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Research Article

EFFECT OF CRY PROTEIN INDUCED TOXICITY ON DIFFERENT BIOCHEMICAL PARAMETERS OF THE TISSUE OF INTEREST

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ABSTRACT

The Cry proteins produced by *Bacillus thuringiens* is are viewed as profoundly explicit insecticidal proteins. Decided to be of no risk for mankind and livestock because of their particular poisonousness, the proteins have been used as an organic pesticide and brought into hereditarily adjusted plants. The immediate impacts of enacted Cry proteins on mammalian cells have not yet been completely affirmed. Therefore, in this study we employed primary tissue homogenates for biochemical analysis preferences in several spheres like enzyme activities for oxidative stress, total protein concentration, alkaline phosphatase activity, serum glutamic oxaloacetate and pyruvate concentrations etc. There were no such significant differences observed between the weights of the organs of the treated and control rats, though the increase in total protein concentration and catalase activity indicates onset of oxidative stress and increase in Acid phosphatase levels in spleen and stomach comprises ceased enzyme activities for prostate.

INTRODUCTION

Bacillus thuringiensis (Bt) is a Gram-positive, soil-staying bacterium, motile with peritrichous flagella and ordinarily utilized as an organic pesticide. *Bacillus thuringiensis* additionally happens normally in the gut of caterpillars of different sorts of moths and butterflies, too on leaf surfaces, sea-going conditions, creature defecation, bug rich conditions, and flour factories and grain-storerooms.

Cry proteins are a large family of crystalline toxins produced by Bt. Individually, the family members are highly specific, but collectively, they target a diverse range of insects and nematodes. The mammalian intestinal epithelium has been found, based on *in vivo* experiments, to be resistant to insecticidal Cry toxins, which are derived from *Bacillus thuringiensis* and fatally damage insect midgut cells. Thus, the toxins are commonly used as a genetic resource in insect-resistant transgenic plants for feed. However, Cry toxins bind to the cellular brush border membrane vesicle (BBMV) of mammalian intestinal cells (23).

The Cry proteins do not become biologically active toxins until they have been dissolved in liquid and activated. Normally this occurs in the highly alkaline midgut environment of lepidopteran insects. The toxin is activated by the insect's gut enzymes. Most mammalian guts are acidic and do not produce a favorable environment for the Cry toxin. It is general accepted that the toxin recognizes certain receptors on the surface of insect gut epithelial cells. A precomplex forms through the cell membrane, resulting in the loss of potassium ions which affects the insect's ability to regulate osmotic presure. Eventually the animal dies due to massive water uptake (24).In this context more studies are required to completely understand the parasporal effect of Cry proteins in the kidney and liver tissues regarding oxidative stress, enzymatic activity along with protein concentration and other biochemical activities. Hence, the present study was conducted with four newly identified strains of *Bacillus thuringiensis* (1953, 6941, 4714, 4715)(25) administered with the diet provided to the male albino rats in the form of Crystal protein for 90 days and observing the effects of different enzyme activities, biochemical parameters and the organ weights of both the groups of treated and untreated rats.

MATERIALS AND METHODS

Biochemical tests

Different biochemical tests were performed to analyze the primary stress situations and the deprived contents of protein, bilirubin, aspartate, oxaloacetate for some significant organs.

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Lowry method for estimation of Protein concentration

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.

Diazo method for the estimation of Bilirubin concentration

Total (conjugated and unconjugated) bilirubin couples with a diazo reagent in the presence of a surfactant to form azobilirubin. The diazo reaction is accelerated by the addition of surfactant as a solubilizing agent. The increase in absorbance at 548 nm is due to azobilirubin is directly proportional to the total bilirubin concentration.

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. The resulting rate of decrease in absorbance at 340 nm is directly proportional to ALT activity.

Catalase Test

20micro lit tissue homogenate taken in a test tube and 2 ml of H_2O_2 added carefully without any type of shaking. Then 0.5ml of PBS added in the test tube and the OD value was measured at 240nm.

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. Optimized concentrations of zinc and magnesium ions are present to activate the alkaline phosphatase in the sample.

Acid Phosphatase test

Citrate buffer(R1) was prepare with Sodium citrate 110 mmol/L, 1,5-pentanediol 220 mmol/L, pH 5.2. Citrate/Tartrate buffer (R2) made with Sodium citrate 110 mmol/L, 1, 5-pentanediol 220 mmol/L, L-tartrate 110 mmol/L, and pH 5.2. ACP substrate Powder (R3) formed with α-Naphtyl phosphate 12.5 mmol/L, Fast Red TR 1.25 mmol/L and lastly Stabilizer (R4) was prepared Acetate buffer 5 M/L, pH 5.2.respectively.Added 10 mL of R1 (total ACP) or 10 mL of R2 (non-prostatic ACP) into a vial of R3.After mixing with the working reagent the sample was incubated and measured for abosrbance in spectrophotometer.

