Advances in Bioresearch
Adv. Biores., Vol 13 (4) July 2022: 149-154

Print ISSN 0976-4585; Online ISSN 2277-1573
Journal's URL:http://www.soeagra.com/abr.html
CODEN: ABRDC3
DOI: 10.15515/abr.0976-4585.13.4.149154

Advances in Bioresearch

ORIGINAL ARTICLE

Effect of Cry Protein Induced Toxicity on Different Biochemical Parameters Of Spleen along with Histological and Histopathological Changes Observed in Male Albino Rats after A 90 Day Feeding Study

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ABSTRACT
Spleen being an important immune responsive organ it has so many works to function as filtering blood in much the way that the lymph nodes filter lymph. The spleen, alongside the liver, eliminates old and harmed erythrocytes from the circulating blood. Like other lymphatic tissue, it produces lymphocytes, particularly because of attacking microbes. Several pathogenic interactions take place in response to the spleen itself. Here in this study protein acquired from Bacillus thuringiensis (Bt) is the main trigger to which has initiated the interactions. The reason that Bt is vastly cultured because of a group of proteins that these microbes produce explicitly to target bug stomach, spleen, liver etc. These proteins are formed like crystals so they are normally called "glasslike crystals". These Cry proteins stay idle until devoured by a bug. When it binds the gut walls naturally it punctures their way to the stomach, spleen causing the substance to adhere. Critically human don't have similar receptors or stomach conditions as insects which implies that Cry proteins have no such degenerately effects on stomach cells though our study showed some histopathological changes involving protein and carbohydrate contents of spleen tissues. Finally, it was observed that various biochemical parameters such as SGOT and ALP of spleen significantly increased in treated rat than control and SGPT value showed no difference in treated and control one.
Keywords*: Spleen, Bacillus thuringiensis, Cry proteins, Histopathological, Gut*

Received 12.05.2022 Revised 21.06.2021 Accepted 23.07.2022

How to cite this article:
Arpita Rani Khamrai, Tanushree Samanta. Effect of Cry Protein Induced Toxicity on Different Biochemical Parameters Of Spleen along with Histological and Histopathological Changes Observed in Male Albino Rats after A 90 Day Feeding Study. Adv. Biores. Vol 13 [4] July 2022. 149-154

INTRODUCTION

In pigs and calves, Cry protein pieces are perceptible yet are dynamically decreased in size as they travel down the GI tract. None were distinguished in the liver, spleen, or lymph hubs demonstrating they were too huge to even think about being foundationally assimilated from the GI lot [1]. It is remarkable that livestock for the most part have a lot higher extent of maize in their daily diet plans than people; for instance, on account of oven chickens, maize represents around 65% of their eating routine all through their useful lifetime. Furthermore, maize took care of to creatures is by and large not handled, other than crushing. Human dietary exposure to Cry proteins is a lot of lower than that of livestock inferable from the eating routine and the handling (e.g., cooking) used to plan most human food containing maize [2]. Reviewing that hazard is a component of both danger and disclosure; the lower vulnerability experienced by people puts them at lower hazard than creatures. This is especially essential when one thinks about that the creatures didn't exhibit solid indications of poisonousness following the utilization of GM crops, not withstanding significantly higher dietary manifestations. *Bacillus thuringiensis* microbials are not viewed as a human allergen, on the grounds that notwithstanding the broad use as a bio-pesticide throughout the most recent few years, there has been just one report of hypersensitive responses in laborers who apply Bt microbials [3]. One more evaluation of allergenic capability of the embedded

protein is to decide the exposure to the protein. This is pertinent to the allergenicity evaluation since it is large acknowledged that expanded manifestations to a protein builds the likelihood that the protein could turn into an allergen. Vulnerability is evaluated by estimating the protein wealth in the grain and the strength of the protein i.e. Protein Digestibility. Taken together, the heaviness of-proof methodology used to survey the Cry proteins present in Bt recommends the allergenic capability of these proteins is low and that they present minimal allergenic danger [4]. In this study we have designated a few organs along with spleen as it contributes a huge part in the immunological exercises. Spleen being a principal organ for applying impacts in both biochemically and histological a few perceptions was made utilizing the Cry proteins. There were less weak impacts incasing of spleen noticed compared to other objective organs (for example liver, kidney, and intestine) and cystic appearances were likewise seen less in number contrasted with different organs.

