodity and not on cotton. For example, most of the previous studies were conducted on Bt corn, Bt brinjal, Bt soybean etc. [18-21]. Reports on risk assessment of Bt cotton are yet limited as to prove the safety based on the product base [26,27]. It is, therefore, case experiments with species of animals (wild and farm animals, ruminants and monogastrics) are needed to generate comprehensive dietary risk assessment database. Secondly, neither study addressed DNA integrity test (genotoxicity test) [36-38] to generate additional information for food safety assessment studies as DNA-based tests are vital for thorough investigations of of diets containing transgene.

Experimental

Animal and animal feeding

In the present study, Forty-two albino healthy male rabbits at 12-14 weeks old with a weight of 900-1100 gms were housed individually in stainless steel standard rabbit cages in a controlled environment room (28 ± 2°C temperature, 50-70% humidity and light from 6:00 am to 6:00 pm).

7 days of acclimatization, the rabbits were divided into seven groups and six individuals were allocated for each group. rabbits in group I was fed on normal diet. rabbits in group II and III were reared on diet containing 20% and 30% of Bt cotton seeds, respectively. Groups IV and V were fed with a diet containing 20% and 30% non-Bt cotton seeds, respectively. Rabbits in group VI and VII were fed leaves from Bt and non-transformed cotton plants, respectively. All the groups were fed ad Libitum in the morning, while in the evening the rabbits were fed green fodder to nutritional requirements. Groups of rabbits were fed with crashed cotton seeds and fresh leaves of Bt containing local cotton strain IR-NIBGE-901 for 90 days.

Biochemical analysis of blood

Blood samples were collected from jugular vein of each rabbit at 0, 45 and 90 days the treatment (DAT). Haematological parameters were determined as described by Dacie et al. [39]. serum was separated by centrifugation at 3,000 rpm for 15min and was stored at -20°C for biochemical analysis.

All biochemical parameters were analyzed using an automated analyzer Microlab 200, Merck, Germany. Serum glutamate pyruvate transaminase (SGPT) and serum glutamate oxolacetate pyruvate transaminase (SGOT) were estimated by the International Federation of Clinical Chemistry (IFCC) method [40,41] using a kit (Diasys, Germany). Alkaline phosphatase (ALP) was estimated by kinetic test optimized method [42] using a kit (Biocon, Germany). Serum bilirubin was measured by a method [43] with a kit manufactured by Biocon, Germany. Enzyme photometric test was applied to estimate cholesterol using a kit (Diasys, Germany). Random blood sugar (RBS) was measured by enzymatic kinetic method [44] using a kit (Biocon, Germany). Serum lactate dehydrogenase LDH was estimated using a commercial kit of Diasys, Germany [45].

Histopathology of liver and kidney samples

exposing the rabbits for 90 days, the rabbits were Liver and kidney samples were collected in 10% formaline solution and preserved in 10% formaline solution, while, for DNA damage studies the samples were collected in separate tubes and stored at –20°C until analysis.

Comet assay

DNA-based test called Comet Assay was undertaken to estimate sharing of total genomic DNA, an indicator of genotoxicity at molecular level. Genotoxicity studies were conducted by comparing the DNA of the treated organism understudy with the control [46]. Forty-eight hours prior to the end of experiment, a new group of six rabbits was administered intraperitonial with a known genotoxic nitrosoguanidine in 13 mg/kg body weight as a positive control for genotoxicity studies. Liver and kidney samples were collected and homogenized by mixing in dimethyl sulphoxide (DMSO). Approximately, 0.5 gram of tissue was taken in a tube and 2.0 ml DMSO was added, and homogenized by an electric homogenizer. Microscopic slides were used, with each slide containing three independent gels. In the three-layer procedure, the cells contained in agarose were placed on a slide protected with a layer of regular agarose. adding the gel-containing layer, another layer of low melting point (LMP) agarose was added to any residual holes in the second agarose layer and to increase the distance between the cells and the gel surface. the agarose gel was the slides were placed for one hour in a chilled lysis solution (2.5 M NaCl, 100 mM EDTA, 1% Na laural sarcocinate) at 4°C. At the end of the lysis period slides were incubated in a (1 mM EDTA solution with 300 mM NaOH of pH 13) for 20 min [47] to produce single strand DNA breaks, and electrophoresed in the same for one hour at 25 V and 300 mA at laboratory temperature 25°C to produce comets [48]. electrophoresis the alkali in the gel was neutralized by rinsing the slides in neutralization (24.5 g of trimza HCl in 500 mL of distilled water). gels were dried and the slides were stored. gels were stained with ethidium bromide (0.04 mg/mL) and comets were scored [49].

