



HISTOLOGICAL STUDY OF THE KIDNEY OF ALBINO MALE RATS FED WITH BT AND NON BT COTTON SEED

Zoology

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ABSTRACT

The development of GE crops like Bt cotton is one of the main achievement of modern plant biotechnology. The present study was performed to investigate the histological effects of Bt cotton seed and non Bt cotton seed in the kidney of male albino rat. The male albino rats were divided into 3 equal groups depends upon the diet given to them. The control group was given normal diet with 50% of carbohydrate (wheat), 30% protein (casein) and 20% fat (groundnut powder). Experimental and non-experimental groups were given same proportion of diet but fat content was replaced by 20% of powdered Bt and non Bt cotton seeds respectively for 60 days. The animals were anesthetized; kidneys were extracted for histological studies. In the kidney of experimental group male rats the changes were noted i.e. focal glomerular atrophy, multifocal necrotic changes, and multifocal congestion with focal hemorrhages.

KEYWORDS

Histological study, Bt cotton, albino rat, kidney.

INTRODUCTION

Though traditional Plant breeding method is playing role as partner of farmer for centuries, the development of recombinant DNA techniques also helpful as it allow the desired genes to be inserted into the plant genomes resulting in genetically modified plant.

GM cotton is one of the most important transgenic crops which found globally. From last 13 years i.e. from 1996 to 2008 the area under genetically modified crops has increased around 73.5 fold [1]. *Bt* cotton (gene of *Bacillus thuringiensis* incorporated by recombinant DNA technology) was introduced into India in 2002 as Bollgard I (one gene cry 1 Ac) while in 2005 Bollgard II (two gene cry 1 Ac and cry 1 Ab). According to the report of cotton association of India there was steady increase in number of *Bt* cotton hybrid. Up to 2012 there were 1128 hybrid of *Bt* were available in the market. *Bt* gives protection against lepidopteran insect pests. Hence it reduces the risk of crop failure [2]. Genetically modified crops technology improves the economy of the nation so there is continuously increasing interest in GM foods. GM foods are distributed all over the world and became part of animal diet.

Cottonseed contains 4% carbohydrates, 7% moisture, 23% protein, 23% fat, 3% minerals, and 24% fiber. It also contains phosphorus, calcium, iron, potassium, magnesium, manganese, aluminum, sodium, silica, sulphur, chlorine, copper (traces), boron, zinc, iodine. It also contains vitamins such as B- complex A, D, E. Other than vitamins nine flavonol glycosides identified in it. Gallic acid quercetin and dihydroxybenzoic acid is present in cottonseed meal. Composition analysis showed there were no difference in carbohydrate, protein and other content in *Bt* and non *Bt* cotton seed. According to report of Mukherjee, Rawal Debyani and Sinha Neeta, 8 percent farmers said that *Bt* cotton is harmful to their cattle. Due to gazing *Bt* cotton their immune system got highly affected. There was a rise in the mortality and infertility rates in cattle. Previous few studies indicated metabolic differences caused by GM diet. So the potential impact of *Bt* and non *Bt* cotton seeds on detoxifying organ like kidney were compared in this study.

2. MATERIALS AND METHODS

Healthy male albino rat, 4-5 week age, weighing 90-100 gms were obtained from The Bharat Serum Laboratory. The animals were housed in plastic cages under controlled conditions of temperature (24 ± 2°C) and light cycle (14h light: 10h dark). Floors of all cages were covered with soft crushed wood shaving; all cages were washed two times per week with 70% alcohol throughout the period of the study. Animals were kept one week to acclimatize before starting the treatment and then weighed. Rats were fed with the standard balanced pellet diet that obtained from the above Institute and given tap water *ad libitum* during acclimatization period. The experimental protocol approved by The Institutional Animal Ethical Committee (IAEC).

2.1. Preparation of diet:

The *Bt* and non *Bt* cotton seeds were procured from Mahyco Company, Maharashtra. The *Bt* has the strain MRC 7351 BG II and commercially available in market. Seeds were washed and dried. The seeds were coarsely powdered. All three groups were given following diet regularly for 60 days.

Control group type 1 diet: 50% carbohydrate (wheat) + 30% protein (casein) + 20% groundnut powder.

Experimental group type 2 diet: 50% carbohydrate (wheat) + 30% protein (casein) + 20% *Bt* cotton seed.

Non experimental group type 3 diet: 50% carbohydrate (wheat) + 30% protein (casein) + 20% non *Bt* cotton seed.

2.2 Experimental design:

The animals were randomly divided into 3 groups (n=6) depending on different doses of diet i.e. Control group which fed with type 1 diet, Experimental group fed with type 2 diet while non-experimental group animals were fed with type 3 diet regularly for 60 days.

2.3 Schedule of sacrifice and collection of organs:

On completion of experiments, the animals were anesthetized by exposure to amount of diethyl ether. The kidneys were quickly removed, blotted free of blood. After being cleaned from the accessory connective and adipose tissue, kidneys were fixed in buffered neutral formalin for 48 hrs.

2.4 Histological sections:

After the fixation of the organs, they were dehydrated in ascending series of alcohol, cleared in xylene and embedded in paraffin wax. 5µm thick sections were obtained by rotary microtome, slides stained with Harris hematoxylin and Eosin [3].