MATERIAL AND METHODS

Cry Protein:

Bacterial isolates used in the experiment include *Bacillus thuringiensis* (1953, 6941, 4714, 4715) which have been bought from IMTECH, Chandigarh. Previously characterization with various studies like morphological, staining, growth curve analysis and various biochemical tests has been done [5]. Afterwards the protein isolated from the Bt strains were incorporated into a group of male albino rat's diet for 90 days.

Lowry method for estimation of total protein:

A standard curve was prepared first. Bovine serum albumin (BSA) powder was dissolved in distilled water and diluted to a concentration of 1 µg/ml. A series of dilutions (0, 1, 2.5, 5, 10, and 20 µg/well) were made in replicates of 4 with a final volume of 100 µl. Samples were diluted such that they would fall within the BSA standard range (0-25 µg / 100 µl) and 100 µl placed in each well. After standards and samples were diluted and transferred to the microplate, 200 ul of biuret reagent was added to each well and mixed thoroughly with repeated pipeting. Biuret reagent was prepared by mixing 0.5 ml of 1% cupric sulfate with 0.5 ml of 2% sodium potassium tartrate, followed by the addition of 50 ml of 2% sodium carbonate in 0.1 N NaOH. The mixture was then allowed to incubate at room temperature for 10-15 minutes prior to the addition of 20 µl per well of 1.0 N Folin & Ciocalteu's reagent. Samples were mixed immediately with repeated pipeting with each addition. Color was allowed to develop for 30 minutes at room temperature and the absorbance measured at 650 nm and blanked on the water only control.

Estimation of Serum glutamic oxaloacetate transaminase (SGOT) and Serum glutamic pyruvic transaminase (SGPT) :

Aspartate aminotransferase (AST) catalyzes the transfer of the amino group from L-aspartate to α-ketoglutarate to yield oxaloacetate and L-glutamate. Malate dehydrogenase (MDH) catalyzes the reduction of oxaloacetate with simultaneous oxidation of NADH+ to NAD. The resulting rate of decrease in absorbance at 340 nm is directly proportional to the AST activity. Lactate dehydrogenase (LDH) is added to prevent interference from endogenous pyruvate which is normally present in serum. Alanine aminotransferase (ALT) catalyzes the transfer of the amino group from L-alanine to α- ketoglutarate resulting in the formation of pyruvate and L-glutamate. Lactate dehydrogenase (LDH) catalyzes the reduction of pyruvate and the simultaneous oxidation of NADH+ to NAD.

Alkaline phosphatase test:

Alkaline phosphatase in the sample catalyzes the hydrolysis of colorless p-nitrophenyl phosphate (p-NPP) to give p-nitrophenol and inorganic phosphate. At the pH of the assay (alkaline), the p-nitrophenol is in the yellow phenoxide form. The rate of absorbance increase at 404 nm is directly proportional to the alkaline phosphatase activity in the sample.

Histological (Hematoxylene and Eosin) staining:

The hematoxylin and eosin (H&E) stained tissue segment is the foundation of physical pathology finding. The H&E strategy stains the core and cytoplasm differentiating shadings to promptly separate cell parts. In any case, staining results are reliant upon appropriate handling, which includes tissue conservation, lack of hydration, clearing, and paraffin penetration [6, 7].

Histochemical staining:

Used for the detection of glycogen in tissues such as liver, cardiac and skeletal muscle on formalin-fixed paraffin-embedded tissue sections, and may be used for frozen sections as well [8].

