Original Research Paper

VOLUME-6 | ISSUE-3 | MARCH - 2017 • ISSN No 2277 - 8179 | IF : 4.176 | IC Value : 78.46

Histological and biochemical alterations in female albino rats fed with Bt and Non-Bt cotton seeds during pregnancy and lactation

Zoology

KEYWORDS: Bt cotton, Liver, Histopathology, Biochemical, albino rat

Megha Kansal — Department of Zoology, College of Basic Sciences & Humanities, Punjab Agricultural University, Ludhiana - 141004, India.

Gurinder K Sangha — Department of Zoology, College of Basic Sciences & Humanities, Punjab Agricultural University, Ludhiana - 141004, India.

ABSTRACT

Bt cotton, a genetically engineered form of natural cotton, has broad spectrum use in agriculture due to its higher agronomic productivity and increased pest resistance. But still there is debate on GM crops with respect to health and other bio-safety issues. The objective of this study was to investigate the Bt and Non-Bt induced changes in vital organs of female albino rats. The female rats of the experimental groups were fed with either the diet containing 20% Bt-cotton seeds or 20% Non-Bt cotton seeds or standard rat diet depending on their groups during the periods of gestation and lactation. After weaning the parental female rats were sacrificed and vital organs and glands excised and weighed. Significant increase in relative weight of stomach was observed in Bt and Non-Bt cotton seed fed rats as compared to control rats. Significant increase in plasma total proteins, albumin and creatinine level was recorded in Bt-cotton seed fed rats. Histopathological changes such as slight enlargement of sinusoids and degeneration in hepatic cords was observed in liver of Bt treated rats. Therefore, the results suggested that in a short term feeding of rats fed with GM cotton showed some effects in female rats fed during pregnancy and gestation.

Interest in genetically modified (GM) crops is continuously increasing due to the possibility of higher agronomic productivity and more nutritious food without the use of pesticides [1-2]. Bt cotton is a genetically engineered form of natural cotton. This transgenic cotton, engineered to continuously express *δ*-endotoxin, encoded by 'Cry' genes from the soil bacterium, *Bacillus thuringiensis* Berliner [3], is more successful in controlling the bollworms [4-7]. The safety issues of GM food are crucial for their acceptance into the market. Although several studies have been conducted to evaluate the safety of GM crops, there is still a debate on the risk of GM consumption and a demand for additional evidence of GM food safety [8-9]. 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 [10-12]. Several detrimental effects of GM- crops have been reported on the metabolism of animals. Some minimal histopathological changes occurred in liver and kidney of the rats fed with GM corn through three generations [13]. Decreases in weight of kidney, tubular changes and inflammation in male rats fed with 33% MON 863 Bt corn in a 90-day study have also been reported [14]. However animal feeding studies may provide additional and useful information to complement safety and nutritional value assessments of whole GM feed and food especially when unintended effects are suspected[15]. The present study was aimed to evaluate the effects of Bt cotton seeds through feed on mortality, body mass, organ weight, to observe histomorphological and biochemical alterations in female albino rats. Data generated after these studies will elucidate possible landmark in the safety assessment of GM food.

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- Bt and Non-Bt were procured from Plant Breeding and Genetics Department of PAU. Rats were divided into three groups. Rat in first group I was 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 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 cotton seeds and Bt cotton seeds were analyzed by Department of Animal nutrition, Guru Angad Dev University and Animal Sciences University (GADVASU), Ludhiana. Composition list of experimental diets were given at Table 1.

Experimental design and treatment- Eighteen female albino rats (6 rats/each group) were mated with 9 male rats (one male for two female rats) overnight. Pregnant rats were fed during gestation and lactation period with either the diet containing 20% Bt cotton seeds or 20% Non Bt- cotton seeds or standard rat diet depending on their groups.

