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Original Research Article

Gastrointestinal helminths infection of *Rattus norvegicus* (brown rats) from Pakistan

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ABSTRACT

Aim: This study was designed to study the gastrointestinal helminths in *Rattus norvegicus* to assess the relevance of rats as the carrier of zoonotic parasites for public health.

Material and Methods: A study of the effects of helminth parasites on rats' gastrointestinal tract was conducted with the help of Randox kits. *Rattus norvegicus* used in the study were trapped from different locations in Pakistan and then maintained and raised in live traps under natural temperature. The rats received a daily ration of barley and water. Gastrointestinal tract organs were separated from the body cavity of dissected rats (intestine, stomach, pancreas, liver, lungs, and rectum). Thereafter, total protein & lipase determination was carried out from these organs.

Results: The activity of the total protein in the intestine, stomach, and lungs was increased in infected *Rattus norvegicus* as compared to the non-infected, while the activity of the total protein in the pancreas, liver, and rectum was found significantly decreased in infected rats as compared to non-infected rats. The activity of lipase in the intestine, stomach, pancreas, and lungs in infected *Rattus norvegicus* was higher as compared to the non-infected. While the activity of lipase in the liver and rectum was found lower in infected rats as compared to the non-infected rats.

Conclusion: It was observed that helminth infection increased the activity of lipase in the intestine, stomach, pancreas, and lungs; while the decreased activity of lipase was observed in the liver and rectum.

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INTRODUCTION

Among mammals, the order "Rodentia" has many species, diversely known as the feeble folks, and characterized by their size and strength. Rats and mice are commensals of man and are found all over the world except "Antarctica" (Merritt, 2010). Family Muridae includes rats and mice; the most common and most important murid rodent is *Rattus norvegicus*, the Brown rat, Norway rat, and Sewer rat.

Brown rats are hazardous to human and animal health. Brown rats play important role in spreading host to a plethora of diseases, among which plague and typhus are important. Bacteria are present in the salvia that causes rat-bite fever. They also destroy electrical wires in buildings. Brown rats are pathogens that are responsible for many serious diseases, such as Hantavirus, pulmonary syndrome, rat-bite fever, Q fever, Weil's disease, and cryptosporidiosis. In America brown rats are responsible for Q fever and Toxoplasmosis. Q fever is caused by *Coxiella burnetii* and Toxoplasmosis is caused by *Toxoplasma gondii*, the parasite that causes Toxoplasmosis. Toxoplasmosis is transferred from rats to humans when cats prey on infected sewer rats. *Trichinella pseudospiralis* a parasite is pathogenic in humans and carried by brown rats. This rat is sometimes a major reservoir of bubonic plague, *Yersinia pestis* found in a few endemic species of rodents such as ground squirrels, black rats, and wood rats. *Yersinia pestis* is mostly transmitted zoonotically by rat fleas. Brown rats may have through non-infected rodent species and also the dog, cats, and humans (Barbieri *et al.,* 2020).

Several scientists have researched helminths found in rats, and the physiology of the host (Von Brand, 2013). Goodchild and Vilar-Alvarez (1962) reported *Hymenolepis diminuta* in surgically altered hosts. Bogitsch (1966) reported the histochemical observations on *Hymenolepis microstoma* reaction in the bile duct of the mouse. Havivi *et al.* (1968) studied histology and chemical composition of the small bowel of hypophysectomized rats. Arme and Read (1970) reported a surface enzyme present on *Hymenolepis diminuta*. Ruff and Read (1973) studied the inhibition of pancreatic lipase by *Hymenolepis diminuta*. Stewart and Read (1973) reported deoxyribonucleic acid metabolism in mouse trichinosis. Siddiqui and Podesta (1985) studied subcellular fractionation of *Hymenolepis diminuta* with special reference to the localization of marker enzymes. Bagheri *et al.* (1986) studied the muscle fiber selectivity of *Trichinella pseudospiralis*. Dunn and Wright (1987) reported the response of the intestinal epithelium in B10, mice to infection with *Trichinella spiralis*. Broaddus *et al.* (1987) reported suppression of *Nippostrongylus brasilienis* (Nematoda) caused lysophospholipase activity and peripheral eosinophilia by *Eimeria nieschulzi* (Apicomplexa). Bortoletti *et al.* (1989) studied the kinetics of mast cells, eosinophils, and phospholipase B activity in the spontaneous–cure response of two strains of mice (fast and low effectors) to *Hymenolepis nana*. Arizono *et al.* (1996) reported the lungs' granulomatous reaction caused by infection with the intestinal nematode, *Nippostrongylus brasiliensis* is suppressed in mast cell-deficient Ws/Ws rats.