Statistical Analysis:

Microsoft Excel (Redmond, WA) was used to compute and tabulate experimental results. For statistical interpretation, student t-test was performed and the results were analysed andgraphically represented. Data were expressed as mean $\pm$ SD and a P value <0.05 was considered significant.

RESULT

The work was conducted in relation to the study of the biological impact on male albino rats,with a range of combined parameters including total protein, SGOT, SGPT, ALP, histology and histochemical staining etc. In addition, we also noted the differences in histological sectioning of spleen tissue after treatment of male albino rats with the Cry protein. The sections were then routinely stained with haematoxylin eosin (HE) to visualized the overall morphological variation (Fig 6) showed macrophotography of sacrificed rat after 3 months feeding of Bt cry protein. . It was also observed that degenerated cell membrane of the respective lineage cells along with, cystic appearance as compared to control rat which exhibit normal morphology. SGOT (AST) catalyses the transfer of amino group between LAspartate and a-ketoglutarate to form oxaloacetate and glutamate. The oxaloacetate formed reacts with NADH in the presence of Malate Dehydrogenase to form NAD. The rate of oxidation of NADH to NAD is measured as a decrease in absorbance which is proportional to the SGOT (AST) activity in the sample. The latter SGOT reading was studied and it showed notable differences with the control group. SGPT (ALT) catalyses the transfer of amino group between L-Alanine and a-ketoglutarate to form pyruvate and glutamate. The pyruvate formed reacts with NADH in the presence of Lactate Dehydrogenase to form NAD. The rate of oxidation of NADH to NAD is measured as a decrease in absorbance which is proportional to the SGPT (ALT) activity in the sample. Here in our study the difference in SGPT level are negligible. Alkaline phosphatase (ALP) catalyze the hydrolysis of 4- nitrophenylphosphate (4-NPP) with the formation of free 4- nitrophenol and inorganic phosphate, acting the alkaline buffer as a phosphate-group acceptor. The reaction is monitored kinetically at 405 nm by the rate of formation of 4-nitrophenol, proportional to the activity of ALP present in the sample. ALP levels were drastically elevated after treating with Cry protein. All the data showed statistically significant difference at a P value <0.05.

1.1. Macrophotograph of dissected rat and spleen: (Fig 1)
1.2. Representing the Total protein concentration of spleen: (Fig 2)
1.3. Representing the SGOT value of spleen: (Fig 3)
1.4. Representing the SGPT value of spleen: (Fig 4)
1.5. Representing the ALP value of spleen: (Fig 5)
1.6. Histological observations: (Fig 6)
1.7. Histochemical observations(PAS Staining): (Fig 7)

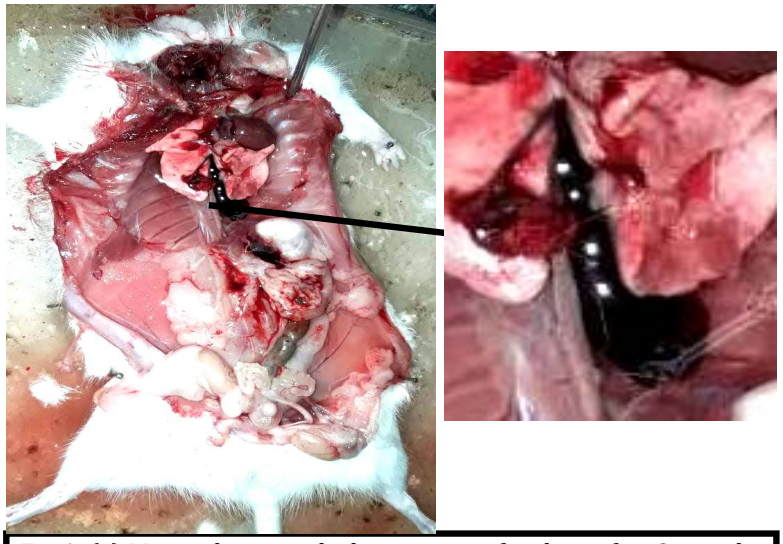

Fig 1: (a) Macrophotograph showing sacrificed rat after 3 months feeding of Bt cry protein
(b) Demonstrating spleen in the Cry protein treated rat

