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Effects of Cry Protein Based Diet on the Intestinal Motility and Histopathological Changes in Male Albino Rats

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Abstract

Gastrointestinal motility was assessed *in vitro* using Dale's assay on isolated rat small intestine after three months of dietary exposure to Bt Cry protein. Treated animals exhibited significantly altered intestinal contractility compared to controls, with the T3 cohort showing the most pronounced effects. Gastrointestinal motility reflects coordinated interactions among smooth muscle, enteric neurons, and extrinsic neural circuits and is essential for chyme propulsion. Evaluating motility can reveal functional changes, assess the impact of pathological states on gastrointestinal transit, and determine the therapeutic potential of prokinetic or antispasmodic agents. Chronic Bt Cry protein exposure increased intestinal motility, potentially disrupting nutrient digestion and absorption by enhancing parasympathetically driven contractions while inhibiting peristalsis, thereby predisposing to diarrhea and malabsorption. Histological examination of the stomach, small intestine, and colon—stained with hematoxylin–eosin—revealed no structural damage. However, gastric tissue from treated rats exhibited significantly elevated catalase activity relative to controls, suggesting an adaptive antioxidant response.

Keywords

Motility, Intestine, Cry, Chyme, Gastrointestinal Tract

Introduction

Cry proteins are specifically toxic to several insect orders, including Lepidoptera, Coleoptera, Hymenoptera, and Diptera, as well as to nematodes. These proteins, produced by *Bacillus thuringiensis* (Bt), comprise at least 50 subgroups with over 200 known variants. Cry proteins are defined as parasporal inclusion proteins from Bt that exhibit toxic effects on target organisms or share obvious sequence similarity with known Cry proteins (Parker & Feil, 2005).

A previous study described how Cry toxins exert their effects when activated in the alkaline pH environment of the larval digestive tract. However, the somatic structure of the mammalian gastrointestinal system does not support such activation. Despite this, the study revealed that Bt spore-crystals could induce hematotoxic effects, particularly targeting erythroid precursors. Hemolysis was observed in the erythrocyte cell lines of rats, mice, sheep, horses, and humans, suggesting that the plasma membrane of these vulnerable cells is the primary target of the toxins

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(Hofte & Whiteley, 1989).

An in vitro evaluation of gastrointestinal motility using Dale's method on small intestinal segments, following a three-month treatment with Bt Cry protein, indicated greater disruption in digestive activity among treated animals compared to the control group. Histological sections of the small intestine, large intestine, and stomach were stained with hematoxylin and eosin (H&E) and examined microscopically. Bt Cry protein did not cause visible damage to the stomach tissue; in fact, an increased catalase activity was observed in the stomach tissue of treated rats compared to controls.

This study aims to identify the histological differences in the gastrointestinal system between male albino rats treated with Bt Cry proteins and those in the control group.

Methods and Materials

Hematoxylin and eosin (H&E) are the principal stains used for visualizing cellular structures, particularly the nucleus and cytoplasmic inclusions. Harris' hematoxylin, the primary stain, contains alum, which acts as a mordant to bind the stain to nuclear material, rendering the nucleus light blue. In the presence of acid, this color shifts to red. Tissue differentiation is achieved by treating the sample with an acid solution. Eosin serves as the counterstain, imparting a pink hue to the cytoplasm and extracellular matrix.

Rats were sacrificed, and the small intestine was carefully harvested. The intestinal segments were cut into small pieces and immersed in Dale's solution. One end of each intestinal segment was secured to a hook inside a bubbler in a Dale's bath, while the other end was attached to a lever system. The bath, containing Dale's solution, was continuously aerated, and the temperature was maintained at approximately 37°C. Intestinal motility was then recorded to assess gastrointestinal function (Hukuhara & Fukuda, 1965).

Aspartate Aminotransferase (AST/SGOT): AST catalyzes the transfer of an amino group from L-aspartate to α-ketoglutarate, yielding oxaloacetate and L-glutamate. Malate dehydrogenase (MDH) reduces oxaloacetate while simultaneously oxidizing NADH to NAD^+. The rate of decrease in absorbance at 340 nm is directly proportional to AST activity. Lactate dehydrogenase (LDH) is added to minimize interference from endogenous pyruvate.

Alanine Aminotransferase (ALT/SGPT): ALT catalyzes the transfer of an amino group from L-alanine to α-ketoglutarate, forming pyruvate and L-glutamate. LDH then catalyzes the reduction of pyruvate and the oxidation of NADH to NAD^+. As with AST, the rate of absorbance decline at 340 nm indicates ALT activity.