RESULTS

Organ Weights

The absolute and relative organ wet and dry weights were not significantly different between the rats fed the Cry diet and those on the control diet. On the other hand, the small intestine, heart, kidneys, and lungs of rats fed the NP diet were significantly larger than those of cry protein diet.

Relative organ wet and dry weights (g/100 g wet or dry body weight) of rats fed the nonprotein (NP) and test (Cry Protein) diets.

Organ		NP	Cry Pro.
Stomach	wet	1.44 ± 0.23	1.93 ± 0.07
	dry	0.70 ± 0.08	0.27 ± 0.04
Intestine	wet	6.28 ± 0.42	3.49 ± 0.51
	dry	2.52 ± 0.38	2.36 ± 0.27
Liver	wet	5.41 ± 0.36	4.55 ± 0.40
	dry	4.23 ± 0.70	3.41 ± 0.40
Pancreas	wet	0.79 ± 0.07	0.16 ± 0.07
	dry	0.30 ± 0.05	0.13 ± 0.08
Heart	wet	0.29 ± 0.04	0.42 ± 0.03
	dry	0.11 ± 0.03	0.34 ± 0.02
Kidneys	wet	2.36 ± 0.08	1.89 ± 0.16
	dry	0.95 ± 0.08	0.76 ± 0.11
Lungs	wet	0.88 ± 0.10	0.79 ± 0.16
	dry	0.99 ± 0.06	0.50 ± 0.07

Effect of Cry protein on total protein of Liver & Kidney

Protein is an essential macronutrient that plays an important role in the growth and maintenance of the human body. It is also one of the energy-giving components of the diet, along with carbohydrates and lipids.

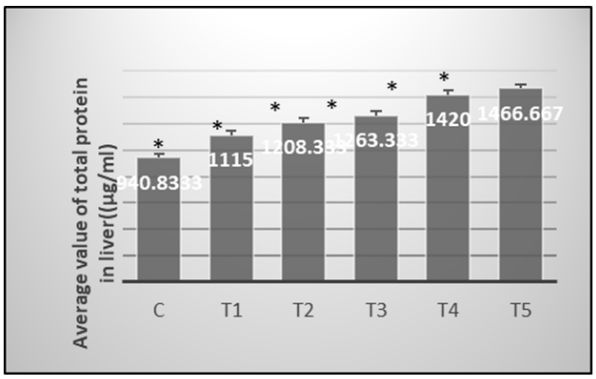

Figure 1 Graphical representation of changes of total protein in liver (*30.211)

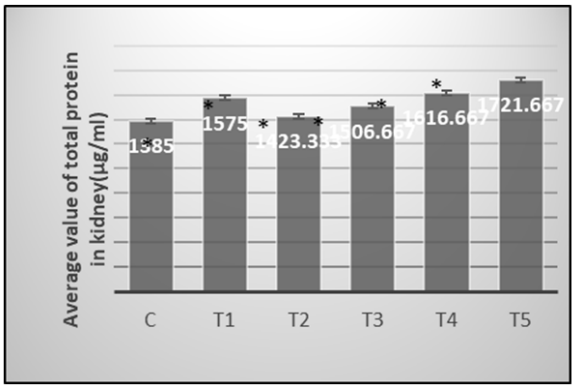

Figure 2 Graphical representation of changes of total protein in kidney (*19.59843)

Furthermore, proteins perform a variety of substitute functions such as enzymatic activity, nutrient transport, and other biochemical activities throughout the cell (1, 2, and 3).Total protein was estimated in tissue homogenate by Lowry method (4). A steeper increase in protein concentration of the liver tissue homogenates were observed along with negligible effect incase of kidney tissue homogenates.

Effect of Cry protein on bilirubin

Bilirubin is a synthetic part inside red platelets (RBCs). It's anything but a yellowish-earthy colored shading that adds to the shade of pee and stool. Undeniable degrees of bilirubin can cause yellowing of the skin and eyes. Raised bilirubin is destructive to the body, and it is anything but a sign of a few genuine ailments. The bilirubin content have been measured by Diazoreagent (5, 6, 7, 8, 9). As a result, it was observed that bilirubin levels significantly decreased in the treated rats.

