ORIGINAL RESEARCH PAPER

Zoology

A HISTOLOGICAL STUDY ON INTESTINES OF FEMALE ALBINO RAT TREATED WITH GENETICALLY MODIFIED FOOD

KEY WORDS: Histological study, Bt cotton, albino female rat, intestine villi.

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ABSTRACT

The present study was performed to investigate the histological effects of genetically modified diet on intestine of female albino rats. Bt cotton is the only GM crop available in India. Bt cotton seeds were incorporated into the diet of female albino rats along with 50% carbohydrate and 30% protein. The study was conducted on 18 female albino rats; the animals were divided into 3 equal groups. The control group was given normal diet with wheat, casein and groundnut powder. The second and third groups were given 2 g /kg of body weight of Bt and non Bt cotton seed powder along with wheat and casein respectively for 60 days. The animals were anesthetized; intestines were removed for histological studies. Histological changes which observed were hemorrhages with degenerative changes, destructive changes in intestinal walls.

INTRODUCTION

Soybean, Maize, Cotton and Canola are the major crops that have got approved as GM foods while Eggplant, Papaya, Potato, Rice, Sugar beet, Sunflower, Sugarcane, and Tomato are also in production around the world. There is debate on genetically modified (GM) food grows in intensity, despite the undeniable benefits of using biotechnology for genetic modification of crops bred for human and animal consumption. Objections to GM plants fix on various aspects technical ones, concerning the construction of transgenes, abuse of constitutive promoters, and lack of control over the insertion of a transgene into the genome structure, about the medical consequences, *i.e.* deleterious effects of GM crops on humans or animals.

During the last few years, the objective of many studies was to determine the nutrient value of GM feeds compared to their conventional counterparts. The available results to date reveal no significant differences in the nutritional value of feedstuffs containing GM and non-GM varieties. They are also reassuring as to the safety of transgenic feed.

One of the most widespread GM plant in India is *Bt* (*Bacillus thuringiensis*) cotton. Cotton has been modified by the introduction of specific DNA sequences containing either the cry1Ac gene (Bollgard cotton) or both the cry1Ac and cry2Ab genes to produce Bollgard II cotton; the production of the CP4 EPSPS protein by the plant provides tolerance to glyphosate which is active ingredient in Roundup (Harrison et al., 1996). However there is concern about genetically modified foods as more amount of vitamins and proteins leads to major health issues.

Many long-term single-generation studies on rats fed GM plant feed have not demonstrated any negative effect of these plants on animal metabolism or health.

Animal feeding studies should also be undertaken to provide further assurance of nutritional safety and value of GM foods. The optimisation and improvement of safety assessment methods will benefit crop developers, regulators including other scientists as it will be shown that they not only demonstrate the safety of currently available GM products but also can readily be applied to newly developed GM crops as well.

Therefore, the aim of our study was to test the possible influence of feeding of genetically modified *Bt* cotton on the intestine of rats. It also suggested that further research should be carried out in order to clarify all doubts.

2. Collection and Preparation of powder:

The *Bt* and non *Bt* cotton seeds were collected from Mahyco, Maharashtra which is easily available in market. The specimens were washed, dried and powdered coarsely.

2.1. Experimental animals:

Albino rats of female sex, weighing 100-110 g were kept in separated cages under standard environmental conditions of temperature 22 to 26°C and humidity. Animals were provided with standard pelleted diet and water ad libitum during acclimatization period of one week prior to experiment. The experimental procedures were carried out in strict compliance with the institutional animal ethical committee regulations (IAEC).

2.2. Treatment protocol: The animals were divided into 3 groups of 6 animals each and treated as per the protocol given below:

Control diet Group I: The animals were given normal diet of wheat, casein and groundnut powder for 60 days.

GM *Bt* cotton seed diet Group II: The animals were given 2g of *Bt* cotton seed powder with wheat and casein.

Cotton seed diet Group III: The animals were given 2g of non *Bt* cotton seed powder with wheat and casein.

Fresh feed was given daily basis at 09:00 h in an excess of 10% of previous days intake after discarding the residue.

2.3. Schedule of sacrification and collection of organs: On completion of experiments, the animals were anesthetized by exposure to mild amount of diethyl ether. The intestines from different groups were dissected out, blotted free of blood and preserved for histological studies.

2.4. Histological studies: Intestine sections were preserved in bouin's fixative. They were dehydrated in ascending series of alcohol, cleared in xylene and embedded in paraffin wax. 5µm thick sections were obtained by rotary microtome. They were stained with haematoxylin and eosin, observed under the microscope to estimate the effect of *Bt* cotton.

3. RESULT

Fine structural modifications of cellular components in relation to GM feed intake have been described in the fig. (2) showing significant histological changes which were noted in intestine of female rat fed with *Bt* cotton i.e. multifocal congestion, focal MNC infiltration with hemorrhages and disruption, multinucleation, swelling, increased degradation

of ileal surface cells in GM *Bt* cotton seed diet fed group II as compared to Control group (1) and Cotton seed diet group III as showed in Fig. (1) and Fig. (3).

