Scholars Academic Journal of Biosciences (SAJB)
Abbreviated Key Title: Sch. Acad. J. Biosci.
ISSN 2347-9515 (Print)
ISSN 2321-6883 (Online)

A Unit of Scholars Academic and Scientific Society, India
www.saspublishers.com

Zoology

A Three Generational Feeding Study of Genetically Modified Cottonseeds on Biochemical and Histological Parameters of Kidney in Albino Rats

Megha Kansal[*], Gurinder Kaur Sangha
Department of Zoology, Punjab Agricultural University, Ludhiana, Punjab, India

Original Research Article

***Corresponding author**
Megha Kansal

Article History
Received: 18.12.2018
Accepted: 26.12.2018
Published:30.12.2018

DOI:
10.36347/sajb.2018.v06i12.005

Abstract: Genetically modified (GM) process in relation to the consumption was known to be highly mutagenic and a debate is ongoing regarding its acceptance in market. The present study was designed to evaluate the impact of feeding a line of genetically modified cotton seeds (Bt cotton seeds) to that of standard diet and reference diet (Non-Bt cotton seeds) on kidney in albino rats. Non-Bt and Bt cotton seeds were incorporated into the diets of the albino rats at a concentration of 20% continuously for three generations. Light microscopic observation of the kidney sections of cotton seeds fed female and male rats showed some variable histopathological changes. Kidney sections showed increase in the diameter of bowman's capsules which may be due to invagination of fatty globules, diffused hyaline and changes in the proximal convoluted tubules which consisted of cytoplasmic vacuolation in Bt treated male and female rats. The effect of Bt cotton seeds on kidney was more in evident in female rats as compared to male rats in all the three generations. A statistically significant increase in the glomerular diameter was observed in cotton seeds fed rats. Amount of uric acid in plasma was statistically higher in Bt fed female and male rats. The studies reviewed showed although no threat on the metabolism of the male and female rats throughout three generations.
Keywords: Bt cotton seeds, albino rats, kidney, histology, biochemical.

INTRODUCTION

Intensive cultivation practices and indiscriminate use of pesticides has created resistance among some of the key pests of cotton including American Bollworm Kumar *et al.*, [1].

An effective control measure for preventive damage by insects and pests has been evolved by the application of transgenic technology, which is also environment friendly. Genetically modified plants expressing *Bacillus thuringiensis* (Bt) genes have been developed in different crops for resistance to insect pests, and some of them have been developed successfully on a commercial scale for pest control [2, 3]. A genetically modified cotton variety commonly known as Bt cotton is being used by large number of farmers in India and many other countries since its commercial use from 2002 Manjunath [4].

Genetically modified (GM) plant products are being widely used now in the farm industry, and thus by consumers Haryu *et al.*, [5]. Many trials with animals fed different GM foods such as maize, potatoes, rice, soybeans, and tomatoes have been conducted, and parameters such as body weight, food consumption, organ weight, blood chemistry, and histopathology have been measured. The majority of these experiments did not indicate abnormalities in such parameters [6, 7]. But several detrimental effects of GM-crops had been shown on the metabolism of animals indicating serious health risks like immune dysregulation, accelerated aging, dysregulation of genes associated with cholesterol and insulin synthesis and changes in liver, kidney, spleen and gastrointestinal system [8-10].

There is an ongoing international debate as to the necessary length of mammalian toxicity studies in relation to the consumption of genetically modified (GM) plants including regular metabolic analyses Seralini *et al.*, [11]. However, animal feeding studies may provide additional and useful information to complement safety and nutritional value assessments of whole GM food and feed, especially when unintended effects are suspected. Therefore, the present study was carried out to assess the possible renal toxicity in rats with diet containing Bt cotton seeds through three generations.

MATERIALS AND METHODS

Animals and housing

The study was conducted on albino rats weighing 100-110 gms obtained from Guru Angad Dev

Veterinary and Animal Sciences University (GADVASU), Ludhiana. The rats were maintained in laboratory under standard conditions of temperature (25±2°C) providing them laboratory pelleted feed and water *ad libitum.* The rats were acclimatized to new quarters for one week before starting the treatment. The experimental protocol met the National guidelines on the proper care and use of animals in the laboratory research. This experimental protocol was approved by the Institutional Animal Ethics Committee (IAEC).