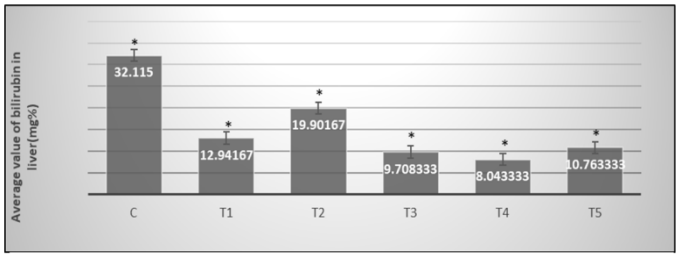

Figure 3 Graphical representation of changes ofbilirubin in liver (*1.409016)

Effect of Cry protein on SGOT & SGPT of Liver & Kidney

Aminotransferase are a group of enzyme that catalyse the transfer of an amino group from on α-axo acid. This is an important step in the metabolism of amino acids.

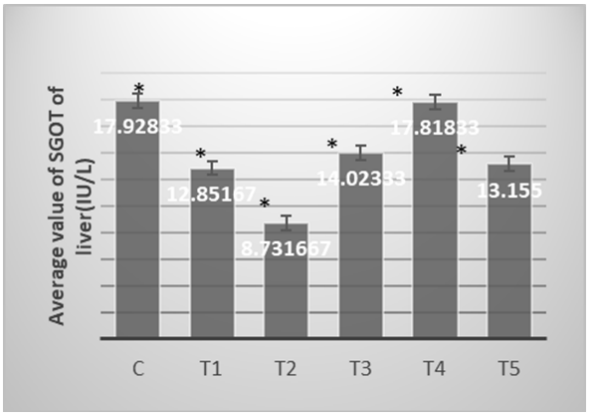

Figure 4 Graphical representation of changes of SGOT in liver (*0.53898)

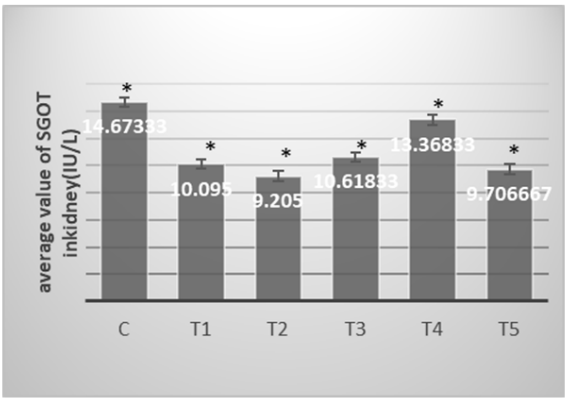

Figure 5 Graphical representation of changes ofSGOT in kidney (*0.361185)

All naturally occurring α-amino acid can take part in such reactions. Aminotransferase require pyridoxal -5'- phosphate as a cofactor. Both these specific serum enzymes are of diagnostic significance and arise from different tissue. Heart, liver, skeletal muscle and kidney are rich sources of AST (in that order) whereas ALT is found in high concentration in liver (10, 11, 12, 13). Here both the tissue homogenates if liver and kidney tissues shows a decrease SGOT and SGPT level respectively.

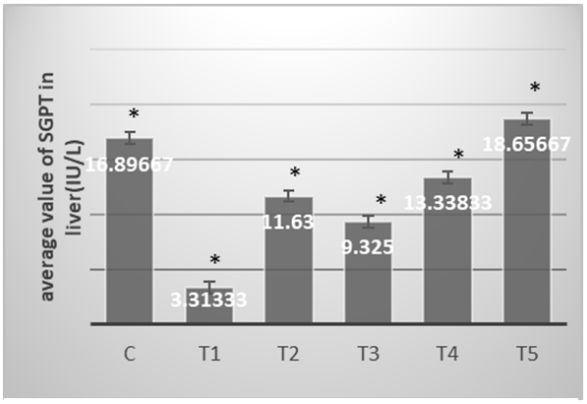

Figure 6 Graphical representation of changes of SGPT inliver (*0.527127)

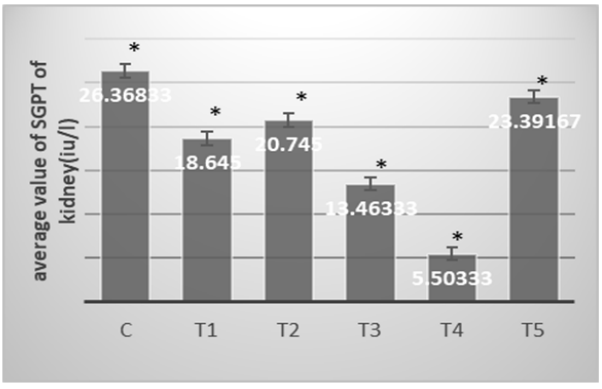

Figure 7 Graphical representation of changes of SGPT in kidney (*0.746713)