Processing of tissues for histopathology- The female rats at the end of lactation i.e. after one month of parturition were weighed and sacrificed. Their vital organs (liver, heart, spleen, stomach and lungs), endocrine glands (adrenal, thyroid and parathyroid) and reproductive organs (ovary, oviduct, uterus and vagina) were excised and relative organ weights were calculated. The tissues of liver and kidney were immediately fixed in Bouin's fixative. After complete fixation, the tissue was dehydrated in graded series of ethanol, cleared in xylene and embedded in paraffin wax (Melting point between 58-60°C). The 7µm thick sections were cut serially with the help of microtome and after usual de-waxing and rehydration in descending series of ethanol to water, the sections were stained in heamatoxylin, counterstained with eosin, dehydrated in ascending ethanol series, cleared in xylene and mounted in DPX. All tissue sections were observed and were photographed.

Biochemical analysis- Plasma was separated from blood samples taken from the heart of female rats, after centrifugation at 3000 rpm for 15 min. Plasma samples were analyzed for determination of the amounts of total proteins, albumin, globulin and creatinine.

Statistical Analysis- All statistical comparisons for body weight, organ weight and biochemical analysis 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 and Discussion

Clinical observations, body and relative organ weights- No toxicological signs were observed in all the three groups during the experimentation period. The relative weights of organs of female rats in Group III showed decreased values of relative weight of heart, spleen and lungs whereas the relative weight of liver, stomach, adrenal, thyroid, parathyroid, ovary, uterus and vagina was observed to be higher as compared to control group. A statistically significant increase was determined in the relative of stomach in female rats of Group III. In Group II female rats the increase in relative weight of stomach, lungs, thyroid, ovary and vagina while relative weight of liver, heart, spleen was observed to be decreased as compared to control group rats (Table 2).

No differences were observed in body weights and weights of kidney and liver in a 90-day feeding study with Bt (Cry1Ab protein) corn in rats [16]. 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 [17] 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 [10]. 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 [18].

Biochemical analysis

Results of biochemical analysis for female rats are presented in Table 3. Statistically significant increase in values of total proteins, albumin was recorded Group II and Group III female rats as compared to control group. Creatinine amount was observed to be higher in cottonseeds fed rats and it was statistically significant in Bt group rats. There was non-significant increase in the amount of creatinine in Group II females rats was recorded. No statistically significant differences were noted for globulin in plasma samples of female rats.

Lambs fed non Bt cotton seeds diet had higher serum protein, creatinine content as compared to control group and Bt diets whereas the albumin content was significantly high in Bt diet fed lambs as compared to control and Non-Bt diet fed lambs[19]. The mean globulin content was high in Non-Bt diet fed lambs, while control and Bt cotton seeds-diet fed lambs had similar globulin levels for 123 days[19]. Female rats fed with diet containing GM and non GM soybeans for 14 weeks had increased in the level of plasma proteins compared to control group[20]. Significant increase in the amount of globulin and total proteins in GM corn fed rats [12]. 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 [12]. Ingestion of 40% of GM quail meat meal induced liver and kidney toxicity indicated by increased serum creatinine. Meanwhile, 20% GM quail elevate only serum creatinine [21].

Histopathology

Liver-Light microscopic observation of the liver sections of the control group showed a normal histological architecture of liver with clearly outlined figs of anastomosing hepatocytes along with adjacent sinusoids radiating from the central veins towards the periphery of the liver lobules. Normal outlines of the central vein (CV) can be clearly visualized (Fig 1A). While slight loosening in arrangement of hepatic cords around central vein and minimal granular degradation was recorded in Non-Bt fed female rats (Fig 1B). The liver of Bt fed cotton seeds of females rats had large vacuolization or empty spaces and showed infiltration of large mass of lecocytes inflammatory cells in CV and sinusoids (Fig 1C).

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 [22]. 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 Non-Bt groups not showing health problems. Additionally, nuclear border changes found statistically significant in female rats in Group III. Irregular shaped hepatocyte nuclei and increase in number of nuclear pore at electron microscopy in offspring's of GM soybean fed pregnant mice was observed [23]. Thirty-five-day feeding study with GM corn in porcine showed the presence of transgene Cry1A(b) in tissues of liver, kidney and in blood but not in muscle [24]. A higher number of nuclear pore, in the hepatocytes of GM fed mice suggested intense molecular trafficking in the liver tissue. Hepatocytes of GM fed mice showed mitrochondrial and nuclear modification indicative of reduced metabolic rates[25]. Control and Non-Bt cotton seeds fed lambs had mild fatty infiltration in liver and such lesions were not seen in Bt cotton seeds fed lambs[19].