Twenty microliters of tissue homogenate were transferred into a test tube. Two milliliters of hydrogen peroxide (H_2O_2) were added carefully without agitation, followed by the addition of 0.5 mL of phosphate-buffered saline (PBS). The optical density (OD) was measured at 240 nm to determine catalase activity.

Results and Discussion

The *in vitro* assessment of gastrointestinal motility using Dale's method on isolated small intestine segments from both control and Bt Cry protein-treated rats, following three months of dietary exposure, revealed notable alterations in intestinal movements. The treated animals, particularly those in the T3 test group, exhibited more pronounced disruptions in gastrointestinal motility compared to the control group. These disruptions indicate that Bt Cry protein exposure may influence the coordination of chyme movement along the gastrointestinal tract (GIT), which relies on the interplay between the spontaneous activity of smooth muscles and both intrinsic (enteric) and extrinsic neural circuits. In this study, distinct motility patterns were also observed among the treatment groups.

The study of gastrointestinal motility is valuable for detecting alterations in gut function, assessing the pathological effects on gastrointestinal transit, and evaluating the therapeutic potential of compounds in treating motility disorders. Histological sections of the small intestine, large intestine, and stomach were routinely stained with hematoxylin and eosin (H&E) and examined microscopically to assess morphological changes.

Both AST (SGOT) and ALT (SGPT) tests are widely used to evaluate tissue damage caused by drugs, diseases, or physical injury. Elevated levels of these enzymes in the blood typically indicate damage to liver or heart tissues. In this study, however, the enzymes were measured in stomach tissue. The SGOT values were decreased in the T1 and T2 treatment groups compared to the control but were elevated in the T3, T4, and especially T5 groups. These inconsistent findings make it difficult to draw definitive conclusions regarding SGOT activity in response to Bt Cry protein.

In contrast, Figure 11 demonstrates a decreased level of SGPT in treated rats compared to the control group. This reduction suggests that Bt Cry protein does not adversely affect stomach tissue or cause significant damage at the histological level.

Further studies are necessary to examine the effects of Bt Cry protein on small intestinal motility more thoroughly, particularly regarding the cystic structures observed post-mortem in albino rats. The results of the intestinal motility analysis are presented in Figure 1, while kymograph recordings of intestinal movements are shown in Figures 2, 3, and 5.

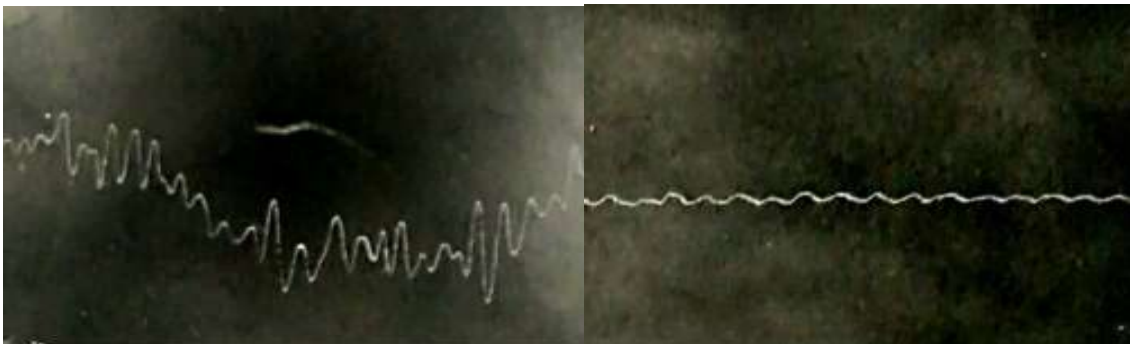

Figure 1. Visualizing the recordings from colon part. Contractions frequency and amplitude increased after administration of cry protein in diet.