Effect of Cry protein on Catalase activity of Liver & Kidney

The catalase enzyme serves to neutralize the bactericidal effects of hydrogen peroxide (17). Catalase expedites the breakdown of hydrogen peroxide (H_2O_2) into water and oxygen ($2H_2O_2$ + Catalase → $2H_2O + O_2$). This reaction is evident by the rapid formation of bubbles (18, 19). Catalase levels raised significantly producing reactive oxygen species in both liver and kidney tissues (14, 15, and 16)

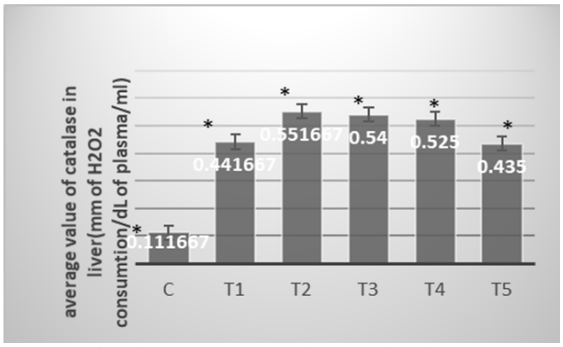

Figure 8 Graphical representation of changes of catalase activity in liver (*0.025944)

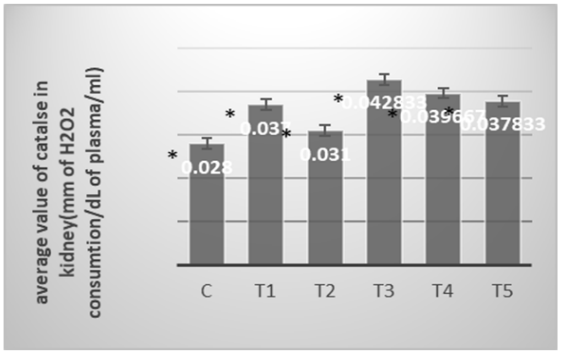

Figure 9 Graphical representation of changes of catalase activity in kidney (*0.001232)

Effect of Cry protein on ALP of Liver & Kidney

ALP blood test evaluates a gathering of chemicals found in a few portions of the body. Rises in ALP may show an issue with the liver, gallbladder, bile channels, bones, or some other organ frameworks(20,21). Here in this context the elevated level of ALP marks the presence of cirrhosis, liver cancer, bile duct obstruction etc.

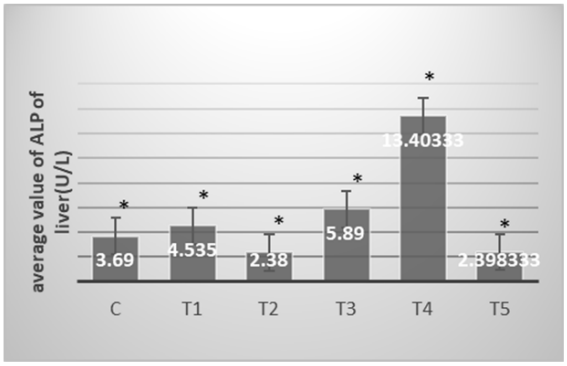

Figure 10 Graphical representation of changes of ALP in liver (*1.43398)

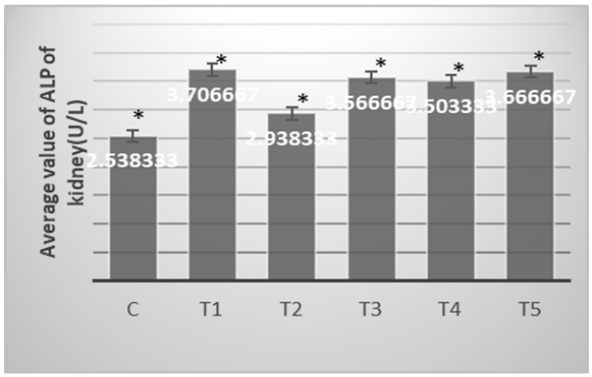

Figure 11 Graphical representation of changes of ALP in kidney (*0.10623)

Effect of Cry protein on ACP of Liver, Kidney, Stomach& Spleen

Elevated serum values of ACPhave been accepted as an indication of extraprostatic disease. But here the liver and kidney tissue homogenates showed lower levels of ACP whereas stomach and spleen showed higher levels of ACP indicating comparatively lower risk of prostate problems(22).