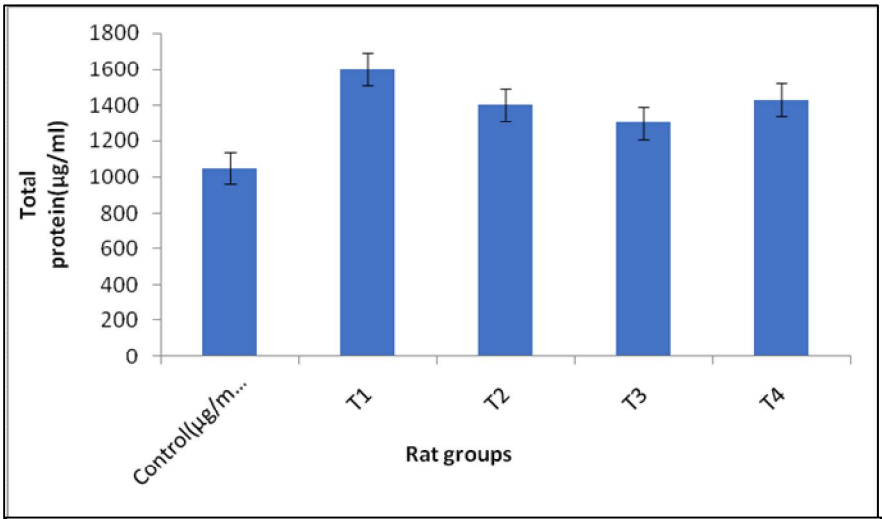

Fig 2: Diagram representing the differences observed after the feeding of Cry protein in caseof total protein. (*29.91357)

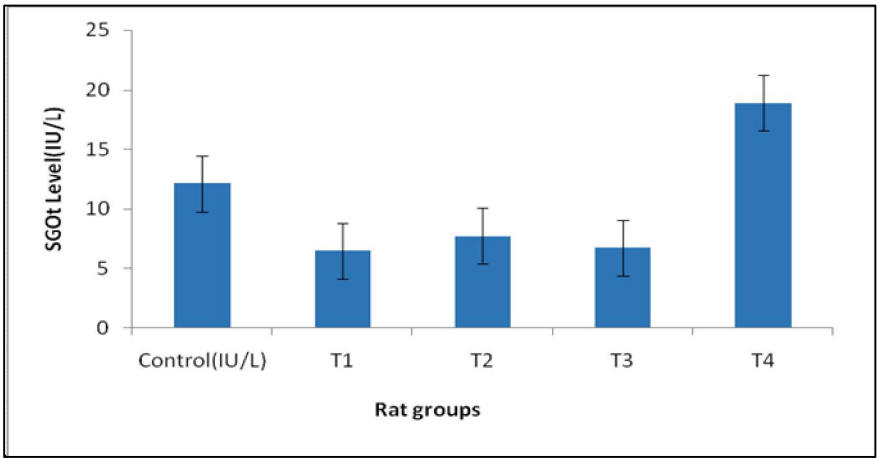

Fig 3: Diagram representing the differences observed after the feeding of Cry protein in case ofSGOT. (*0.725113)