Experimental diets

Non-Bt and Bt seeds were procured from Plant Breeding and Genetics Department of PAU, Ludhiana, Punjab, India. Rats were divided into three groups. Rats in group I were considered as control and fed only with standard diet, i.e. wheat:grams (50:50 w/w); rats those were in group II were considered as Non- Bt group fed with diet containing wheat: gram :Non-Bt cotton seeds (50:30:20 w/w) and the rats in group III were considered as Bt group was fed with diet containing 20% transgenic Bt cotton seeds, i.e wheat: gram: Bt cotton seeds (50:30:20 w/w). The rats were fed according to their group by soaking the diet overnight.

Analysis of Diet composition

Supplemented diets given to rats i.e. Control, Non-Bt and Bt group were analyzed by Department of Animal nutrition, Guru Angad Dev University and Animal Sciences University (GADVASU), Ludhiana, India. Composition list of experimental diets were given at Table-1.

Experimental design and treatment

The parental generation (F0) was fed with either standard diet or diet having 20% Non-Bt cotton seeds or diet with 20% Bt cotton seeds depending on their groups and three generations were bred. Eighteen female albino rats (6 rats/each group) were mated with 9 male rats (one male for two female rats) overnight. Vaginal smears of the females were taken on the following morning. The presence of spermatozoa was considered day 0 of gestation. Dams and their offspring were fed with the same diets during the periods of mating, gestation, lactation, offspring care and pubescence. The off springs of F1 and F2 generation in a group of each generation were mated among themselves to obtain subsequent generation. F3 rats were also fed with either standard diet or experimental diets until they attain maturity. The six male and six female rats from each group of each generation were dissected for histological and biochemical studies.

Processing of Tissues for Histopathology

The kidney of both male and females of F1, F2, F3 generation were fixed and dehydrated in graded series of ethanol, cleared in xylene and embedded in paraffin wax. The haematoxylin-eosin stained 5µm thick sections were observed for histo-morphological alterations and photographed.

Forty glomeruli from kidney sections of each animal were selected and the diameter of glomerulus was calculated at major and minor axis using ocular pre-caliberated with stage.

Biochemical Analyses

Biochemical parameters were studied in plasma of male and female rats of control, Bt and Non-Bt group male and female rats of F_1, F_2 and F_3 generations. The animals (six male and six females from each group of each generation) were mildly anaesthetized using chloroform and the blood was collected directly from heart in heparinized vials. Plasma was separated from blood by centrifuging the blood at 3000 rpm (rotations per minute) for 15 minutes in cold centrifuge at 4°C. The supernatant was used for biochemical estimation of uric acid Sharma and Sangha [12], urea and creatinine Hawk *et al*., [13] in plasma.

Statistical Analysis

All statistical comparisons were presented as the mean ± standard error of mean (S.E.M). Comparisons were made between control, Non-Bt and Bt groups on computer using “Analysis of Variance (ANOVA)” as a statgraphics statistical package. A “P” value of 0.05 was selected as a criterion for statistically significant differences.

RESULTS

Histological Studies

Light microscope examination of renal sections of control rats showed intact capsule with well formed glomeular tuft in F1– F3 generation (Fig 1A-C). The arrangement of tubules and blood vessels were normal with no degenerative or infiltration like changes in both male and females.

In female animals fed with Non-Bt cotton seeds showed some variable pathological changes in glomeruli and in some parts of the urinary tubules in all the three generations were observed (Fig 1D-F). The kidney sections of Bt group showed significant increase in the diameter of bowman’s capsules which may be due to invagination by fatty globules, diffused hyaline and thickening of capillary endothelium in F1 and F2 generation female rats (Fig 1G-H). However, in F3 generation female rats of Bt group the glomerulus was observed to be contracted (Fig-2).

In F2 and F3 generation male rats of cotton seeds fed groups either with or without Bt gene showed significant changes in the proximal convoluted tubules which consisted of cytoplasmic vacuolation (Fig-2F, 2H) and lining of tubular epithelial cells with pyknotic nuclei (Fig-2I). The effect of lobulations in some glomerular tufts with haemorrhagic lesions was seen in F1 (Fig-2G) generation male rats.