Results and Discussion

Effect on body growth

General observation was made daily whether there is sign of toxicity and allergenicity, and the body weight was measured once a week. All rabbits in each group remained alive, behaved normally and exhibited normal signs during the course of experiment. Food and water intake was proper throughout the experimental period. are compatible with the earlier reports [33,50,51].

Weekly weight gain by each rabbit was not by the treatments (data not shown). results are in accordance with earlier reports indicating no between cows fed on conventional and Bt cottonseed [52,53]. Such commonalities have also been shown by exposing and Northern Bobwhite Quail to Bt cottonseed meal [50,51,54].

Effects on biochemical parameters

average white blood cell count (× 102/mm3) in the normal group is 55.6 ± 4.33, 58.6 ± 10.4 and 64.0 ± 9.53 at zero, 45 and 90 days, respectively. Similar values were recorded from treated groups exposing for 0, 45 and 90 days, and these values were in normal range (Table 1). Total leucocytes count (mean+ SEM) was 5566+433, 5866+1047 and 6400+953 count/ mm3 in control group 0, 45 and 90 days of treatment. In the 30% Bt cottonseed treated group the total leucocytes count was 6800+1101, 6200+869 and 6666+774 count/ mm3 at 0, 45, 90 DAT, respectively. All these values were from each other as well as from the control group. Similar trend was observed with the other treated groups (Table 1).