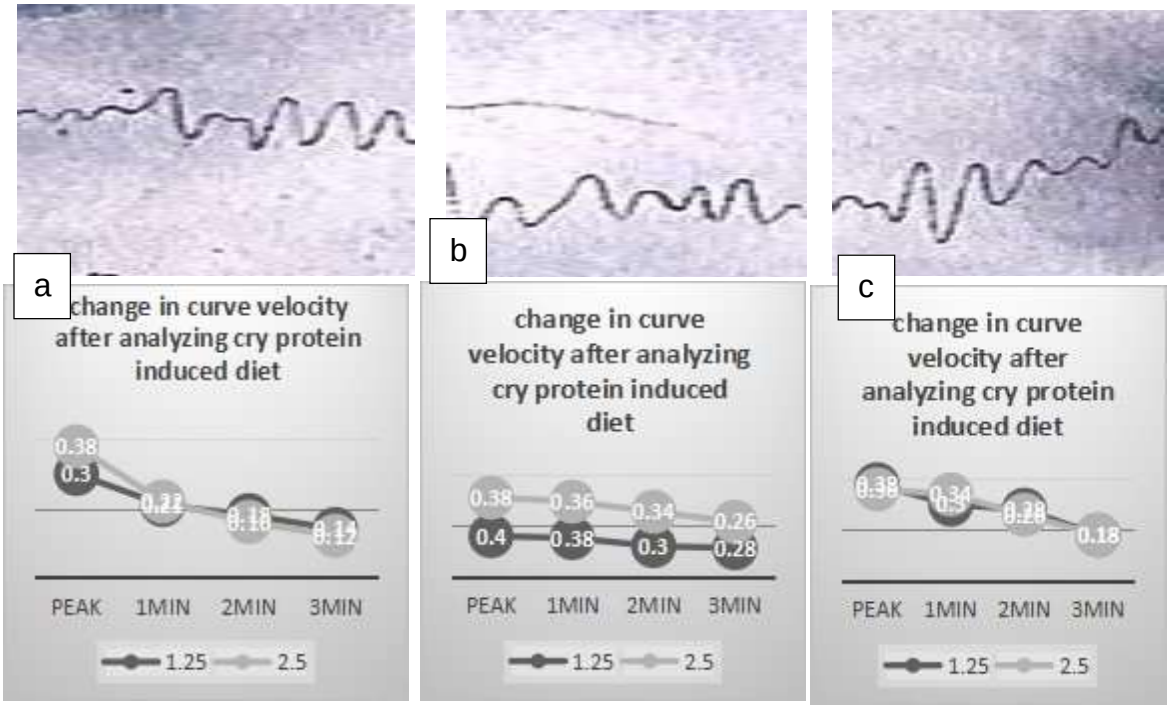

Figure 2. Different parts of colon showed different amplitudes varying with drum speed changes with the change in time. (a) Frontal part, (b) intermediate part and (c) peripheral part.