The major axis diameter of glomerulus increased signficantly in Non-Bt and Bt cotton seeds fed female and male rats of F1 and F3 generations. In F3 generation the diameter of glomerulus in females was comparable to control rats while it increased significantly in treated male rats. The diameter of minor axis of glomerulus also increased in cotton seeds fed male and female rats in all the three generations as compared to control rats (Table-2).

Biochemical Studies

Amount of uric acid (mg/dl) increased significantly in F1 and F2 generation in females and in F1-F3 generation in male rats of Bt group. However, the levels of urea and creatinine did not vary differently in Bt fed male and female rats as compared to control rats (Table-3). Non- significant change in the levels of uric acid, urea and creatinine was observed in animals of Non-Bt group.

Table-1: Analysis of diet composition given to rats during multigenerational study

Name of Feed	Nutrient (%)								
	Fresh /DM basis	Moisture /Dry Matter	Crude Protein	Crude Fiber	Ether Extract	Ash	Acid Insoluble Ash	Calcium	Phosphorus
Control	Fresh	9.65	18.81	5.90	3.20	3.42	0.11	0.20	0.06
	DM Basis	90.35	20.82	6.52	3.54	3.79	0.12	0.22	0.07
Non-Bt Cotton Seeds	Fresh	9.60	20.56	8.80	5.93	3.14	0.27	0.13	0.13
	DM Basis	90.40	22.74	9.73	6.56	3.47	0.30	0.14	0.14
Bt Cotton seeds	Fresh	9.11	17.50	6.25	6.20	3.19	Nil	0.19	0.14
	DM Basis	90.89	19.25	6.88	6.82	3.51	Nil	0.21	0.15

Table-2: Effect of Bt and Non-Bt cottonseeds on diameter of glomerulus (µm) of F1, F2, F3 generation albino rats dissected on attainment of maturity

Animal	Diameter (µm)	F1			F2			F3		
		Control	Non-Bt	Bt	Control	Non-Bt	Bt	Control	Non-Bt	Bt
Female	Major Axis (Y)	69.26 ± 2.86	83.25± 3.89*	85.58±4.94*	70.26 ± 1.86	93.91±2.70*	86.25±3.25*	68.00± 2.80	57.61±4.85	63.00±7.09
	Minor Axis (X)	64.27 ±2.11	69.93± 3.94	72.59±3.05	65.27±2.01	79.25±4.73*	76.26±2.88	66.07±1.01	59.61±2.38	60.94±4.32
Male	Major Axis (Y)	53.94±3.79	78.92 ± 4.57*	60.94 ± 3.62*	50.14 ± 3.09	70.61 ± 4.80*	89.24 ± 3.82*	53.44 ± 4.79	69.26 ± 1.75*	56.94 ± 3.03
	Minor Axis (X)	52.61±3.55	71.26 ± 4.79*	48.95 ± 3.27	49.11± 2.55	57.95 ± 4.00	72.93 ± 3.60*	51.11± 4.55	66.27 ± 4.65*	55.28 ± 3.27

Values are Mean ± SE

*Significant difference at (p≤0.05) as compared to control

Table-3: Effect of Bt and Non-Bt cottonseeds on concentrations of uric acid, urea, creatinine, in the plasma of female and male rats of F1, F2 and F3 generation.

