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Cry Protein Induced Toxicity Study of Blood Parameters in Male Albino Rats

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Abstract

Blood being one of the most important connective tissues, hematological study was done after isolating blood sample from Bt cry protein fed rats thereby determining the effect of the protein if any. Blood cell count was done by using Neubauer's Haemocytometer slide and it was observed the total count of erythrocytes gradually decreased as compared to the control. This can be either by, degrading RBCs or by, causing an infection. Study on differential count of leukocytes showed that there was an increase in the neutrophil, eosinophil and monocyte count in blood along with a drastic decline in basophil count as compared with the control. This might again be an indication of certain infection in the toxin fed rats as neutrophil, eosinophil & monocyte acts as a first responder to infection. Further, the PBMCs (Peripheral Blood flow Mononuclear Cells) showed that normal morphology of monocytes and lymphocytes were distorted. The cells were found to be ruptured and the degraded cell membrane had certain black dots that are yet to be inferred.

Keywords: Bacillus Thuringiensis (Bt), Cry Protein, TC, DC, Gradient Centrifugation, Neutrophil, Monocyte

Introduction

Cry proteins are specifically toxic to several insect orders like Lepidoptera, Coleoptera, Hymenoptera and Diptera, and also to nematodes. The Cry proteins comprise at least 50 subgroups with more than 200 members. Cry proteins are defined as: a parasporal inclusion protein from Bt that exhibits toxic effects to a target organism, or any protein that has obvious sequence similarity to a known Cry protein [1]. A previous study explains Cry toxins exert their toxicity when activated at alkaline pH of the digestive tract of exposed larvae, and, because the somatic morphology of the digestive system in mammalian body does not allow the activation. However, the study demonstrated that Bt spore-crystals induced hematotoxicity, particularly to the erythroid ancestry. Bt spore-crystals caused haemolysis in cell lines of rat, mouse, sheep, horse, and human erythrocytes and suggested that the plasma membrane of vulnerable cells (erythrocytes in this case) may be the primary target for these toxins [2].

Bt spore-crystals can induce haematological problems in vertebrates, and their toxicity increases with time [3-5]. According to the studies carried out, aggrandized work is needed to understand the mechanism behind the hematotoxicity seen in mice.

Experimental Design and Structure

The design of the experiment carried out for analysis is described below (Fig.2.1)

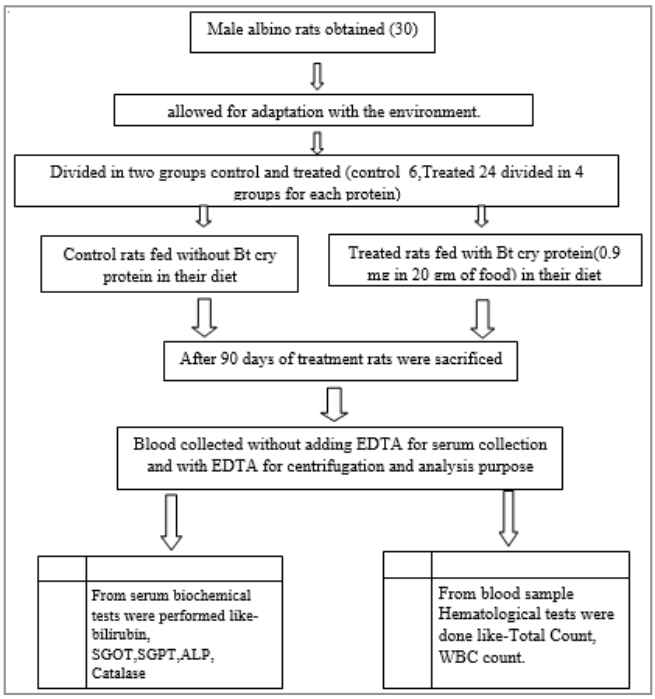

Figure 1: Representing the Experimental Structure of the Study

Results including changes in blood parameters along with serum analysis

Result representing the total count of RBCs after feeding with cry protein

Table 1: Representing Differences Observed after Treatment of Bt Cry Protein for 90 days in Case of Total Count of RBC from the Blood Collected