Webb and Xue (1998) reported a comparison between spatial and temporal concentrations of choline in the gut contents of the small intestine of non-infected and infected rats with *Hymenolepis diminuta*. Kim *et al.* (2006) reported cysteinyl leukotrienes to regulate Th2 cell – Dependent pulmonary inflammation. Suzuki *et al.* (2008) reported the effectors' reaction for the gastrointestinal nematode parasites, *Trichinella spiralis* expulsion in rats. Lagapa *et al.* (2008) observed the immunohistochemical specialization of cellular proliferation in small intestinal hyperplasia of rats with hepatic *Strobilocercus fasciocularis* disease spread by parasites. Yu *et al.* (2009) studied the Th17: A new participant in gut dysfunction in mice disease spread with *Trichinella spiralis* mediators of inflammation. Kosik-Bagacka *et al.* (2009) observed *Hymenolepis diminuta* effect of disease spread by parasites onion transport in the colon and hematological parameters of rats. Takedu *et al.* (2010) studied the direct effects of IL-4/IL-13 and the nematode *Nippostrongylus brasiliensis* on intestinal epithelial cells *in vitro*. Similar studies were conducted by Coakley *et al.* (2016). Zaph *et al.* (2014) and Zvinorova *et al.* (2016).

Rats are widely distributed and thus are associated with disease organisms. These include hemiparasites, protozoans, cestodes and nematodes, and trematodes borne diseases. The present investigation deals with helminth parasites (cestodes, cestodes larva, and nematodes) present in *Rattus norvegicus* in a different area of Pakistan, and determination of physiological changes in infected and noninfected organs i.e., intestine stomach, pancreas, lungs, rectum, and liver of rats.

MATERIALS AND METHODS

Rattus norvegicus used in the study were trapped from different locations in Pakistan, then maintained and raised in live traps under natural temperature, and brought to the Abdul Wali Khan University and Kohat University of Science & Technology, Pakistan for performing laboratory work. The rats received a daily ration of barley and were given water. In the present study physiology of rats' gastrointestinal tract (intestine, stomach, pancreas, liver, lungs, and rectum) was carried out with the help of Randox kits to study of effects of some helminth parasites (cestodes, nematodes, and cysticercus larva) on rat's gastrointestinal tract. Intestine, stomach, pancreas, liver, lungs, and rectum samples of six infected *Rattus norvegicus* and of six noninfected *Rattus norvegicus* were selected for the estimation of total protein and lipase. Virtually many organs of rats were found infected by many species of helminth parasites. Endoparasites were obtained by postmortem observations of the host. The steps involved were the exposure of the internal organs. Removal and separation of the organs. Study of body cavity and study of the organs. Infected samples with helminth parasites cestodes, nematodes, and cestodes larva (*Hymenolipis dimunita*, *Physaloptera* spp., and *Cysticercus* larva) were identified and preserved. Infected and noninfected organs including intestines, stomach, pancreas, liver lungs and rectum were separated and extracted. Total protein and lipase determination were carried out from these organs. Homogenate preparation was done as suggested by (Akhtar *et al.*, 2006) and (Nisar *et al.*, 2012).

For the biochemical analysis specimens (intestine, stomach, pancreas, liver, lungs, and rectum) weighing about 50 mg, were crushed in 2 ml of distilled water with a mortar and pestle. These were homogenized in Teflon Pyrex tissue grinder for 5 minutes at 1000 rpm. The homogenates were centrifuged at 5300 rpm for 10 minutes in labofuge 15000 supernatants were taken in a separate tube and were used for biochemical estimation, during estimation the homogenate and reaction mixture was kept in ice at 5°C approximately. This homogenate was used for the estimation of total protein and lipase. For the estimation of total protein contents, the colorimetric technique was used which is based on by biuret method Kingsley (1939) and Reinhold (1953). Three test tubes were taken and labeled as the sample, standard, and reagent blank. Then 0.02 ml sample, 0.02 ml standard, and in the same way 0.02 ml distilled water was taken in tests tube marked as reagent blank then 1.0 ml of biuret reagent added in each tube. Mixed and read the initial absorbance and start the timer simultaneously. The tubes were incubated for 30 minutes at 20-25°C. Then measure the absorbance of the sample against the reagent black at 546 nm on Shimadzu spectrophotometer UV-120. For the estimation of total protein concentration following formula was used. Tot. Prot. Conc. = 19 × A Sample (g/dL). Estimation of Lipase: The activity of lipase was determined by the turbidimetric technique which is based on the method Lott *et al.* (1986). For the estimation set, 2 test tubes were taken and marked as standard samples. In each test tube

1.0 ml of reagent was taken and 0.04 ml of the standard was added to the tube marked as the sample. After 4 minutes the first reading (A1) was noted and after 5 minutes second reading (A2) was noted against air blank at 340 nm on Shimadzu spectrophotometer UV-120. Finally, these values were kept in the following formula to calculate the lipase activity.