Test Name	Sampling Time (Days)	Groups						
		1	2	3	4	5	6	7
WBC (102/mm3)	0	55.6 ± 4.33	90.0 ± 1.15	68.0 ± 11.0	78.0 ± 7.57	71.3 ± 8.76	66.3 ± 9.4	81.0 ± 5.56
	45	58.6 ± 10.4	58.6 ± 11.7	62.0 ± 8.69	70.3 ± 10.8	60.0 ± 9.29	63.0 ± 2.08	50.0 ± 4.72
	90	64.0 ± 9.53	58.6 ± 1.45	66.6 ± 7.74	75.0 ± 8.73	68.3 ± 6.17	74.3 ± 5.33	67.0 ± 4.35
TLC (Count/mm3)	0	5566.0 ± 433	9000.0 ± 115	6800.0 ± 110	7800.0 ± 757	7133.0 ± 876	6633.0 ± 94	8100 ± 556
	45	5866.0 ± 105	5866.0 ± 117	6200.0 ± 869	7033.0 ± 108	6000.0 ± 929	6300.0 ± 208	5000.0 ± 472
	90	6400.0 ± 953	5866.0 ± 145	6666.0 ± 774	7500.0 ± 873	6833.0 ± 617	7433.0 ± 533	7000.0 ± 435
DLC %age								
Neutrophils	0	63.0 ± 4.0	59.0 ± 5.0	58.3 ± 7.0	58.3 ± 8.0	61.0 ± 9.0	54.0 ± 5.0	65.0 ± 10
	45	50.3 ± 6.0	56.3 ± 6.0	59.6 ± 6.0	56.0 ± 4.0	48.0 ± 4.0	53.3 ± 8.0	57.0 ± 11
	90	55.0 ± 8.0	58.6 ± 7.0	64.0 ± 5.0	58.0 ± 6.0	60.0 ± 7.0	55.6 ± 6.0	60.0 ± 9.0
Lymphocytes	0	34.0 ± 8.0	39.3 ± 3.0	40.0 ± 6.0	39.0 ± 6.0	37.0 ± 5.0	45.0 ± 7.0	34.0 ± 7.0
	45	42.3 ± 6.0	40.0 ± 8.0	35.3 ± 6.0	39.6 ± 2.0	44.6 ± 5.0	39.6 ± 4.0	37.0 ± 8.0
	90	40.0 ± 4.0	38.0 ± 2.0	33.0 ± 4.0	38.6 ± 3.0	38.0 ± 2.0	40.3 ± 6.0	35.0 ± 4.0
Monocytes	0	2.00 ± 0.0	2.00 ± 0.0	1.66 ± 0.0	1.66 ± 0.6	1.66 ± 0.3	1.00 ± 0.0	1.00 ± 0.0
	45	4.00 ± 1.0	4.00 ± 0.0	2.33 ± 0.0	3.33 ± 1.0	4.00 ± 0.0	2.00 ± 1.0	4.00 ± 1.0
	90	2.00 ± 0.0	2.33 ± 1.0	2.00 ± 0.0	2.00 ± 0.0	2.00 ± 0.0	3.00 ± 0.0	3.00 ± 1.0
Eosinphils	0	1.00 ± 0.0	0.00 ± 0.0	0.00 ± 0.0	1.00 ± 0.0	1.00 ± 1.0	0.00 ± 0.0	0.00 ± 0.0
	45	3.00 ± 1.0	1.00 ± 0.5	1.00 ± 1.0	1.00 ± 1.0	3.00 ± 4.0	2.00 ± 1.0	2.00 ± 1.0
	90	1.00 ± 0.0	1.00 ± 0.0	1.00 ± 0.0	1.00 ± 0.0	1.00 ± 0.5	2.00 ± 1.0	2.00 ± 1.0
ESR (mm/hr)	0	5.00 ± 3.0	4.00 ± 2.0	6.00 ± 2.0	4.00 ± 1.0	7.00 ± 2.0	8.00 ± 1.0	4.00 ± 1.0
	45	4.00 ± 2.0	5.00 ± 3.0	7.00 ± 1.0	3.00 ± 2.0	5.00 ± 1.0	7.00 ± 1.0	3.00 ± 1.0
	90	7.00 ± 2.0	8.00 ± 2.0	8.00 ± 0.0	5.00 ± 2.0	6.00 ± 2.0	6.00 ± 2.0	5.00 ± 0.0
Hb (mg/dl)	0	13.3 ± 1.5	14.8 ± 1.1	14.9 ± 0.4	14.9 ± 0.9	12.3 ± 0.9	13.4 ± 0.4	11.2 ± 1.0
	45	15.0 ± 8.8	14.3 ± 0.2	14.8 ± 0.5	14.4 ± 0.8	13.2 ± 0.6	15.8 ± 0.4	14.8 ± 0.7