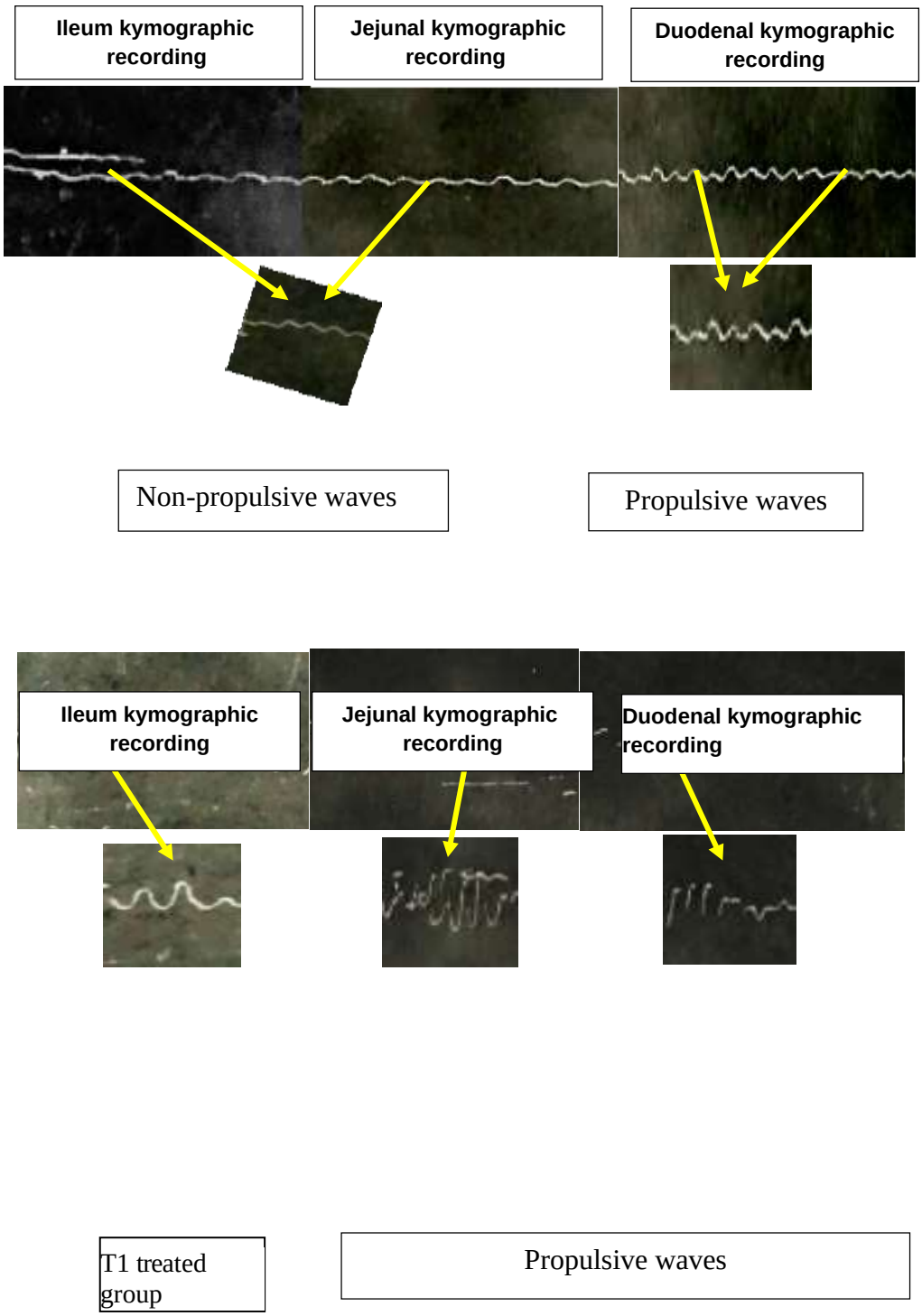

Figure 3. Kymographic recordings showing the propulsive and non- propulsive waves occurring during the experimental recordings of different parts of small intestine in Dales apparatus. The duodenal recording differs from the jejunal an ileum recording of the small intestine incase of thee propulsion reflex in case of normal recordings.