Statistical analysis

The data obtained from different investigations and determinations were analyzed by Analysis of Variance Technique (ANOVA) and Generalized Linear Model (1988) to find the significant difference between total protein and lipase activity were measured in six infected and six noninfected samples of *Rattus norvegicus* according to Walpole et al. (1998) and Rayan et al. (1982).

RESULTS

Based on our results helminth parasites directly affect the concentration (Table 1-4) of total protein, and lipase. The activity of the total protein in the intestine, stomach, and lungs was increased in infected *Rattus norvegicus* as compared to the noninfected *Rattus norvegicus*, while the activity of the total protein in the pancreas, liver, and rectum was found significantly decreased in infected rats as compared to non-infected rats. The activity of lipase in the intestine, stomach, pancreas, and lungs in infected *Rattus norvegicus* was higher as compared to the noninfected *Rattus norvegicus*. While the activity

of lipase in the liver and rectum was found lower in infected rats as compared to the noninfected rats. The concentration of total protein in the intestine, stomach, pancreas, liver, lungs, and rectum of noninfected rat samples (7, 8, 9, 10, 11, and 12). The estimated concentration of total protein in the intestine was 3.6 g/dL, the stomach was 3.44 g/dL, the pancreas was 3.1 g/dL, the liver was 4.5 g/dL, lungs were 3.27 g/dL and the rectum was 3.8 g/dL. While the estimated concentration of total protein in infected samples (1, 2, 3, 4,5, and 6) intestine was 6.1 g/dL, the stomach was 5.42 g/dL, the pancreas was 1.33 g/dL, the liver was 1.935 g/dL, lungs were 4.8 g/dL and rectum was 2.27 g/dL. In infected samples, total protein was found to increase in the intestine, stomach, and lungs and decreased in the pancreas, liver, and rectum as compared to the noninfected samples of rats. Infestation of cestodes, nematodes, and cysticercus larval parasites affect the concentration of total protein in rat organs. The concentration of lipase in the intestine, stomach, pancreas, liver, lungs, and rectum of noninfected rat samples (7, 8, 9, 10, 11, and 12). The estimated concentration of lipase in the intestine was 1047.33 U/L, the stomach was 760 U/L, the pancreas was 1382.6 U/L, the liver was 1650 U/L, and the lungs were 806.66 U/L, and rectum was 868 U/L. While in infected samples (1, 2, 3, 4, 5, 6, and 7) of rats, the estimated concentration of lipase in the intestine was 1736 U/L, the stomach was 1984 U/L, the pancreas was 1991 U/L, the liver was 940 U/L, lungs were 1628 U/L and rectum was 553.33 U/L. In infected samples, lipase was found to increase in the intestine, stomach, pancreas, and lungs and decreased in the liver and rectum as compared to the non-infected samples. So the presence of cestodes, nematodes, and cysticercus larva in rats affected the level of lipase.

Table 1. Activity of total protein in organs of noninfected *Rattus norvegicus*.

S. No.	Name of Organs	Mean of Enzyme unit (g/dL)	S.D.	S.E	Range = mean ± S.E × 95% confidence unit
1	Intestine	3.6	0.84	0.35	4.296–2.924
2	Stomach	3.44	2.3	0.98	5.53–1.638
3	Pancreas	3.1	0.608	0.25	3.6–2.6
4	Liver	4.5	3.11	1.29	7.06–2.063
5	Lungs	3.27	1.07	0.44	4.31–2.4
6	Rectum	3.8	0.636	0.265	4.3–3.361

Table 2. Activity of total protein in organs of infected *Rattus norvegicus*.

S. No.	Name of Organs	Mean of Enzyme unit (g/dL)	S.D.	S.E.	Range = mean ± S.E × 95% confidence unit
1	Intestine	6.1	2.213	0.922	8.4–4.80
2	Stomach	5.4	3.1	1.2	7.7–3.04
3	Pancreas	1.33	0.45	0.188	1.69–0.98
4	Liver	1.93	0.54	0.22	2.37–1.49
5	Lungs	4.8	1.7	0.69	6.1–3.4
6	Rectum	2.27	1.248	0.52	3.2–1.2

Statistical analysis activity in infected and noninfected samples of *Rattus norvegicus*

Activity of total protein: Comparison of mean of enzyme unit g/dL total protein activity in intestine in infected and noninfected samples is shown in Fig. 1. Mean of enzyme unit g/dL of total protein activity in intestine was found significantly different in the noninfected and infected samples ($t = 1.94$, $p < 0.05$). Comparison of mean of enzyme unit g/dL total protein activity in stomach in noninfected and infected samples is shown in Fig. 2. Mean of enzyme unit g/dL of total protein activity in stomach was found significantly different in normal and noninfected samples ($t = 1.94$, $p < 0.05$). Comparison of mean of enzyme unit g/dL of total protein activity in pancreas in infected and normal samples is shown in Fig. 3. Mean of enzyme unit g/dL of total protein activity in pancreas was found significantly different in the infected and normal samples ($t = 1.89$, $p < 0.05$).