	90	14.5 ± 0.7	14.1 ± 0.4	14.7 ± 0.4	14.8 ± 0.4	14.2 ± 0.8	15.4 ± 0.6	15.5 ± 0.6
Liver Enzymes tests								
SGPT (IU/l)	0	53.0 ± 6.42	66.6 ± 10.8	64.0 ± 9.0	70.3 ± 1.76	61.0 ± 21.3	43.6 ± 8.22	43.0 ± 2.30
	45	57.0 ± 10.5	95.0 ± 13.0	62.3 ± 13.0	63.0 ± 7.62	88.0 ± 19.6	84.6 ± 14.6	87.3 ± 2.33
	90	46.6 ± 8.57	67.6 ± 6.33	64.6 ± 7.83	70.6 ± 4.97	72.0 ± 6.11	73.3 ± 5.12	71.0 ± 6.55
SGOT (IU/l)	0	28.3 ± 2.90	46.0 ± 4.72	62.3 ± 5.66	86.0 ± 4.35	86.0 ± 21.1	78.0 ± 22.7	57.6 ± 22.3
	45	62.3 ± 20.0	50.6 ± 13.8	40.6 ± 8.68	55.0 ± 7.23	65.6 ± 36.7	42.3 ± 4.84	79.0 ± 31.6
	90	66.6 ± 7.44	53.3 ± 6.38	75.3 ± 7.05	75.6 ± 12.3	77.0 ± 13.0	71.3 ± 7.68	75.0 ± 11.5
ALP (IU/L)	0	286.0 ± 38.6	337.6 ± 68.6	315.3 ± 33.3	281.0 ± 15.8	265.0 ± 100	350.6 ± 33.4	242.6 ± 65.0
	45	340.0 ± 102	341.3 ± 44.4	304.0 ± 21.9	285.3 ± 40.5	278.3 ± 43.7	297.6 ± 50.8	361.6 ± 27.6
	90	370.3 ± 45.6	312.3 ± 28.5	293.6 ± 4.09	373.0 ± 6.42	294.0 ± 8.71	318.6 ± 38.1	261.6 ± 28.6
Bilirubin (mg/dl)	0	0.54 ± 0.03	0.51 ± 0.01	0.54 ± 0.03	0.53 ± 0.02	0.51 ± 0.04	0.47 ± 0.03	0.43 ± 0.04
	45	0.61 ± 0.05	0.59 ± 0.04	0.58 ± 0.03	0.53 ± 0.01	0.57 ± 0.01	0.52 ± 0.01	0.47 ± 0.04
	90	0.45 ± 0.02	0.47 ± 0.04	0.53 ± 0.04	0.44 ± 0.03	0.43 ± 0.04	0.38 ± 0.01	0.47 ± 0.02
FBS (mg/dl)	0	81.6 ± 2.96	85.6 ± 9.37	80.3 ± 2.84	80.6 ± 4.63	83.6 ± 8.88	76.0 ± 2.88	66.6 ± 4.80
	45	96.6 ± 4.41	84.0 ± 12.0	90.3 ± 8.76	83.3 ± 7.44	92.3 ± 8.25	82.0 ± 9.53	98.3 ± 5.04
	90	86.6 ± 7.66	91.3 ± 2.66	83.6 ± 5.36	90.0 ± 5.19	91.3 ± 4.17	83.3 ± 6.11	82.6 ± 5.45
Cho (mg/dl)	0	53.0 ± 7.55	53.6 ± 12.1	56.3 ± 9.49	47.6 ± 6.06	72.0 ± 17.6	99.0 ± 6.50	68.3 ± 8.41
	45	61.3 ± 3.75	56.6 ± 5.23	49.0 ± 2.73	54.0 ± 5.17	41.3 ± 4.97	42.3 ± 2.33	45.0 ± 3.05
	90	60.3 ± 1.45	64.0 ± 2.30	56.3 ± 4.41	51.0 ± 4.93	56.0 ± 2.51	56.0 ± 7.50	69.3 ± 4.91
LDH (IU/l)	0	410.0 ± 34.0	442.0 ± 6.36	699.6 ± 109	468.6 ± 140	612.6 ± 197	472.3 ± 125	433.6 ± 37.7
	45	436.0 ± 35.8	454.6 ± 17.5	558.6 ± 104	448.0 ± 108	448.3 ± 148	423.6 ± 69.5	491.0 ± 104
	90	437.0 ± 54.3	434.3 ± 33.3	538.0 ± 45.8	468.0 ± 22.3	524.6 ± 54.8	461.0 ± 35.6	434.3 ± 57.0

Table 1: Biochemical analysis of blood of various groups of rabbits fed on Bt cotton variety IR-NIBGE-901 and its conventional counterpart FH-901. Each value is presented as mean ± SD. WBC, white blood cells; DLC, leucocyte count; ESR, erythrocyte sedimentation rate; Hb, haemoglobin; SGPT, serum glutamate pyruvate transaminase; SGOT, serum glutamate oxolacetate transaminase; ALP, alkaline phosphatase; FBS, fasting blood sugar; Cho, cholesterol; LDH, lactate dehydrogenase.

Treatment on leucocytes count (neutrophils, lymphocytes, monocytes and eosinophils) in the normal and treated groups are presented in (Table 1). Bt cottonseed meal/or leaves did not the leucocytes count. Values of erythrocyte sedimentation rate (ESR mm/hr) in the control group of rabbits are 5.00 ± 3.0, 4.00 ± 2.0 and 7.00 ± 2.0 mm/hr at zero 45 and 90 DAT, respectively. values in the 30% Bt cottonseed treated group are 6.00 ± 2.0, 7.00 ± 1.0 and 8.00 ± 0.0 mm/hr at zero, 45 and 90 days, respectively. All the values including the values of other groups (Table 1) were

values for haemoglobin are identical between the control and treated groups. values of haemoglobin in the control group of rabbits were 13.3 ± 1.5, 15.0 ± 8.8 14.5 ± 0.7 g/L at 0, 45 and 90 DAT, respectively. values in the 30% Bt cotton treated groups were 14.9 ± 0.4, 14.8 ± 0.5 14.7 ± 0.4 g/L, respectively at 0, 45 and 90 DAT. Values for the other groups are presented in the Table 1 indicating no impact of Bt cottonseed and leaves exposed rabbits.