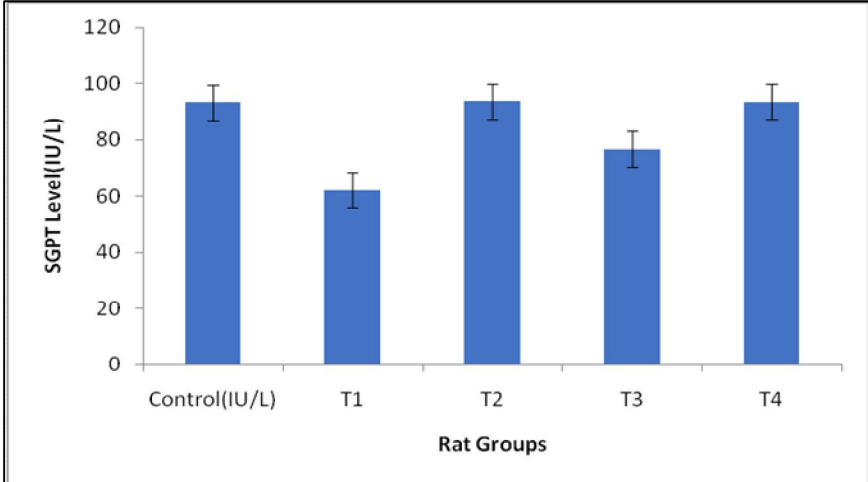

Fig 4: Diagram representing the differences observed after the feeding of Cry protein in case of SGPT. (*2.028077)

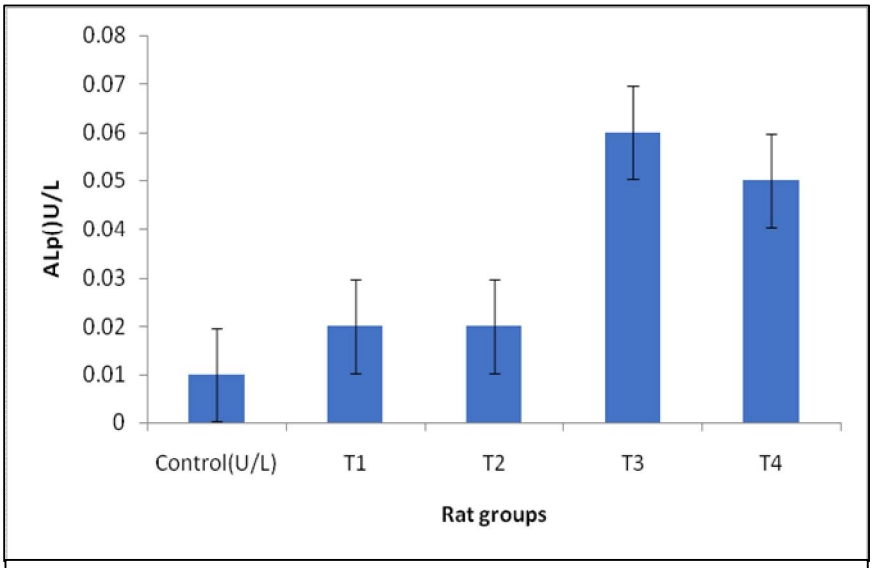

Fig 5: Diagram representing the differences observed after the feeding of Cry protein in case of ALP(*0.003645)

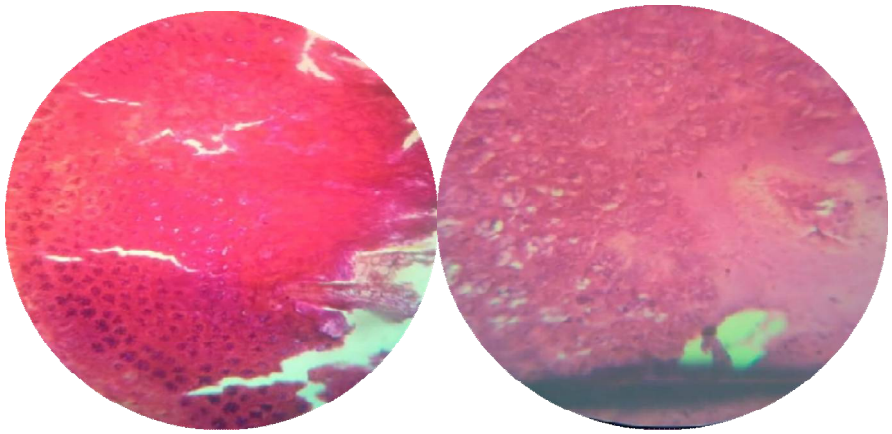

Fig 6: Representing the haematoxylin eosin stained histological slides of normal and treated albinorats. **A** denotes the normal histology of spleen of the control rats whereas ; **B** denotes the treated ratshistology after feeding of Cry protein which shows degenerated cell appearances of the respective lineage cells (Magnification- 10X*40X).