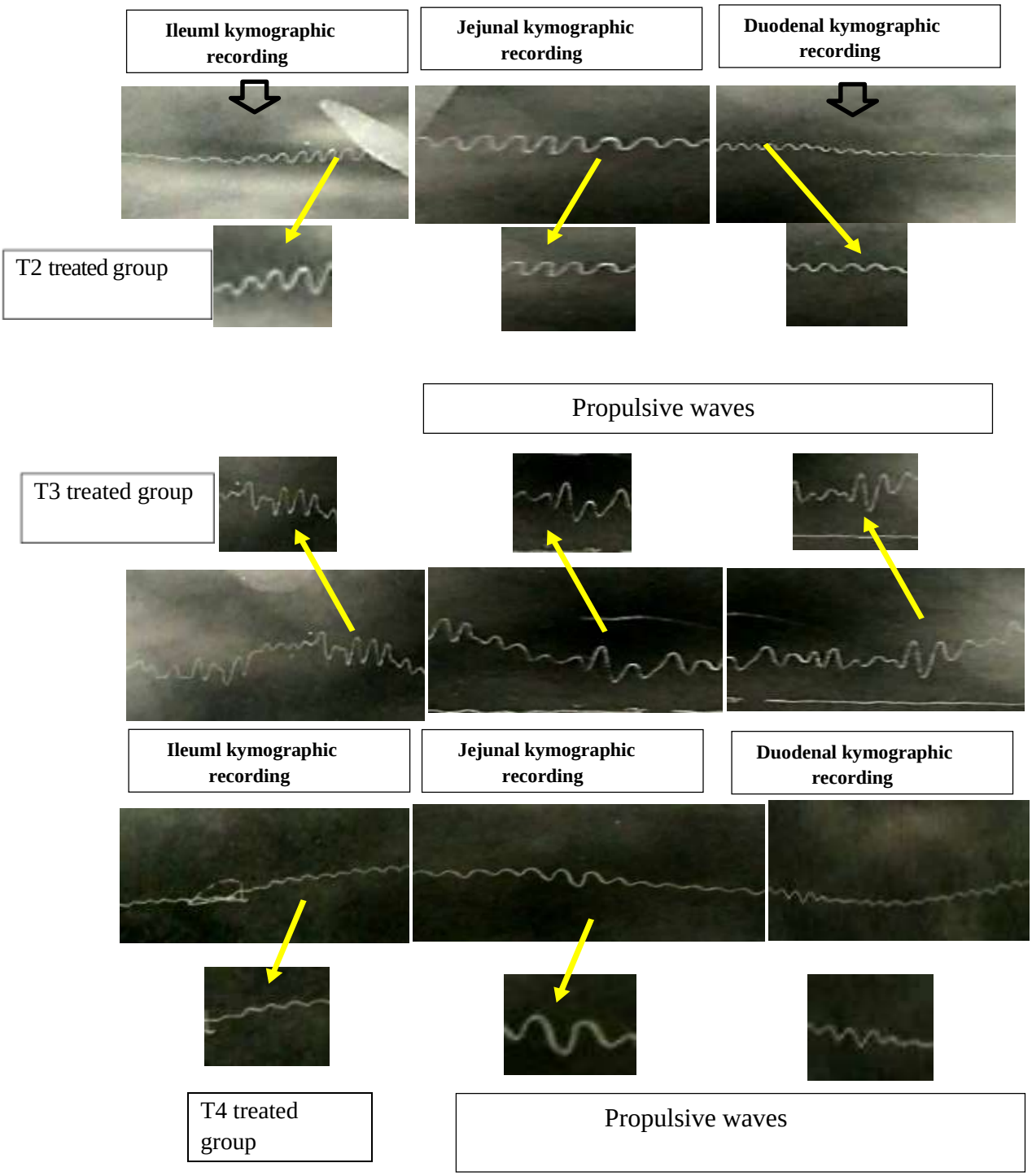

Figure 4. All the intestinal segments, including the duodenum, jejunum, and ileum, showed propulsive reflexes during the kymographic recording in the case of the group of Cry protein-treated rats.

Cry toxins bind to the cellular brush border membrane vesicle (BBMV) of mammalian

intestinal cells. It was found that Cry toxin did bind to the bovine and porcine BBMV, but far weaklier. The results of histological and histopathological changes are shown in Figures 5, 6, 7, and 8.

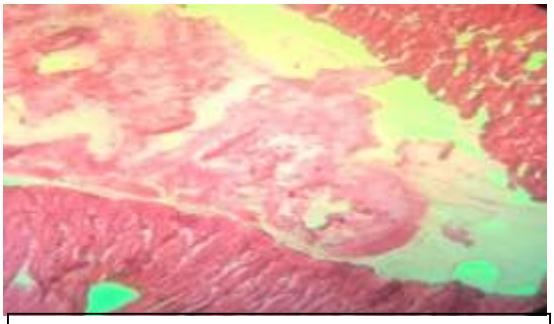

Figure 5. Histology of large intestine of the control rat.

Magnification 100X

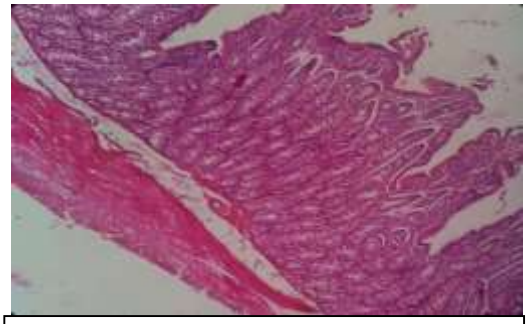

Figure 6. Denotes the treated rat's histology after feeding cry protein which shows degenerated cell membranes of the respective intestinal lineage cells along with degenerated and lobular appearance.

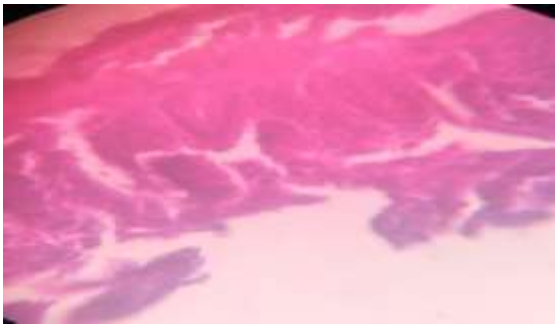

Figure 7. Histology of small intestine of the control rat.