values of serum glutamate pyruvate transaminase (SGPT) in the control group of rabbits were 53.0 ± 6.42, 57.0 ± 10.5 and 46.6 ± 8.57, respectively at 0, 45 and 90 DAT. observations in the 30% Bt cotton treated group were 64.0 ± 9.0, 62.3 ± 13.0 and 64.6 ± 7.83 at 0, 45 and 90 days. All these values in the control and the remaining treated groups were statistically same (Table 1). SGPT is very to liver, its statistically values in all the groups exhibited hepatoprotectivity of rabbits exposing them to Bt cotton for a long period. Similarly, all the treated groups behaved randomly for SGOT (Table 1). values recorded of alkaline phosphatase in the control group were 286.0 ± 38.6, 340.0 ± 102 and 370.3 ± 45.6 on 0, 45 and 90 DAT, and these values in 30% Bt cotton treated group were 315.3 ± 33.3, 304.0 ± 21.9 and 293.6 ± 4.09, respectively. values of Bilirubin in the control group were 0.54 ± 0.03, 0.61 ± 0.05 and 0.45 ±

0.02 mg/dl at 0, 45 and 90 DAT. values in the 30% Bt cotton treated rabbits were 0.54 ± 0.03, 0.58 ± 0.03 and 0.53 ± 0.04 mg/dl at 0, 45 and 90 DAT. Similar type of data was recorded from all the other groups (Table 1). Similarly, all values of blood glucose, cholesterol and lactate dehydrogenase (LDH) found statistically similar among and within each group at 0, 45 and 90 DAT, respectively (Table 1).

tissues were dehydrated in ascending grades of ethyl alcohol, cleared xylene and Sections of 4-6 µm thick were cut and stained with Harris haematoxylin and Eosin (H & E) stain to study the histopathological changes as described by Humason [55] and kidney samples collected from Bt and non-Bt cottonseed and leaves treated groups showed no remarkable histopathological changes and were found normal (Figure 1 depicting liver cells only).

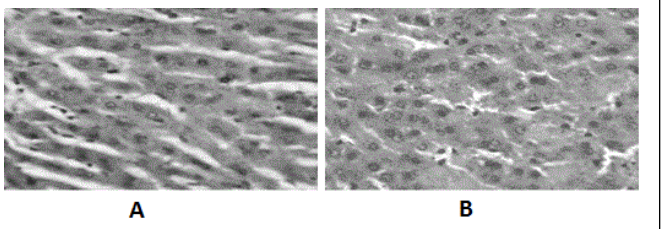

Figure 1: Histopathological studies of liver tissues of rabbits exposed on Bt cotton variety IR-NIBGE-901 and its counterpart FH-901. Both Figure 1A (treated with 30% Bt cotton) and Figure 1B (control) showing no of Bt cotton on liver tissues.

In the present studies, hematological parameters (total leucocytes count, leucocytes count, erythrocyte sedimentation rate and haemoglobin) of treated and control groups were similar. It is the case for liver enzymes, bilirubin, cholesterol, random blood sugar and lactate dehydrogenase level of all the treated and control rabbits. are in line with Hashimoto et al. and Momma et al. [56,57]. Similarly, liver and kidney of rabbits treated with Bt and non-Bt cotton seeds and leaves showed no remarkable histopathological changes which are compatible to earlier reports [29,56-58]. Weight gain is another important parameter to study changes in body exposing novel food [30-33,56-58] which showed no among the treated and control groups. Similar results were reported in previous [58,59].