Animal	Biochemical Parameters	F1			F2			F3		
		Control	Non-Bt	Bt	Control	Non-Bt	Bt	Control	Non-Bt	Bt
Female	Uric Acid (mg/dl)	1.44 ± 0.01	1.56 ± 0.08	1.77 ± 0.01*	1.40 ± 0.01	1.60 ± 0.06*	1.65 ± 0.01*	1.41 ± 0.002	1.49 ± 0.004	1.55 ± 0.01
	Urea (mg/dl)	32.40 ± 0.01	30.10 ± 0.01	25.50 ± 0.07	33.30 ± 0.07	30.20 ± 0.01	32.60 ± 0.07	34.54 ±0.01	31.45 ± 0.086	29.99 ± 0.04
	Creatinine (mg/dl)	0.52 ± 0.01	0.55 ±0.12	0.58 ± 0.004	0.53 ± 0.26	0.54 ± 0.056	0.57 ± 0.19	0.53 ± 0.06	0.56 ± 0.01	0.57 ± 0.01
Male	UricAcid (mg/dl)	1.40 ± 0.09	1.46 ± 0.08	1.78 ± 0.01*	1.45± 0.01	1.50 ± 0.06	1.67±0.01*	1.44±0.002	1.40±0.01	1.75±0.01*
	Urea (mg/dl)	42.80± 0.30	35.14±2.10	35.50±3.07	43.35±0.05	40.70±1.00	39.63±0.27	44.54 ±0.10	41.60± 1.08	39.99±2.04
	Creatinine (mg/dl)	0.41±0.01	0.56±0.03	0.59±0.01	0.42±0.11	0.62±0.02	0.56±0.29	0.44±0.01	0.59±0.01	0.58±0.02