Sl. No.	Control	Treated				
		T1	T2	T3	T4	T5
1	7424	5125	5047	4705	5577	4937
2	7521	5121	5081	4699	5705	4893
3	7399	5234	5110	4765	5687	4965
4	7610	5312	5091	4693	5696	4762
5	7515	5099	5103	4795	5565	4901
6	7448	5211	5015	4599	5496	4888

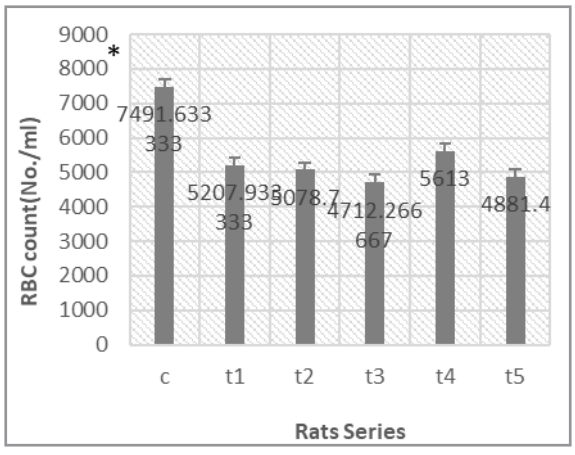

Figure 2: Representing the Changes Observed in Total Count of RBC after Cry Protein Treatment. (*211.072)

Result representing the differential count of rbcs after feeding with cry protein

Neutrophil& Eosinophil Count

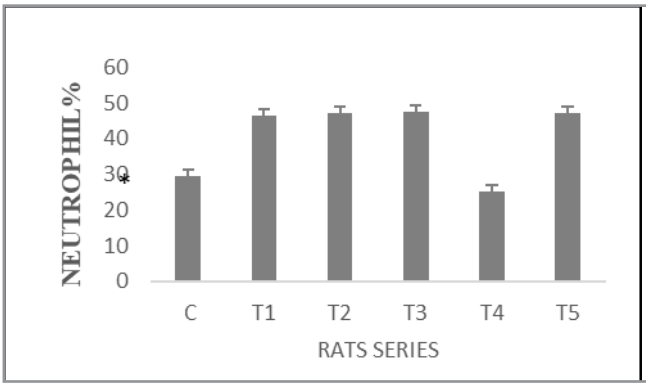

Figure 3: Representing the Changes Observed in Neutrophil % after Cry Protein Treatment. (*1.887)

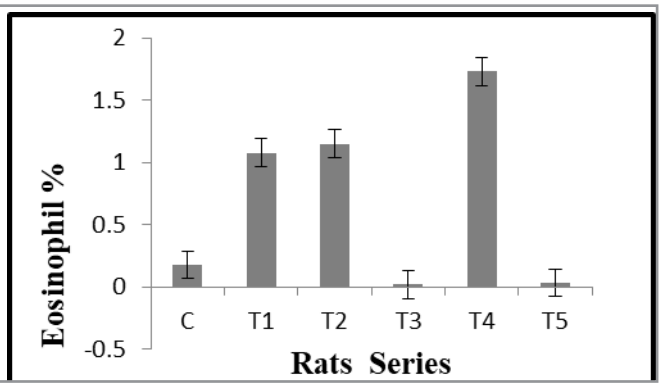

Figure 4: Representing the Changes Observed in Eosinophil % after Cry Protein Treatment. (*0.111)

Basophil Count

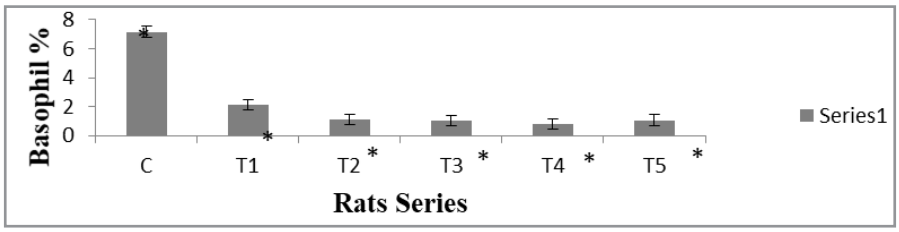

Figure 5: Representing the Changes Observed in Basophil % after Cry Protein Treatment. (*0.375)