Magnification 100X

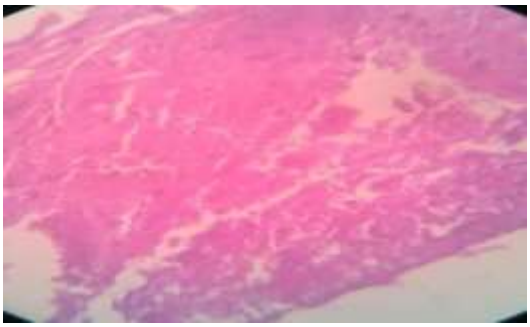

Figure 8. Denotes the treated rat's histology after feeding of cry protein which shows degenerated cell membranes of the respective intestinal lineage cells and teared appearance

The experiments on the effects on SGOT and SGPT concentration of the stomach, and catalase concentration, yield the results as shown in Figures 9, 10, and 11.

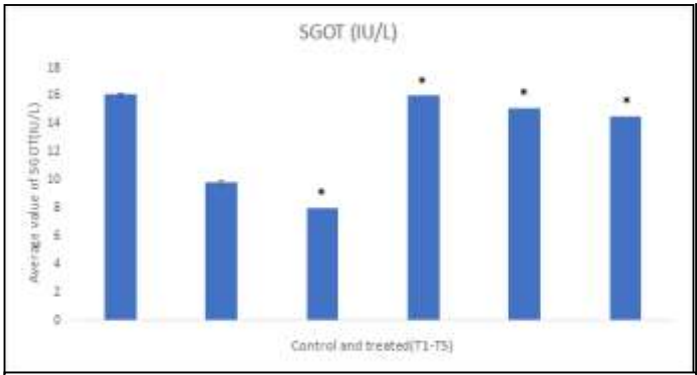

Figure 9:Diagram showing the difference between treated and untreated albino rat in case of SGOT test(IU/L)Se value=0.100047

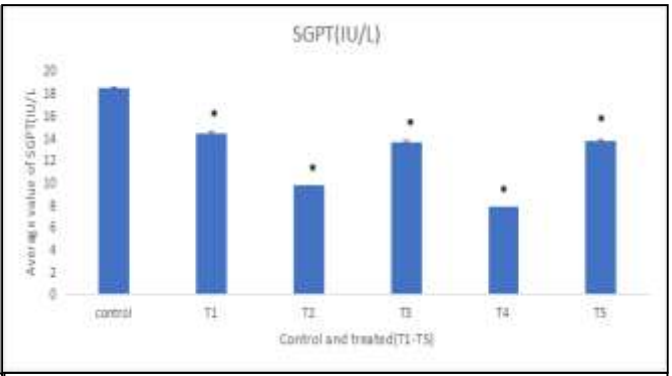

Figure 10:Diagram showing the difference between treated and untreated albino rat in case of SGPT test(IU/L)Se value=0.150918

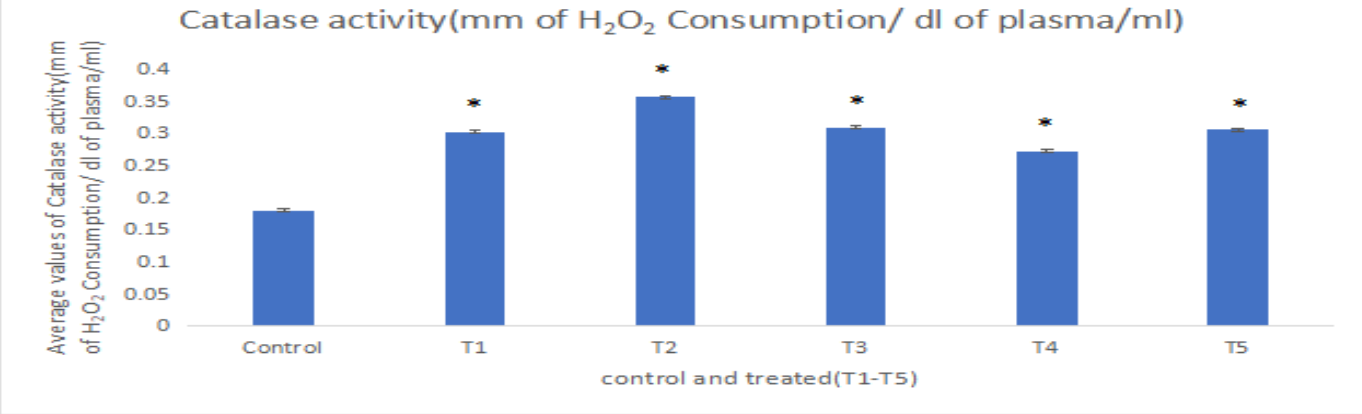

Figure 11: Diagram showing the difference between treated and untreated albino rat in case of Catalase activity of stomach tissue (mm of H2O2 Consumption/ dl of plasma/ml)

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