Values are Mean ± SE

*Significant difference at (p≤0.05) as compared to control

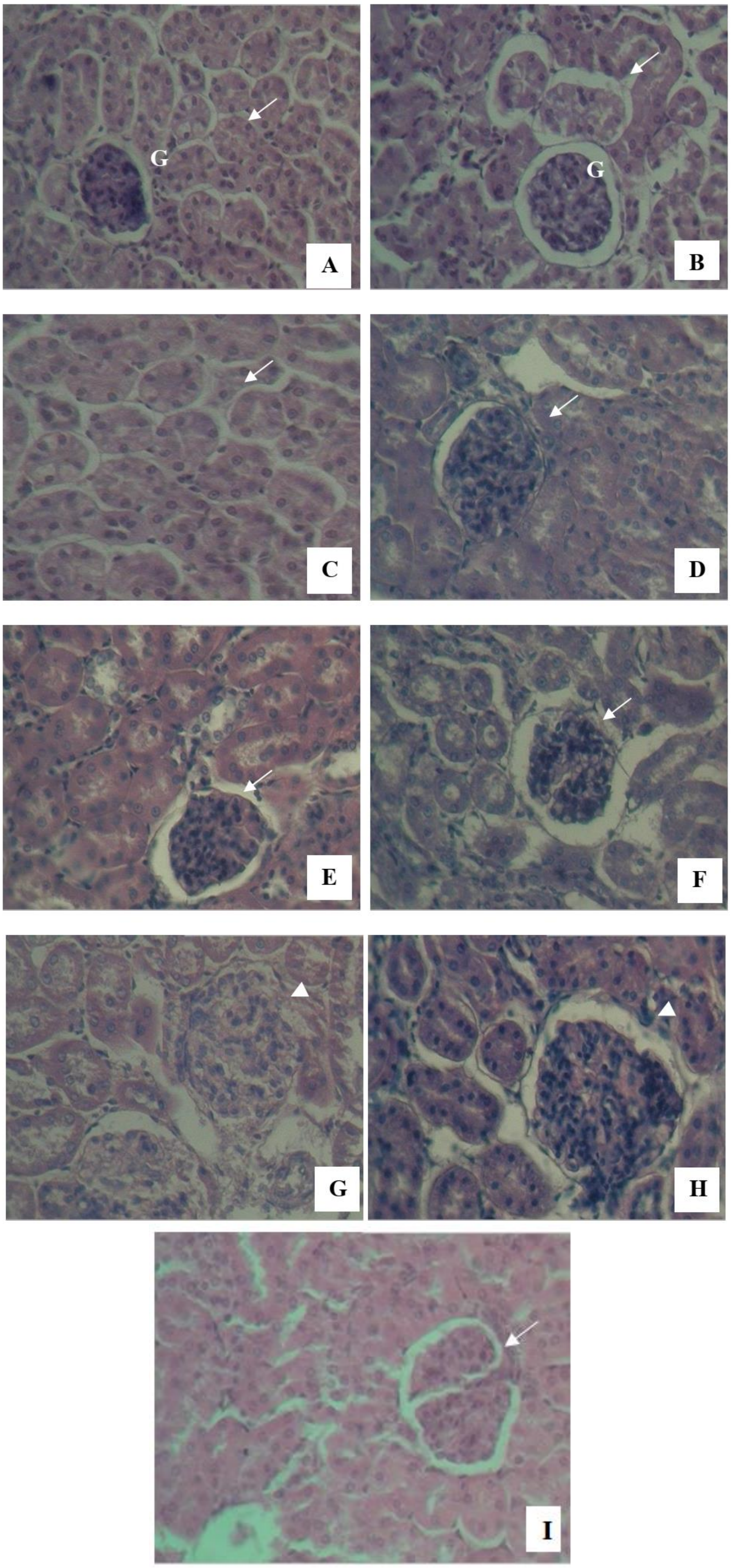

Fig-1: Photomicrograph of a T.S. of kidney of a female albino rat dissected at maturity:
(A-C) Control group showing intact capsule with well demarcated glomerular tuft (G) and normal arrangement of tubules in the cortical portion (arrow) (X400). (D-F) Non-Bt group showing minimal tubular degeneration (arrow) (X400). (G-H) Bt group showing tubular degenerations (arrow) and enlargement in parietal layers of Bowman's capsules (arrow head), (I) Showing tubular degenerations (arrow) and lobulation in the glomeruli (arrow head) (X400).