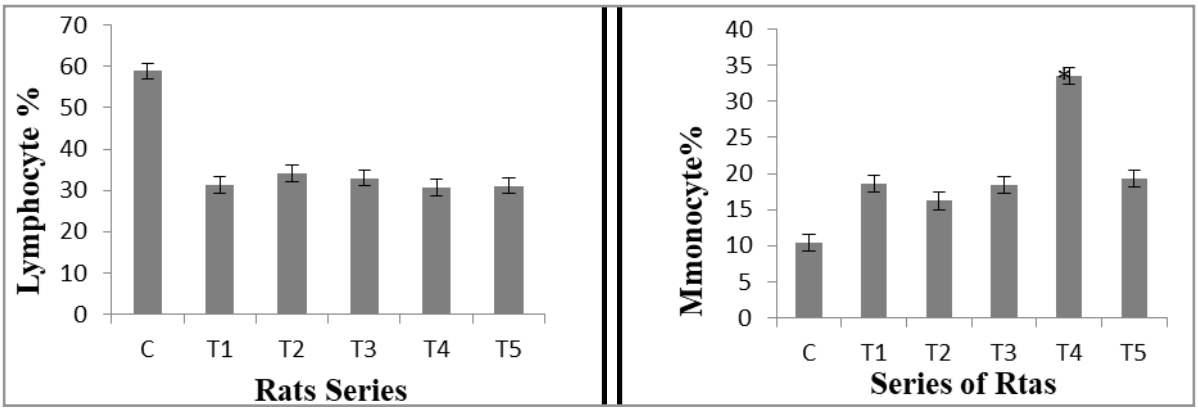

Figure 6: Representing the Changes Observed in Lymphocyte % after Cry Protein Treatment. (*1.8966)

Figure 7: Representing the Changes Observed in Monocyte % after Cry Protein Treatment. (*1.167)

Result of Histopaque Gradient PBMC Isolation

As shown in the figures the PBMC isolation showed that after the treatment of Bt cry protein the buffy layer containing monocytes and lymphocytes has decreased significantly.

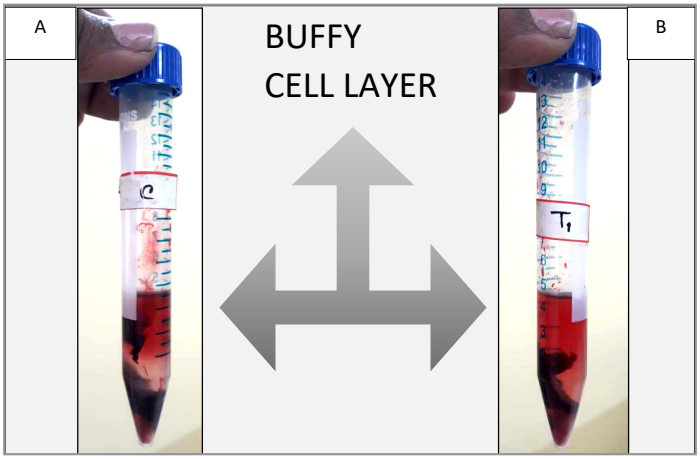

Figure 8: HISTOPAQUE-1077 Gradient Centrifugation
PBMC cell layer for the control and treated rats respectively. The control(A) centrifuge tube denoting the buffy layer i.e. the monocyte and lymphocyte layer is higher than the centrifugedenoting the same for treated rat (B).