Kidney- Kidney sections of control rats showed normal histological structures of the glomruli, and renal tubules in the cortical and medullary portions (Fig.1 D). Enlargements in parietal layer of Bowman's capsule and minimal tubular degenerations were observed at different ratios in groups. Swelling in the lining epithelium of the renal tubules with narrow lumen and the presence of inflammatory cellular infiltration was observed in group II (Fig.1 E) and III (Fig.1 F) while this granular degradation was sever in group III followed by shrunken of glomeruli in Group III.

In a short term safety assessment in rats fed with GM potato showed neither pathological nor histopathological finding in liver and kidney[26]. Another feeding study in rats with MON 863 Bt corn demonstrated inflammation in kidney and lesions in liver and kidney[27]. Decrease in weight of kidney, tubular changes and inflammation in male rats fed with 33% MON 863 Bt corn in a 90-day study was observed [29]. Study conducted on Roundup-tolerant genetically modified maize (from 11% in the diet), cultivated with or without Roundup (R), and Roundup alone (from 0.1 ppb in water), for 2 years in rats reported that degenerating kidneys with turgid inflammatory areas demonstrate the increased incidence of marked and severe chronic progressive nephropathies, which were up to 2-fold higher in the 33% GM maize or lowest dose R treatment groups[29].

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

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.

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

VOLUME-6 | ISSUE-3 | MARCH - 2017 • ISSN No 2277 - 8179 | IF : 4.176 | IC Value : 78.46

Table 2- Effect of Bt and Non Bt cotton seeds on relative organ weight (g/100g BW) of different organs in control, Non-Bt and Bt group rats

[Values are mean ± SE]

Organs	Treatment		
	Gr I	Gr II	Gr III
Liver	4.48 ± 0.12	3.81 ± 0.36	4.62 ± 0.43
Heart	0.39± 0.002	0.34 ± 0.03	0.37 ± 0.07
Spleen	0.28 ± 0.03	0.25± 0.10	0.24 ± 0.09
Stomach	1.30 ± 0.05	1.54 ± 0.12	1.65 ± 0.37*
Lungs	0.62± 0.001	0.63± 0.17	0.53 ± 0.13
Adrenal	0.01 ± 0.01	0.01± 0.002	0.02± 0.001
Thyroid	0.08± 0.01	0.12 ± 0.02	0.13 ± 0.01
Parathyroid	0.02± 0.001	0.01± 0.004	0.02 ± 0.002
Ovary	0.02± 0.001	0.03± 0.003	0.04 ± 0.01
Oviduct	0.01± 0.004	0.01 ± 0.09	0.01 ± 0.01
Uterus	0.12± 0.01	0.10 ± 0.05	0.19 ± 0.02
Vagina	0.05± 0.002	0.07± 0.01	0.07 ± 0.02

*Significantly different from control group, p ≤ 0.05; Gr 1, control; Gr 2, Non-Bt; Gr 3, Bt

Table 3- Effect of Bt and Non-Bt cottonseeds on concentrations of total proteins, albumin, globulin, creatinine in the plasma of male of control, Bt and Non-Bt fed rats.

[Values are mean ± SE]

Biochemical Parameters in plasma sample of females	Treatment		
	Gr I	Gr II	Gr III
Protein (g/dL)	6.66 ± 0.19	7.38 ± 0.05*	7.02 ± 0.02*
Albumin (g/dL)	2.60 ± 0.01	3.45 ± 0.01*	3.30 ± 0.01*
Globulin (g/dL)	3.66 ± 0.04	3.85 ± 0.003	3.70 ± 0.54
Creatinine (mg/dl)	0.41 ± 0.01	0.49 ± 0.03	0.53 ± 0.01*

*Significantly different from control group, p ≤ 0.05; Gr 1, control; Gr 2, Non-Bt; Gr 3, Bt