Genotoxicity (comet assay—dna based test)

Comets assays were conducted using the organ samples of the rabbits were into several categories based on the length of the migration and/or the perceived relative portion of the DNA in the tail. By assigning the numerical value to each migration class, the average extent of DNA migration among cells within a culture was calculated [47,37,38]. To evaluate the genotoxic two parameters were used, comet tail length and the percentage of DNA damaged cells. data were analyzed using analysis of variance. Treatment means were compared using the least (LSD) test at 1% level of [60]. was no for damaged cell (2-3%) within and between the normal and Bt cotton exposed groups. However, the damage in the +ve control was higher (64%) from both the groups (Table 2). comet tail length (Figure. 2) parameter recorded from all the groups (control and treated) were statistically identical, while, in +ve control, it was higher (Table 2). It may be concluded that transgenic cotton containing Cry1Ac gene is quite safe for other than target organisms [34].

Group	Treatment	Dosage	% DNA damaged cells in liver	% DNA damaged cells in kidney	Comet Tail length in liver cells (µm)	Comet Tail length in kidney cells (µm)
1	+ve control (NTG)*	13 mg/kg body weight	64a ± 2	60a ± 2	18a ± 3.2	16a ± 1.8
2	-ve control	None	2.66b ± 0.1	2b ± 0	0.5b ± 0.1	0.5b ± 0.1
3	20% Bt cotton seeds	Mixed in feed	2.66b ± 0.1	2b ± 0	0.5b ± 0.1	0.5b ± 0.1
4	30% Bt cotton seeds	Mixed in feed	2.66b ± 0.1	1b ± 0	0.6b ± 0.1	0.4b ± 0.05
5	20% non-Bt cotton seeds	Mixed in feed	3.33b ± 0.1	2b ± 0	0.5b ± 0.1	0.5b ± 0.1
6	30% non-Bt cotton seeds	Mixed in feed	2.33b ± 0.1	2b ± 0	0.4b ± 0.1	0.5b ± 0.1
7	Bt cotton leaves	Ad libitum	3.33b ± 0.1	1b ± 0	0.6b ± 0.05	0.3 b ± 0.1
8	Non-Bt cotton leaves	Ad libitum	3.33b ± 0.1	1b ± 0	0.5b ± 0.1	0.3 b ± 0.1

Table 2: Percentage of DNA damaged cells and mean comet tail length of DNA damaged cells in liver and kidney of rabbits. Means of +ve control group from –ve control and treated groups (P<0.01). *NTG stands for nitrosoguanidine, a known genotoxic Dissimilar superscripts in columns represent among means (Tukey Test) at P<0.01.

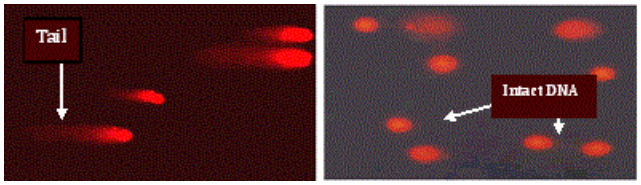

Figure 2: Fluorescent micrograph of comet assay. An assay of organ sample of rabbits treated with a known mutagen nitrosoguanidine. Comet tail length can clearly be seen-an indicator of DNA damage. B assay of tissues of various groups of rabbit exposed to Bt cotton seed (IR-NIBGE-901) and conventional cotton seed (FH-901).

Conclusion

We have indicated that Bt cotton has no adverse on the feeding test and subsequent health condition on rabbits when exposed till 90 days. Also we have that the Bt-gene product has no detrimental impact on genomic DNA integrity.

Cultivation of Bt cotton is safe for environment including for most of taxonomic groups of insects other than *lepidopteran* and animals in various locations in other countries [61,25]. However, the risk characterization of Bt requires careful and thorough evaluation combined with extensive publicizing to avoid of any minimal impact, which may distrust biotech corporations and public organizations [62]. With context, risk communication in food safety aspect is more than cardinal and we have obtained important facts on the safety.

Acknowledgements

We are grateful to Prof Dr. Qaiser Khan, National Institute for Biotechnology & Genetic Engineering (NIBGE) for providing us lab facility to conduct comet assay. study was funded through ADP scheme no. 27, "Cotton Crop Improvement through Genetic Engineering" of the Agriculture Department, Government of the Punjab, Pakistan. We are also thankful to Dr. Rob W. Briddon (Higher Education Commission, Pakistan, funded visiting Professor at NIBGE) for assistance in preparing the manuscript. Collaborative research resources were also provided from JSPS kaken Kiban A #.

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