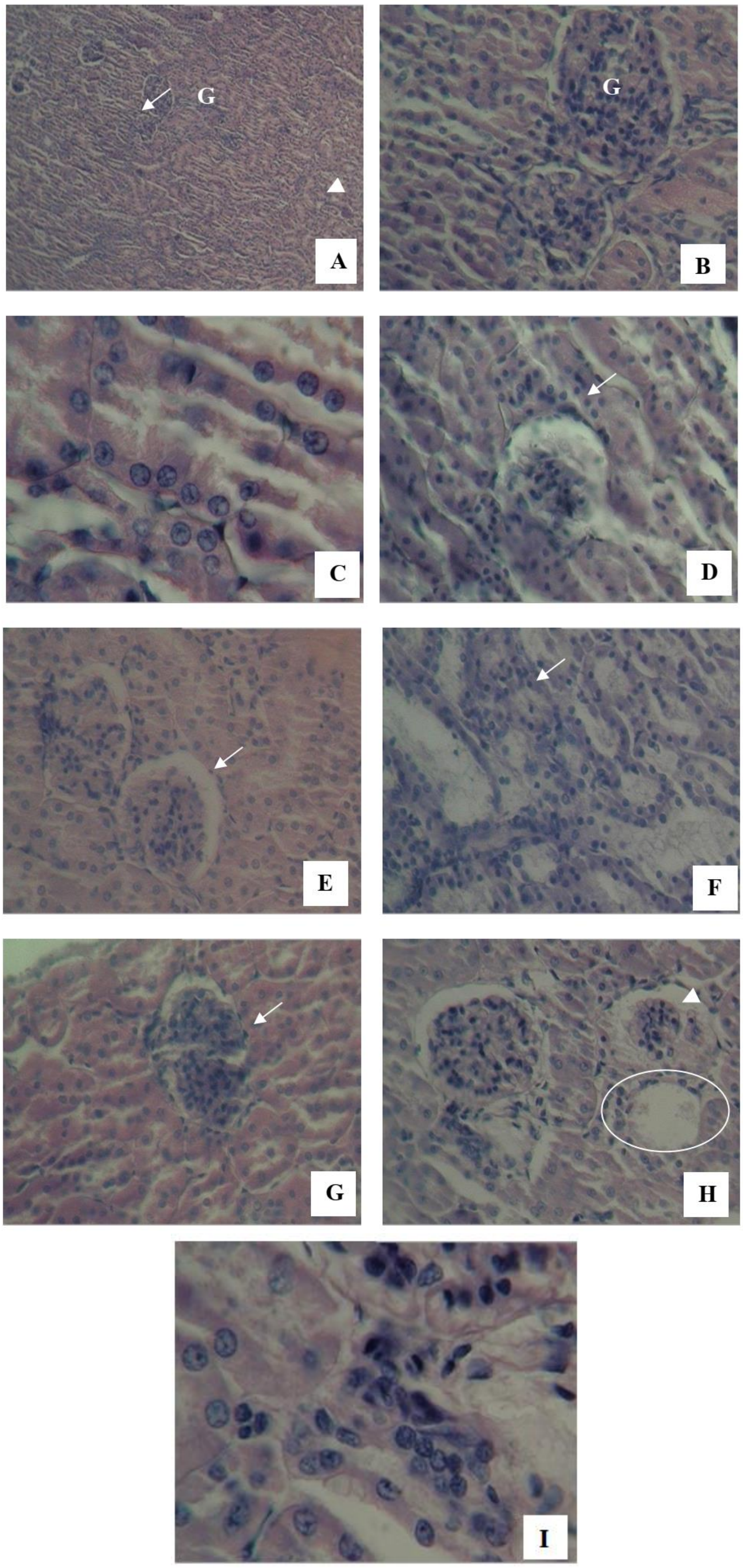

Fig-2: Kidney sections of male rats dissected at maturity:

(A-C) Control group showing intact structures of the glomeruli (G) and renal tubules in the cortical (arrow) and medullary portions (arrow head) (X100), (B) Magnified view showing intact glomeruli (G) (X400), (C) Magnified view showing normal nuclei in the tubules of cortical zone (arrow) (X1000); (D-F) Showing minimal tubular degeneration (arrow) in Non-Bt group (X400) ; (G-H) Bt group with lobulated glomeruli (arrow), tubular degenerations (circle) and enlargement in parietal layers of Bowman's capsules (arrow head) (X400), (I) Magnified view showing lining of epithelial cells in the renal tubules at the cortical zone with pyknotic or karyolysed nuclei (PN) in Bt treated rats (X1000).

DISCUSSION

There is an urgent need to develop comprehensive toxicological/nutritional methods to screen for the unintended potentially deleterious consequences for human/animal health of genetic manipulation to pinpoint the problems of the incorporation of the GM food stuff into the food chain Ewen and Pusztai [14].

One of the most important processes in kidneys is excretion of toxic metabolic waste products by glomerular and tubular filtration which are indicated by parietal layer of Bowman's capsule. Similar to the findings of the present study, Seralini *et al.*, [15] also observed tubular changes and inflammation in male rats fed with 33% MON 863 Bt corn in a 90-day study. Latham *et al*., [16] data strongly suggests that these GM maize varieties induce a state of hepatorenal toxicity possessing unintended metabolic effects due to the mutagenic properties of the GM transformation. Degenerating kidneys with turgid inflammatory areas demonstrating the increased incidence of marked and severe chronic progressive nephropathies were also evident in rats with roundup- tolerant genetically modified maize Seralini *et al.*, [11]. Early signs of toxicity at month 3 in kidney were also observed for 19 edible GM crops containing pesticide residues Seralini *et al*., [17]. Feeding study in rats with MON 863 Bt corn demonstrated inflammation and lesions in kidney Smith [18]. However, in one of the short term safety assessment in rats fed with GM potato showed neither pathological nor histopathological finding in kidney Hashimoto *et al.*, [19].

Th increase in major axis diameter of glomerulus was observed in animals fed with cottonseeds but these pathological alterations were minor changes and did not showed any effect on animal health. Long term intake of GM soybeans in diet also had no apparent adverse effects in histological findings and showed some possible effect on metabolism of rats and goats [20, 21]. Kilic and Akay [22], also observed statistically changes in at tubular and glomerular level in males and females from Non-GM groups. Decreases in short and long glomerular diameter in GM group rats fed with GM corn were statistically significant [22].

The effect of Bt cotton seeds on kidney was more evident in females as compared to male rats in all the three generations. As when there was a low or environmental dose impregnation of the feed (with a pesticide GM plant for instance), the chronic effects could be more differentiated according to the sex, the physiological status, the age, or the number of intakes over such and such a period of time in the case of a drug Seralini *et al*., [17].