Results from the current study showed that in spite of the assuring reports on GM products, GM has profoundly altered the histological structure of the intestinal tissue at many levels and revealed several alarming signs as the proliferative and eroded hemorrhagic lesions in addition to several ultra-structural alterations described here for jejunum under GM food influence. Possible mechanisms have been proposed including inflammation associated with goblet cell overexpression and PCNA upregulation.

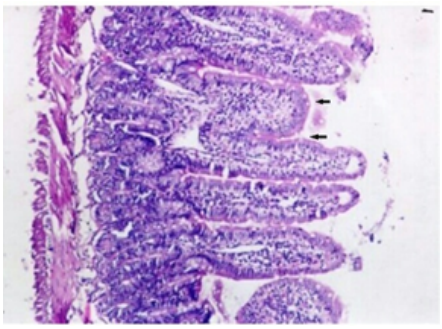

Fig. (1): Microphotograph of intestine showing normal histoarchitecture from Control diet group I female rat treated with (normal) diet (H&E 100X).

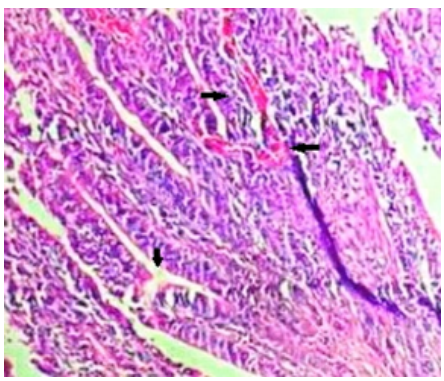

Fig. (2): Microphotograph of intestine showing changes in histoarchitecture showing distruption, multinucleation, swelling, increased degradation of cells from GM *Bt* cotton seed diet group II female rat treated with 2g *Bt* cotton seed diet (H&E100X).

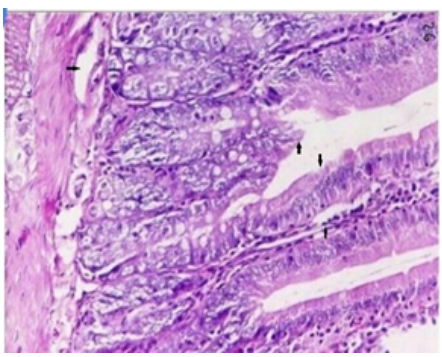

Fig. (3): Microphotograph of intestine showing mild changes in structure of intestinal cell wall from Cotton seed diet group III female rat treated with 2g non *Bt* cotton seed diet (H&E100X).

4. DISCUSSION AND CONCLUSION

Digestive tract is the first site of contact for any ingested compound. The stomach and the intestines are the sites of longest residence for any ingested product; these become the most important sites for the evaluation of an ingested compound's toxicity. Diet can influence the characteristics of the gastrointestinal tract as the intestinal mucous membrane is directly in contact with food and absorbs the substances produced by digestion (Montagne et al., 2003). It has been reported that the diet may affect small and large intestine in terms of mucosal architecture, villous height and crypt depth, epithelial cell proliferation including other features (Seralini et al., 2007; Trabalza-Marinucci et al., 2008).

Despite a number of studies which demonstrated no effect of genetically modified foods on animal health, the claim of the safety of GM feed is still controversial topic in many countries. Therefore, for the public acceptance of these GM materials, safety assessments of new GMO feed products should be strictly performed. The aim of this study was to evaluate the safety of GM feed based on observation of the intestinal histology of rats fed on diets containing conventional and genetically modified *Bt* Cotton seed.

In the present work, focal structural changes including distortion, shortening, flattening and fusion of some jejunal villi were observed in GM *Bt* cotton diet fed group II. In addition, stratification alternating with shedding of the jejunal surface epithelium was detected as was similarly reported by Fares & El-Sayed (1998). These changes could be observed as early as after only 45 days of GM *Bt* cotton seed diet consumption as suggested by El-Shamei et al., (2012). While according to de Vendômois et al., (2009) some researchers have suggested that significant GM food diet linked effects were generally detected either after 14 weeks of consumption and at a high genetically modified feed dose in the diet.

Whereas the finding of our study is contradictory with the result of study conducted by Tripathi *et al* (2011) which concluded that over the period of time, the growth and the histopathology of ileum did not change by *Bt* cotton seeds diet feeding in growing lambs.

Feeding rats diets containing genetically modified *Bt* cotton seed did affect the histology of intestine of the animals. These current findings were confirmed by the results obtained by Velimirov *et al (2008)* in which mice fed with GM insecticide producing maize over four generations showed a buildup of abnormal structural changes in various organs with major changes in the pattern of gene function in the gut, reflecting disturbances in the chemistry of the organ system (Velimirov *et al* 2008). Similarly experimental researches in mice showed that ingested foreign DNA can persist in fragmented form in the gastrointestinal tract, penetrate the intestinal wall and reach the nuclei of leukocytes (Schubbert *et al* 1994).

