In: Multidisciplinary Science … ISBN: 978-1-53618-959-9
Editors: Kaushik Pal et al.

Chapter 11

BIOLOGICAL IMPACT OF FEEDING RATS WITH CRY PROTEIN BASED DIET

Tanushree Tulsian Samanta[1,*], MD and Arpita Rani Khamrai[2]

[1]First Author Affiliation, Assistant Professor, Department of Physiology(UG&PG), Raja Narendra lal Khan Womens College (Autonomous),Gope Palace, Midnapore-721102, West Bengal, India

[2]Second Author Affiliation, Department of Physiology(UG&PG), Raja Narendra lal Khan Womens College (Autonomous),Gope Palace, Midnapore- 721102, West Bengal, India

ABSTRACT

Effect of Bt cry protein depends upon the concentration and length of exposure. The current study suggest that Bt cry protein ingestion induces a time dependent decreases in food intake capacity in treated rats through there is an increase in body weight. This work was conducted on the study of thebiological impact study on albino rats, with a range of

* Corresponding Author's E-mail: tsamanta19@yahoo.co.in.

combined parameters including Total protein & DNA fragmentatin to evaluate the impact of a cry protein based diet on animal health. The procedures were performed in the liver, kidney, and spleen, stomach tissues. The percentage of survival rate thus calculated, exhibited 100%survivability in both groups. Experimental researches in mice showed that DNA can persist in fragmented form in the gastrointestinal tract, penetrate the intestinal wall and reach the nuclei of leukocytes, spleen, liver cells. We studied the percentage of DNA fragmentation of isolated tissue (such liver, spleen, kidney stomach & blood) by DPA assay method and taking absorbance at 600nm by colorimetric method. It was observed that percentage of DNA fragmentation are higher in Bt cry protein treated rats as compared to control for stomach, kidney, liver tissue whereas lower value was obtained in spleen and blood samples. . In addition, oxidative stress induction was observed in the stomach, kidney, liver after 90 days of infection initiation. The elevated DNA fragmentation may be related to increase oxidative stress.

Keywords: Bacillus thuringiensis (Bt), Cry protein, DNA Fragmentation, Colorimetric method, Oxidative stress.

1. INTRODUCTION

Bacillus thuringiensis (Bt) is a unique bacterium in that it shares a common place with a number of chemical compounds which are used commercially to control insects important to agriculture and public health.(1).

This work was conducted in the context of the effects on the male albino rats after feeding them with Crystalized protein based diets. It was designed to evaluate the potential impact of feeding rats with Cry protein based diets for 30, 60, or 90 days starting from weaning. Blood samples and tissue (i.e., liver, spleen, etc.) were collected for analysis of DNA fragmentation ratio and total protein.

2. MATERIALS AND METHODS

The study was conducted on sexually mature male albino rats, weighing 250-280 gm. The rats were maintained under controlled condition of temperature(37°C) and provided standard diet food and water. The rats were divided into five groups in which four group contained treated rats where as other contain control albino rats respectively. The treated rats were named as T1,T2,T3,T4 and T5 and the untreated was named control respectively. Day after the last dose i.e., after 90 days, the animals were mildly anaesthetized by using chloroform and blood sample from each rat was collected directly from heart in centrifuge tube. The animals were dissected and vital organs i.e., liver, spleen, kidney, stomach were excised out, clearing off the adherent tissue. The tissue were homogenized in 2ml of 0.1 mM phosphate buffer (7.4). After that homogenized tissue were centrifuged at 3000 r.p.m for 10 minutes at 4°C and followed by collection of supernatant& pellet separately and used for % DNA fragmentation assay. DNA fragmentation was determined in all of these tissue and blood samples via colorimetric diphenylamine assay. Total protein were estimated from tissue homogenate by Lowry method. Blue colored developed by the biuret reaction of the protein with the alkaline copper tartarate are measured in the Lowry method.

3. RESULTS AND DISCUSSION

From collecting blood & tissue (i.e., liver, spleen, kidney stomach), DNA fragmentation were determined. The % of DNA fragmentation were determined colorimetrically at 600nm absorbance. Total protein were estimated in tissue (i.e., spleen, kidney, liver) homogenate by Lowry method. Amounts of isolated protein were determined colorimetrically at 600nm absorbance.

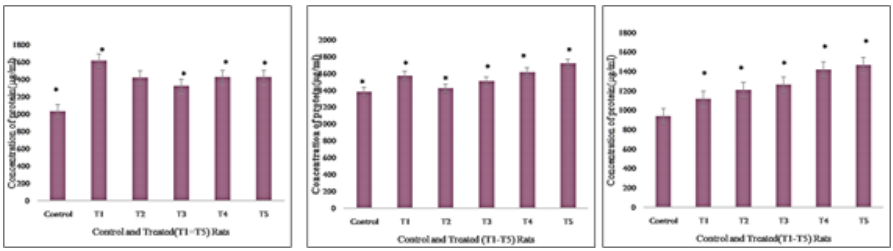

Figure 1. Concentration of protein (µg/ml) significantly compared between control and treated rates after treatment of Bt Cry protein.