Significant higher plasma level of uric acid in Bt group rats were observed although no abnormal physiopathology occurred during the study. Creatinine levels in the present study of female and male plasma samples was non-significantly higher in cottonseeds fed animals in all the three generations. Hammond *et al*., [23] also did not observe any significant difference in the values of serum creatinine in male and female Sprague-Dawley rats following 13 weeks of exposure to Roundup Ready (RR) corn grain in their diet. However, the serum creatinine content of the blood increased linearly with increased experimental feeding of lambs with Bt cotton seeds Tripathi *et al*., [24]. Nahas *et al*., [25] ingestion of 40% of GM quail meat meal induced kidney toxicity indicated by increased serum urea and creatinine. However, 20% GM quail elevate only serum creatinine. Creatinine levels of female serum samples in Non-GM group significantly increased from control and GM corn fed rats, thus relating the cause directly with individual sex and diets [22]. On contrary, Poulsen *et al*., [26] pointed out lower creatinine levels in female rats fed on GM rice.

In one of the study conducted by Seralini *et al*., [17] showed increased level of urea in the urine and decreased levels of creatinine in GM fed groups. In the present study the decreased levels of urea was observed in plasma samples of cottonseeds fed rats, Schrøder *et al*., [27] reported higher concentration of urea was reported in male rats fed with Bt rice in another 90-day study. Lambs fed with cotton seeds diet for 123 days showed serum urea content ranged between 27.5 and 29.4 mg/dl which were similar among control, Non-Bt and Bt group diets Tripathi *et al.*, [24].

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.

ACKNOWLEDGEMENT

Thanks are to the Department of Animal nutrition, Guru Angad Dev University and Animal Sciences University (GADVASU), Ludhiana, for analyzing diet composition used in the experiment and Department of Plant Breeding and Genetics for providing cotton seeds.

REFERENCES

1. Kumar S, Chandra A, Pandey KC. Bacillus thuringiensis (Bt) transgenic crop: an environment friendly insect-pest management strategy. J Environ Biol. 2008 Sep 1;29(5):641-53.
2. Oliveira GR, Silva MC, Lucena WA, Nakasu EY, Firmino AA, Beneventi MA, Souza DS, Gomes JE, de Souza JD, Rigden DJ, Ramos HB. Improving Cry8Ka toxin activity towards the cotton boll weevil (Anthonomus grandis). BMC biotechnology. 2011 Dec;11(1):85.
3. Dhillon MK, Sharma HC. Comparative studies on the effects of Bt-transgenic and non-transgenic cotton on arthropod diversity, seedcotton yield and