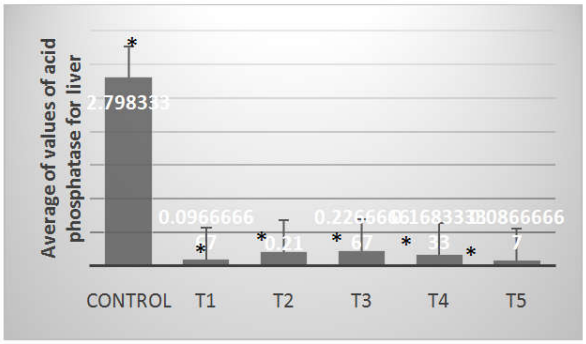

Figure 12 Graphical representation of changes of acid phosphatase levels for liver (*0.466389).

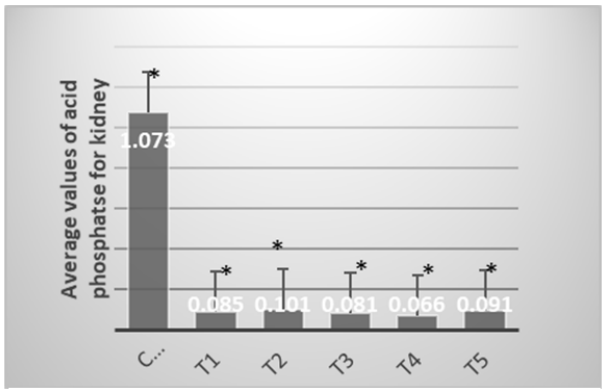

Figure 13 Graphical representation of changes of acid phosphatase levels for kidney (*0.178889).

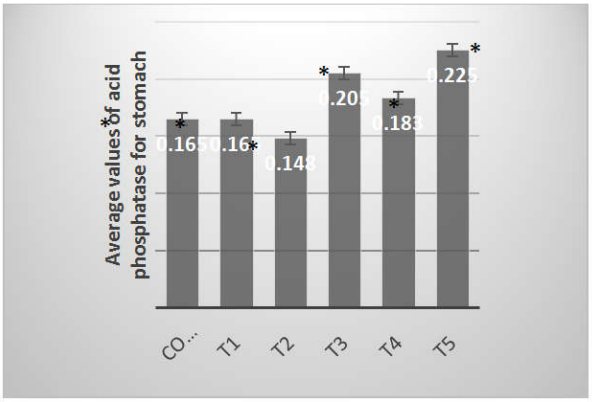

Figure 14 Graphical representation of changes of acid phosphatase levels for stomach (*0.005452).

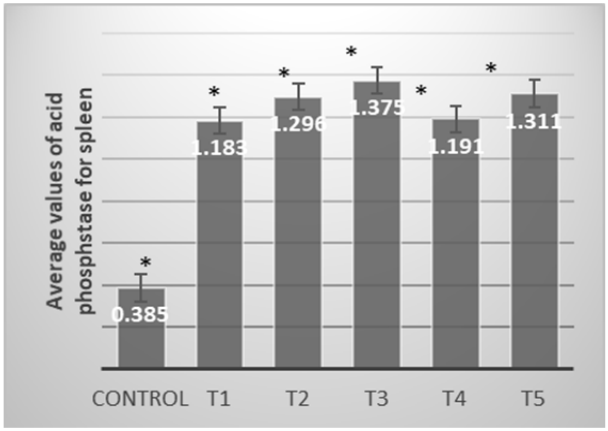

Figure 15 Graphical representation of changes of acid phosphatase levels for spleen (*0.064167).

CONCLUSION

In conclusion, the results obtained from this study can be summarised as, total protein content level uplifted significantly both in liver & kidney tissue of Bt cry protein treated rat as compared to control. Secondly, Bt cry protein does not affect bilirubin excretion as a waste material through liver tissue, as bilirubin activity is less than control group of rats. In addition to this, through biochemical analysis it was observed that SGOT & SGPT levels are decreased significantly for both liver & kidney tissue. Whereas catalase activity increased significantly thus protecting cells preferably from oxidative stress induced by Bt toxin. ALP level gradually increases in case of kidney tissue whereas gives insignificant result for liver tissue, and the other tissue homogenates e.g. stomach and spleen shows greater susceptibility towards the Cry protein.

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