3. RESULTS:

Fig.(1) showing section for kidney from the control animal showing normal histoarchitecture. The Fig.(2) illustrated section of kidney after given the diet of non *Bt* cotton seed 20% showing mild changes in epithelial cells with mild lesions. The Fig. (3) Showing significant histological changes were noted in kidney of male rat fed with *Bt* cotton seed. It showed mild lesion with hemorrhage in interstitial tissue with enlargement of epithelial cells lining renal tubules necrotic changes. There were multifocal congestion with MNC infiltration (fig. i), tubular degeneration (fig. ii), congestion and hemorrhages within interstitial tissue (fig. iii), Focal globular atrophy (fig. iv), necrosis with focal tubular degeneration also seen in some tubule. In some cells the lesions were more severe including patches of hemorrhage.

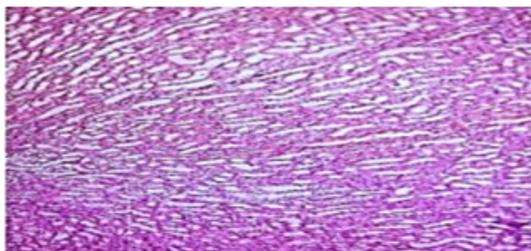


Fig. (1): Microphotograph of kidney showing normal histoarchitecture from control group male rat treated with type 1 (normal) diet (H&E 100X).

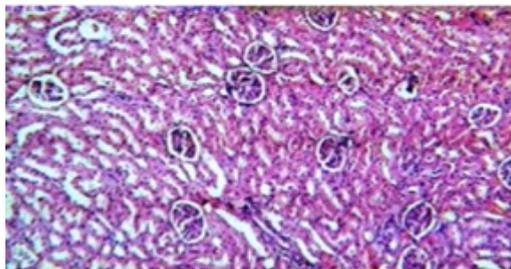


Fig. (2): Microphotograph of kidney showing mild changes in histoarchitecture from non-experimental group male rat treated with type 3 (non Bt) diet (H&E 100X).

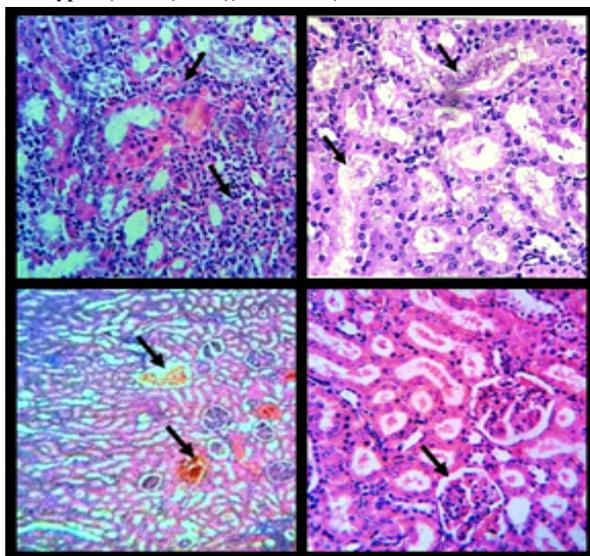


Fig. (3): Microphotograph of kidney showing MNC filtration (fig. i), necrotic changes with tubular degeneration (fig. ii), congestion and hemorrhages (fig. iii), glomerular atrophy (fig. iv) in experimental group male rat treated with type 2 (*Bt*) diet.

4. DISCUSSION

In the present work, significant structural abnormalities observed in the kidneys of experimental group (*Bt* cotton seed) animals. These abnormalities may attribute to presence of *Bt* genes in the diet of animals [4, 5]. Gastrointestinal tract provides route to foreign DNA, protein fragments which comes in animal feed. The foreign DNA-fragments of many plant genes were found in blood, muscles tissues and many other internal organs of many agriculture important animals such as broiler chickens, calves, pigs and cattle [6, 7, and 8]. Two fragments of *cry1Ab* genes i.e. *cp4epsps*, *cry1Ab* and *P35S* were found in heart, liver muscle and kidney tissue of goats [9, 10]. The toxic substance is absorbed from gastrointestinal tract it binds to erythrocytes, distributes widely to soft tissues like liver, kidney, brain and spleen like soft tissues [11]. So we selected the kidney to study histological changes after treated with *Bt* and non *Bt* cotton diet.

In kidney, structural changes were observed such as multifocal congestion with focal hemorrhages, focal tubular degeneration with necrotic changes. In some renal tissue atrophy is seen. These changes are in agreement with the study conducted to compare the effects of

three GM corn varieties on mammalian health showed there is disturbance in kidney function arise from toxic effects caused by mutagenic effects of GM technology. Possibly due to mutant forms of *Bt* toxin produced by MON 863. MON 810 fed females had kidney weight enhancement with mild hyperplasia seen in immune inflammatory process. MON 863 showed more sensitive kidney disturbance at higher GM feeding dose. The study also showed chronic nephropathy and hepatorenal toxicity [12]. Hammond et al (2006) observed that the male rat fed with 33% of MON 863 showed decreased in kidney weight with focal inflammation, regenerative changes as compared to control diet fed male rat. Transgenic *Cry1 AC* was found in the tissue of spleen, kidney when thirty five days GM corn diet given to porcine [13]. According to de Vendomois (2009) MON 810, 863 and NK 603 caused hepatorenal toxicity in rats [12]. Rats were suffered problems in liver and kidney when fed with GM maize [14]. According to Tripathi et al (2011) histological changes did not observed in lamb when *Bt* cotton diet was given [14]. Mice fed on GM soya showed changes in kidney, liver, spleen and pancreas [15]. Feeding studies of [16] transgenic potatoes with glycinins showed no pathologic symptoms, histopathological abnormalities in liver and kidney in treated rats. Rats fed on GM maize showed lower kidney weight [17].

In conclusion *Bt* cotton seed causes some deleterious effects in kidney as compared to non *Bt* seeds. The effects are depends upon duration of feeding *Bt* seeds diet.

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