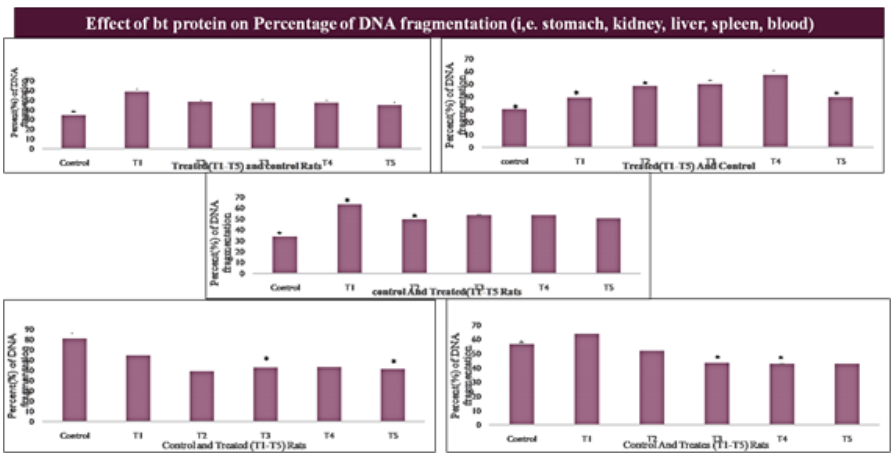

Figure 2. Percentage of DNA fragmentation are higher in Bt cry protein treated rats as compared to control for stomach, kidney, liver tissue whereas lower value was obtained in spleen and blood samples.

It was showed that, experimental researches in mice showed that DNA can persist in fragmented form in the gastrointestinal tract, penetrate the intestinal wall and reach the nuclei of leukocytes, spleen, liver cells(4). We studied the percentage of DNA fragmentation of isolated tissue (such liver, spleen, kidney stomach & blood) by DPA assay method and taking absorbance at 600nm colorimetrically.It was observed that percentage of DNA fragmentation are higher in Bt cry protein treated rats as compared to control for stomach, kidney, liver tissue whereas lower value was obtained in spleen and blood samples. Total protein were estimated for specified tissue homogenate (spleen, kidney, liver) by Lowry method and compared between control and treated groups. Concentration of protein uplifted for

all tissues as compared to control and showed significant differences. In the majority of Statistical analysis of total protein, for all tissue, showed that the p value is less than 0.05. thus we can reject the null hypothesis that there is no significant difference between the mean and conclude that a significance difference does exist within sampling. Changes in protein level suggest either an increased catabolism of the biomolecule to meet the enhanced energy demand of animals under stress or their reduced synthesis due to impaired tissue function.

CONCLUSION

Effect of Bt cry protein depends upon the concentration and length of exposure. The current study suggest that Bt cry protein ingestion induces a time dependent decreases in food intake capacity in treated rats though there is an increase in body weight, thus can be concluded that food intake (Bt cry protein)does not hamper body weight. In addition, oxidative stress induction was observed in the stomach, kidney, liver after 90 days of infection initiation. The elevated DNA fragmentation may be related to increased oxidative stress. However, stomach, kidney, and liver tissues undergo apoptosis to be more sensitive to the Bt cry protein on induction of oxidative stress and apoptosis compared blood and spleen tissue. after 90days of experiment, total protein content of spleen, kidney &liver were gradually increased in a time dependent manner.

ACKNOWLEDGMENTS

This study is ostensibly supported by the Principal of Raja Narendra Lal Khan Women's College (Autonomous) Dr. Jayshree Laha along with providing the lab equipment, reagents etc. However there were no financial support from the institution.

REFERENCES

[1] Bulla LA, Rhodes RA, St Julian G. Bacteria as insect pathogens. *Annu Rev Microbiol*. 1975;29:163–190.

[2] Gatehouse AMR, Gatehouse JA. Identifying proteins with insecticidal activity: use of encoding genes to produce insect–resistant transgenic crops. *J Pestic Sci.* 1998; 52: 165–175. 10.1002/(sici)1096-9063(199802)52:2<165::aid-ps679>3.0.co;2-7.

[3] Cao S., He X., Xu W., Ran W., Liang L., Luo Y., et al., (2010). Safety assessment of Cry1C protein from genetically modified rice according to the national standards of PR China for a new food resource. *Regul. Toxicol. Pharmacol.* 58 474–481 10.1016/j.yrtph.2010.08.018.

[4] du Rand, Nicolette (July 2009). *Isolation of Entomopathogenic Gram Positive Spore Forming Bacteria Effective Against Coleoptera*. Pietermaritzburg, South Africa: University of KwaZulu-Natal. 10413-1235

[5] Wang EH, Yu Z, Hu J, Xu HB.(2013 Dec) Effects of 90-day feeding of transgenic Bt rice TT51 on the reproductive system in male rats. Food and chemical toxicology. 1;62:390-6.

[6] Chilcott CN, Ellar DJ.(1998) Comparative toxicity of *Bacillus thuringiensis varisraelensis* crystal proteins *in vivo* and *in vitro*. *J. Gen. Microbiol.*;134:2551–2558.

[7] Reardon RC, Dubois NR, McLane W (1994). *Bacillus thuringiensis for managing gypsy moth*; *978-0-323-14317-2.*

[8] Tang W, Chen H, Xu CG, Li XH, Lin YJ, et al., (2006) *Development of insectresistant transgenic indica rice with a synthetic Cry1C gene*. 18: 1– 10.

[9] Betz FS, Hammond BG, Fuchs RL (2000) Safety and advantages of Bacillus thuringiensis-protected plants to control insect pests. *Regulatory Toxicology and Pharmacology* 32: 156–173.

[10] McClintock JT, Schaffer CR, Sjoblad RD (1995) A comparative review of mammalian toxicity of Bacillus thuringiensis-based pesticides. *Pesticide Science* 45: 95–105.

[11] Chilcott CN, Ellar DJ(1988). Comparative toxicity of *Bacillus thuringiensis* var*israelensis* crystal proteins *in vivo* and *in vitro*. *J. Gen Microbiol.*;134:2551–2558.