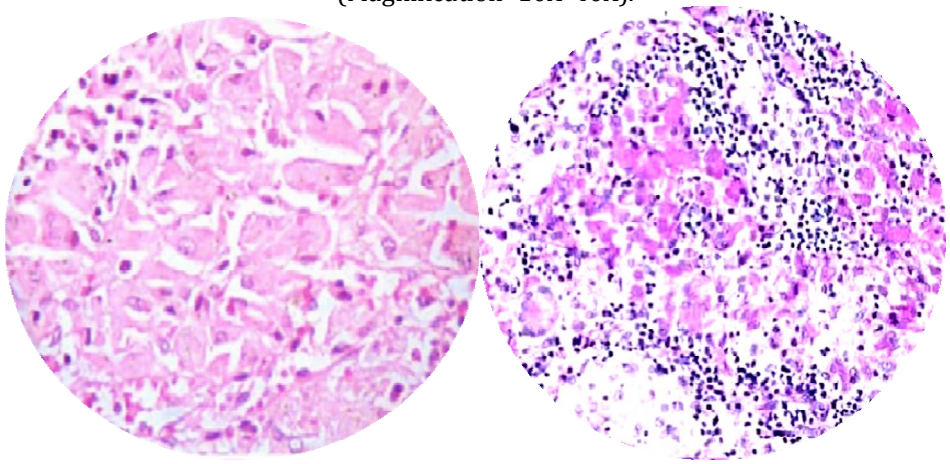

Fig 7: Representing the PAS stained histological slides of normal and treated albino rats. Adenotes the normal histology of spleen of the control rat whereas B denotes the treated rat's histology after feeding of Cry protein (Magnification-10X *40X).

DISCUSSION

Malatesta *et al* [9], did not reveal any significant difference in the total proteincontent of the spleen between control and treated but in our study (respective figure no. 16) it was observed that total protein content of spleen tissue significantly increased as compared to control. Allthe data were statistically analyzed and hence has significant difference at a P value <0.05. According to Aysun & Turan *et al*, [10], in alkaline phosphatase, there were no such statistical significant differences in relative organ. In case of our study, the determined effect of Cry protein on spleen tissue in term of various biochemical parameters such as SGOT, SGPT andALP etc are notable respectively. Fig 3,4 & 5 respectively represent SGOT of spleen which showeda decrease in value ,except T4 sample group of treated rats, whereas SGPT value didn't show any significant difference among control and treated rats except T1 and T3 sample group of treated rats, ,in another content ALP value increased significantly for more than 3-4 times in treated rats except T1 and T2 sample group of treated rats than control. All the data showed statistically significant difference at a P value <0.05.

In conclusion, the results obtained from this study gives several information that can be summarised as histochemical analysis by HE staining of spleentissue fortified the cellular deformities along with cystic appearance observed for spleen. Finally, it was observed that various biochemical parameters such as SGOT and ALP of spleen significantly increased in treated rat than control and SGPT value showed no difference while compared within treated and control one.

ANIMAL ETHICAL COMMITTEE APPROVAL

This research experiment was carried out by the sole permission from IQAC, Raja Narendra Lal Khan Women's College, Autonomous, medinipur-721102, West Bengal, India.

ACKNOWLEDGEMENT

We are very grateful to our Principal Dr.Jayashree Laha for providing us with the Laboratory and we are equally grateful to our institution Raja Narendra Lal Khan Women's College (Autonomous) for the sole assistance and the supporting environment.

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