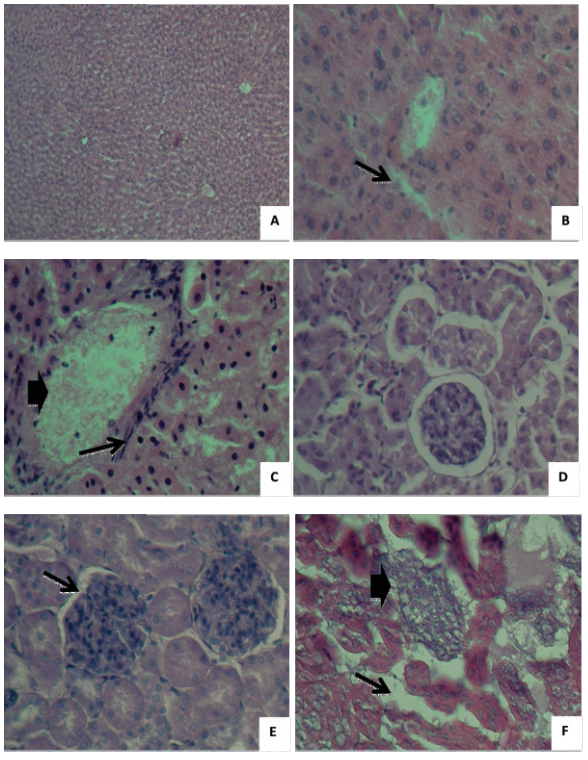

Fig. 1 — (a): Photomicrographs of liver (A–C) and kidney (D–F) tissues of rats stained with H&E. A and D are of control groups; B and E are of Non-Bt groups while C and F are of Bt cotton group. In liver, (B) minor granular degeneration (arrows) and (C) enlargement in central vein (arrow heads), focal mononuclear cell infiltration (arrows) are shown. In kidney, (E) enlargements in parietal layers of Bowman's capsule and (F) minimal tubular degeneration (arrows), shrunken glomeruli (arrow heads) (magnification: A,100X; B–F, 400X).