bollworms control. Journal of Environmental Biology. 2013;34:67-73.
4. Kumar S. Bt-cotton: Protein expression in leaves is most critical. Current Science. 2006;90(3):278.
5. Haryu Y, Taguchi Y, Itakura E, Mikami O, Miura K, Saeki T, Nakajima Y. Longterm biosafety assessment of a genetically modified (GM) plant: the genetically modified (GM) insect-resistant Bt11 corn does not affect the performance of multi-generations or life span of mice. The Open Plant Science Journal. 2009 Sep 7;3(1).
6. Hammond B, Dudek R, Lemen J, Nemeth M. Results of a 13 week safety assurance study with rats fed grain from glyphosate tolerant corn. Food and Chemical Toxicology. 2004;42(6):1003-14.
7. MacKenzie SA, Lamb I, Schmidt J, Deege L, Morrisey MJ, Harper M, Layton RJ, Prochaska LM, Sanders C, Locke M, Mattsson JL. Thirteen week feeding study with transgenic maize grain containing event DAS-Ø15Ø7-1 in Sprague–Dawley rats. Food and Chemical Toxicology. 2007 Apr 1;45(4):551-62.
8. Finamore A, Roselli M, Britti S, Monastra G, Ambra R, Turrini A, Mengheri E. Intestinal and peripheral immune response to MON810 maize ingestion in weaning and old mice. Journal of agricultural and food chemistry. 2008 Nov 14;56(23):11533-9.
9. Smith JM. Alternative Medicine Truth: Genetically modified GM food dangers. Spilling the Beans Newsletter, 2009; 1:1-6.
10. De Vendômois JS, Roullier F, Cellier D, Séralini GE. A comparison of the effects of three GM corn varieties on mammalian health. International Journal of Biological Sciences. 2009;5(7):706.
11. Séralini GE, Clair E, Mesnage R, Gress S, Defarge N, Malatesta M, Hennequin D, De Vendômois JS. RETRACTED: Long term toxicity of a Roundup herbicide and a Roundup-tolerant genetically modified maize, 2012.
12. Sharma RK, Sangha SPS. Basic techniqiues in biochemistry and molecular biology. I K International Publishing house, New Delhi, 2009.
13. Hawk PB, Oser BL, Summerson WH. Practical physiology chemistry. Blackiston Co Inc, Toronto, 1954.
14. Ewen SW, Pusztai A. Effect of diets containing genetically modified potatoes expressing Galanthus nivalis lectin on rat small intestine. The Lancet. 1999 Oct 16;354(9187):1353-4.
15. Séralini GE, Cellier D, de Vendomois JS. New analysis of a rat feeding study with a genetically modified maize reveals signs of hepatorenal toxicity. Archives of environmental contamination and toxicology. 2007 May 1;52(4):596-602.
16. Latham JR, Wilson AK, Steinbrecher RA. The mutational consequences of plant transformation. BioMed Research International. 2006.
17. Séralini GE, Mesnage R, Clair E, Gress S, De Vendômois JS, Cellier D. Genetically modified crops safety assessments: present limits and possible improvements. Environmental Sciences Europe. 2011 Dec;23(1):10.
18. Smith JM. Most Offspring died when mother rats ate genetically engineered soy. Spilling the Beans newsletter,, October. 2005 Oct:1-4.
19. Hashimoto W, Momma K, Katsube T, Ohkawa Y, Ishige T, Kito M, Utsumi S, Murata K. Safety assessment of genetically engineered potatoes with designed soybean glycinin: compositional analyses of the potato tubers and digestibility of the newly expressed protein in transgenic potatoes. Journal of the Science of Food and Agriculture. 1999 Sep;79(12):1607-12.
20. Sakamoto Y, Tada Y, Fukumori N, Tayama K, Ando H, Takahashi H, Kubo Y, Nagasawa A, Yano N, Yuzawa K, Ogata A. A 104-week feeding study of genetically modified soybeans in F344 rats. Shokuhin eiseigaku zasshi. Journal of the Food Hygienic Society of Japan. 2008;49(4):272-82.
21. Tudisco R, Mastellone V, Cutrignelli MI, Lombardi P, Bovera F, Mirabella N, Piccolo G, Calabro S, Avallone L, Infascelli F. Fate of transgenic DNA and evaluation of metabolic effects in goats fed genetically modified soybean and in their offsprings. Animal. 2010 Oct;4(10):1662-71.
22. Kılıç A, Akay MT. A three generation study with genetically modified Bt corn in rats: Biochemical and histopathological investigation. Food and Chemical Toxicology. 2008 Mar 1;46(3):1164-70.
23. Hammond BG, Dudek R, Lemen JK, Nemeth MA. Results of a 90-day safety assurance study with rats fed grain from corn borer-protected corn. Food and Chemical Toxicology. 2006 Jul 1;44(7):1092-9.
24. Tripathi MK, Mondal D, Somvanshi R, Karim SA. Haematology, blood biochemistry and tissue histopathology of lambs maintained on diets containing an insect controlling protein (Cry1Ac) in Bt-cottonseed. Journal of animal physiology and animal nutrition. 2011 Oct;95(5):545-55.
25. El-Nahas AF, Mohamed AA, Zweel HH, El-Ashwamy IM. Hepatorenal and genotoxic effects of genetically modified quail meat in a 90-day dietary toxicity study in mice. International Food Research Journal. 2011 Nov 1;18(4).
26. Poulsen M, Kroghsbo S, Schrøder M, Wilcks A, Jacobsen H, Miller A, Frenzel T, Danier J, Rychlik M, Shu Q, Emami K. A 90-day safety study in Wistar rats fed genetically modified rice expressing snowdrop lectin Galanthus nivalis (GNA). Food and Chemical Toxicology. 20071;45(3):350-63.
27. Schrøder M, Poulsen M, Wilcks A, Kroghsbo S, Miller A, Frenzel T, Danier J, Rychlik M, Emami K, Gatehouse A, Shu Q. A 90-day safety study of genetically modified rice expressing Cry1Ab protein (Bacillus thuringiensis toxin) in Wistar rats. Food and Chemical Toxicology. 2007 Mar 1;45(3):339-49.