Observation after Staining of the Monocytes & Lymphocytes
As shown in the figures the PBMC isolation showed that after the treatment of Bt cry protein the buffy layer containing monocytes and lymphocytes was taken and centrifuged as described previously and stained with Leishman staining procedure the following results were observed-

- The control rat's blood sample showed that the lymphocytes are not in particular shape and with degenerated morphological appearance.
- After treatment the treated rat showed that the monocytes have black dots in its membrane differing significantly from the normal ones.

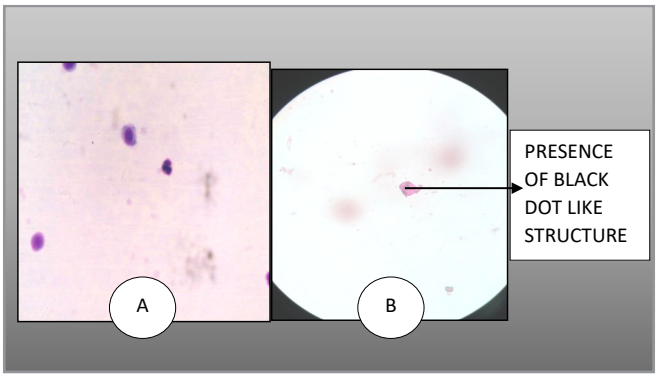

Figure 9: A. Denoting the Normal Monocyte Obtained from the Blood Sample of Untreated Albino Rat after Leishman Staining Procured from Histopaque Gradient Centrifugation. B.Denoting the Ruptured and Degraded Cell Membraned Monocyte obtained from the Blood Sample of Treated Albino Rat after Leishman Staining

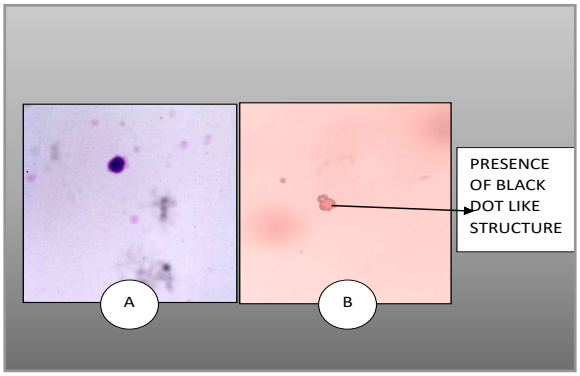

Figure 10: A. Denoting the Normal Lymphocyte Obtained from the Blood Sample of Untreated Albino Rat after Leishman Staining Procured from Histopaque Gradient Centrifugation. B. Denoting the Ruptured and Degraded Cell Membrane along with Presence of Dots on the Membrane of Lymphocyte Obtained from the Blood Sample of Treated Albino Rat after Leishman Staining Procured from Histopaque Gradient

Result of Serum Biochemical Tests

Bilirubin & ALP Test

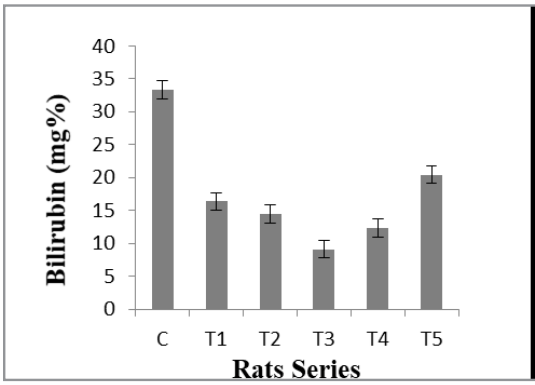

Figure 11: Representing the Changes Observed in Bilirubin (mg%) conc. after Cry Protein Treatment. (*1.3674)

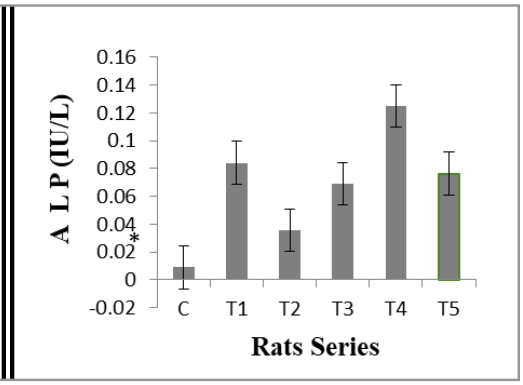

Figure 12: Representing the Changes Observed in A L P (IU/L) after Cry Protein Treatment. (*0.015312)