Sheep fed with *Bt* insecticide- producing GM maize over three generations showed disturbances in the functioning of the digestive system of ewes (Trabalza *et al* 2008). GM DNA was found to survive processing and was detectable in the digestive tract of sheep fed GM maize (Duggan *et al* 2003).

Bt-potatoes and *Bt*-toxin caused the disruption, multinucleation, swelling, increased degradation of ileal surface cells in rats and their intestines were enlarged (Pusztai 2002). The similar disruption of cells found in present study. Preliminary reports have indicated mortality in sheep that grazed on *Bt* cotton plants, post mortems of the animals showed severe irritation and black patches in both intestines, due to a toxin, most probably Bt-toxin (ISIS 2006).

The current work presents a multi-approach histological assessment of a typical 60 days sub-chronic toxicity for a full histological evaluation about the influence of GM food on the intestine in a rat model. Results from the present study could show that in spite of the assuring reports on GM products, GM *Bt* cotton seed diet has profoundly altered the histological

structure of intestine at many levels. Under the influence of GM *Bt* cotton seed diet revealed several alarming signs as the proliferative and eroded hemorrhagic lesions in addition to several ultra-structural alterations described here.

CONCLUSIONS

Although there is a comprehensive set of studies that confirm the absence of unintended effects and the nutritional equivalence of GM *Bt* cotton to existing conventional cotton seed, the current results of studies on model animals, provide convincing evidence of the negative effects of genetically modified feed.

This finding is just basic of should and can be taken forward for more extensive research to reveal the exact mechanism of such unintended effect and to re-modulate the GM crops to avoid their adverse effects.

REFERENCES

1. Harrison L A, Bailey M R, Naylor M W, Ream J E, Hammond B G, Nida D L, Burnette B L, Nickson T E, Mitsky T A, Taylor M L, Fuchs R L and Padgette S R (1996), The expressed protein in glyphosate-tolerant soybean, 5 enolpyruvylshikimate-3- phosphate synthase from Agrobacterium sp. strain CP4, is rapidly digested in vitro and is not toxic to acutely gavaged mice. J Nutr 126: 728–40.
2. Deplancke B, Gaskins HR, Microbial modulation of innate defense globlet cells and the intestinal mucus layer, Am. J. Clin. Nutr. 2001; 73(6) 1131 S -4S.
3. Montagne L, Pluske JR, Hampson DJ., A review of interactions between dietary fibre and the intestinal mucosa, and their consequences on digestive health in young non-ruminant animals. Anim. Feed. Sci. Technol. 2003;108(1–4): 95–117.
4. Seralini GE, Cellier D, de Vendomois JS., New analysis of a rat feeding study with a genetically modified maize reveals signs of hepatorenal toxicity. Arch. Environ. Contam. Toxicol. 2007;52(4):596–602.
5. Fares NH, El-Sayed AK. Fine structural changes in the ileum of mice fed on deltaendotoxin-treated potatoes and transgenic potatoes. Nat. Toxins 1998;6 (6):219–33.
6. El-Shamei ZS, Gab-Alla AA, Shatta AA, Moussa EA, Rayan AM., Histopathological changes in some organs of male rats fed on genetically modified corn (Ajeeb YG). J. Am. Sci. 2012;8(10):684–96.
7. Velimerov A, Binter C and Zentek J (2008), Biological effects of transgenic maize NK603*MON810 fed in long term reproduction studies in mice. Bundesministerium fur Gesundheit, Familie und Jugend Report, Forschungsberichte der Sektion IV Band 3/2008, Austria Pp 1-105.
8. Schubbert R, Lettmann C and Doerfler W (1994), Ingested foreign (phage M13) DNA survives transiently in the gastrointestinal tract and enters the blood stream of mice. Mole Genes and Genetics 242: 495-504.
9. Trabalza-Marinucci M, Brandi G, Rondini C, Avellini L, Giammarini C, Costarelli S, Acuti G, Orlandi C, Filippini G, Chiaradia E, Malatesta M, Crotti S, Antonini C, Amagliani G, Manuali E, Mastrogiacomo AR, Moscati L, Haouet MN, Gaiti A, Magnani MA. Three-year longitudinal study on the effects of a diet containing genetically modified Bt176 maize on the health status and performance of sheep. Livest. Sci. 2008;113:178–90.
10. Duggan P S, Chambers P A, Heritage J and Forbes JM (2003) Fate of genetically modified Maize DNA in the oral cavity and rumen of sheep. British J Nutr 89: 159-66.
11. Pusztai A (2002) Can science give us the tools for recognizing possible health risks of GM food. Nutr and Health 16: 73-84.
12. ISIS (2006) Mortality in Sheep Flocks after Grazing on Bt Cotton Fields. Institute of Science in Society, Andhra Pradesh.