References

1. Shintani, D. & DellaPenna, D. (1998). Elevating the vitamin E content of plants through metabolic engineering. Science, 282, 2098-2100.
2. Ye, X., Al-Babili, S., Kloti, A., Zhang, J., Lucca, P., Beyer, P. & Potrykus, I. (2000). Engineering the provitamin A (-carotene) biosynthetic pathway into (carotenoid-free) rice endosperm. Science, 287, 303-305.
3. Perlak, F. J., Deaton, R. W., Armstrong, T. A., Fuchs, R. L., Sims, S. R., Greenplate, J. T. & Fischhoff, D. A. (1990) Insect resistant cotton plants. Biotechnology, 8, 939-943.
4. Wilson, D. F., Flint, M. H., Deaton, R. W., Fischhoff, A. D., Perlak, F. J., Armstrong, A. T., Fuchs, R. L., Berberich, S. A., Parks, N. J. & Stapp, B. R. (1992). Resistance of cotton lines containing a Bacillus thuringiensis toxin to pink bollworm (Lepidoptera : Gelchiidae) and other insects, J Econ Entomology, 85, 1516-1521.
5. Benedict, J. H., Sachs, E. S., Altman, D. W., Deaton, W. R., Kohel, R. J., Ring, D. R. & Berberich, S. A. (1996). Field performance of cottons expressing transgenic Cry 1 Ac insecticidal proteins for resistance to Heliothis virescens and Helicoverpa zea (Lepidoptera: Noctuidae). Jornal of Economic Entomology, 89, 230-238.
6. Wang, W. G., Jiang, Y. X., Yang, X. M., Guo, Y. Y., Guo, S. D., Ni, W. C., Chen, Z. X. & Tian, Y. C. (1997). Study on evaluation and utilization of Bt transgenic cotton resistant to cotton bollworm. Scientia Agricultural Sinica, 30, 7-12.
7. Wu, K., Guo, Y., Lv, N., Greenplate, J. T. & Deaton, R. (2003). Efficacy of transgenic cotton containing a Cry1Ac gene from Bacillus thuringiensis against Helicoverpa armigera (Lepidoptera: Noctuidae) in North China. Journal of Economic Entomology, 96, 1322-1328.
8. Haslberger, A. G. (2006). Need for an "integrated safety assessment" of GMOs, linking food safety and environmental considerations. Journal of Agricultural and Food Chemistry, 54, 3173-3180.
9. Hug, K. (2008). Genetically modified organisms: do the benefits outweigh the risks. Medicina (Kaunas), 44, 87-99.
10. 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 and Chemical Toxicology, 44, 1092-1099.
11. 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 and Chemical Toxicology, 45, 551-562.
12. Seralini, G. E., Mesnage, R., Clair, E., Steeve, G., Vendomois, J. S. & Cellier, D. (2011). Genetically modified crops safety assessments: present limits and possible improvements. Enviornmental and Science of Europe, 23, 1-10.
13. Kilic, A. & Akay, M. T. (2008). A three generation study with genetically modified Bt corn in rats: Biochemical and histopathological investigation. Food and Chemical Toxicology, 46, 1164-1170.
14. Séralini, G. E., Cellier, D. & de Vendomois, J. S. (2007). New analysis of a rat feeding study with a genetically modified maize reveals signs of hepatorenal toxicity, Archives of Environmental and Contamination Toxicology, 52, 596-602.
15. Snell, C., Brenheim, A., Berge, J. B., Kuntz, M., Pascal, G., Paris, A. & Ricroch, A. E. (2012). Assessment of the health impact of GM plant diets in long term and multigenerational animal feeding trails: a literature review. Food and Chemical Toxicology, 50, 1134-1148.
16. 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 and Chemical Toxicology, 45, 339-349.
17. 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 and Chemical Toxicology, 42, 1003-1014.
18. 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 and Chemical Toxicologyl, 45, 350-363.
19. Tripathi, M. K., Mondal, D., Somvanshi, R. & Karim, S. A. (2011). 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, 95, 545-555.
20. Sakamoto, Y., Tada, Y., Fukumori, N., Tayama, K., Ando, H., Takahashi, H., Kubo, Y., Nagasawa, A., Yano, N., Yuzawa, K. & Ogata, A. (2007). A 52-week feeding study of genetically modified soybeans in F344 rats. Journal of Food and Hygienic Society Japan, 48, 41-50.
21. Nahas, A. F., Mohamed, A. A. A., Zweel, H. H. & Ashmawy, I. M. (2011). Hepatorenal and genotoxic effects of genetically modified quail meat in a 90-day dietary toxicity study in mice. International Food Research Journal, 18, 1313-1319.
22. Schmucker, D. L. (1990). Hepatocyte fine structure during maturation and senescence. Journal of Electron Microscopy, 14, 106-125.
23. Malatesta, M., Tiberi, C., Baldelli, B., Battistelli, S., Manuali, E. & Biggiogera, M. (2005). Reversibility of hepatocyte nuclear modifications in mice fed on genetically modified soybean. European Journal of Histochemistry, 49, 237–242.
24. Mazza, R., Soave, M., Morlacchini, M., Piva, G. & Marocco, A. (2005). Assessing the transfer of genetically modified DNA from feed to animal tissues. Transgenic Resserach, 14, 775-784.
25. 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. Cellular and Structural Function, 27, 173-180.
26. 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, Jounal of Science and Food Agriculture, 79, 1607-1612.
27. Smith, J. M. (2005). Most offspring died when mother rats ate genetically engineered soy, Spilling the Beans Newsletter, 10, 1.
28. Seralini G E, Report on MON 863 GM maize produced by MONSANTO Company. (2005). Controversial Effects on Health Reported after subchronic toxicity test: a confidential rat 90 day feeding study, June.
29. Séralini, G. E., Clair E, Mesnage, R., Gress, S., Defarge, N., Malatesta, M., Hennequin, D., & Vendômois, J. S. (2012). Long term toxicity of a Roundup herbicide and a Roundup-tolerant genetically modified maize. Food and Chemical Toxicology, 50, 4221–4231.