Catalase Test

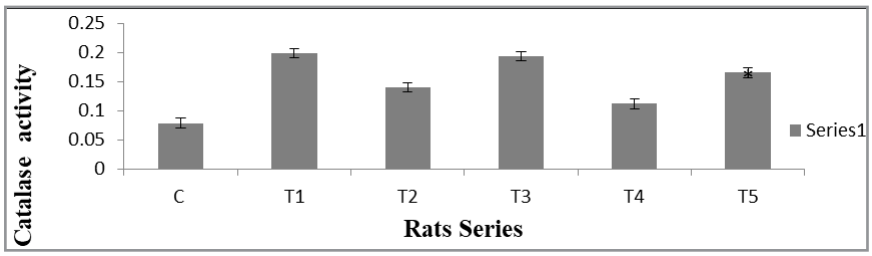

Figure 13: Representing Bar Diagram Regarding the Changes Observed Catalase (mm of H2o2consumption /dl of Plasma/ml Cry Protein Treatment. (*0.014508)

SGOT & SGPT Test

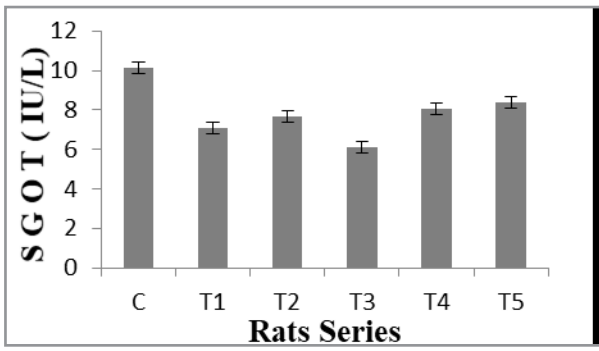

Figure 14: Representing the Changes Observed in S G O T(I-U/L) after Cry Protein Treatment. (*0.2960)

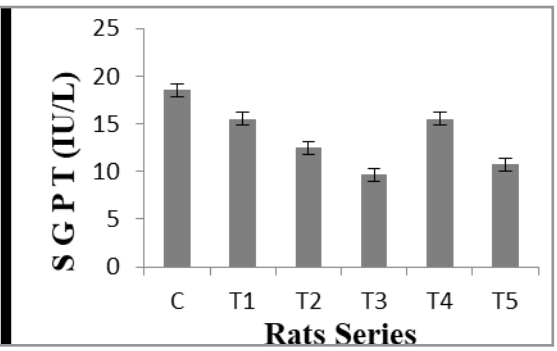

Figure 15: Representing the Changes Observed in S G P T (IU/L) after Cry Protein Treatment. (*0.6838)

Conclusions, Outlook and Future Aspects

In conclusion, the results obtained from this study, gives several information that can be summarized as total count of RBC is decreased in Bt cry protein treated rat as compared to untreated rat while differential count of WBC exhibit an increase in % count of neutrophil, eosinophil and monocyte thus gives a statistically significant difference, on the other end, decline in basophil and lymphocyte count observed in treated rat than control, thus can be concluded that Bt cry protein may be induces infections in treated rats as well as low count of RBC also fortified this as it may be degraded or causing infection with in rat [6-15].

In addition to this, through biochemical analysis it was observed that the levels of bilirubin, SGPT, SGOT are decreased significantly in blood in the treated group from the control. Other serum enzymes such as ALP increased significantly in blood of treated animal and Catalase activity decreased significantly for the same than control [16-21].

Though these deductions conclude that Cry proteins might give rise to certain inflammatory symptoms further investigations are needed in this area to explore the mechanism and to determine the cytotoxicity of Cry proteins in mammalian tissues [22-26].

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