Comparison of mean of enzyme unit g/dL of total protein activity in liver in the normal and infected samples is shown in Fig. 4. Mean of enzyme unit g/dL of total protein activity in liver was found significantly different in infected and noninfected samples ($t = 1.94$, $p < 0.05$).

Table 3. Activity of lipase in organs of noninfected *Rattus norvegicus*.

S. No.	Name of Organs	Mean of Enzyme unit (g/dL)	S.D.	S.E	Range = mean± S.E × 95% confidence unit
1	Intestine	1047.3	81.5	33.4	1112.8–981.81
2	Stomach	760	341.36	139.9	1034.2–485.4
3	Pancreas	1382.66	489.2	200.5	1810.2–989.64
4	Liver	1650	511.2	209.5	2060.6–1239.36
5	Lungs	806.6	237.39	97.29	997.36–615.97
6	Rectum	868	303.73	124.48	1111.98–624.01

Table 4. Activity of lipase in organs of infected *Rattus norvegicus*.

S. No.	Name of Organs	Mean of Enzyme unit (g/dL)	S.D.	S.E.	Range = mean± S.E × 95% confidence unit
1	Intestine	1736	768.399	3.14.89	2353.19–1118.8
2	Stomach	1984	1385.253	567.72	3096.74–871.26
3	Pancreas	1991	611.3	250.5	2482.7–1500.68
4	Liver	940	777.64	318.07	1564.6–315.3
5	Lungs	1628	972.6	398.64	2409.3–846.66
6	Rectum	553.3	209.1	85.7	721.3–385.3

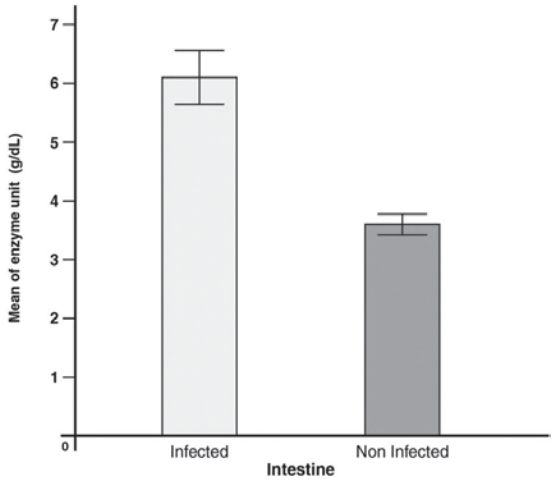

Fig. 1: Activity of total protein in intestine.

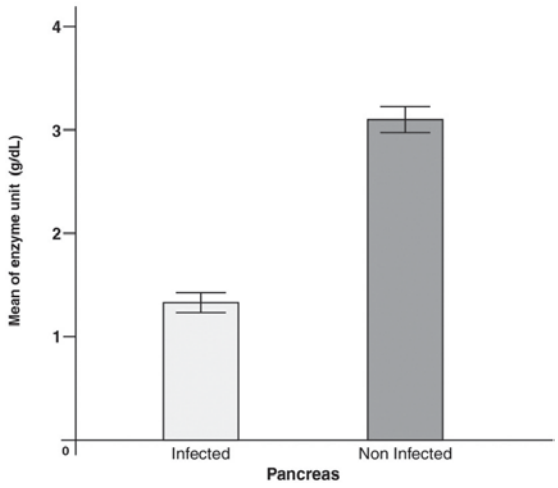

Fig. 3: Activity of total protein in pancreas.

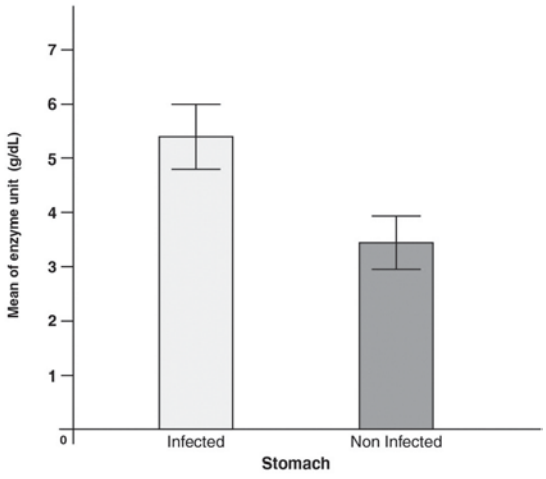

Fig. 2: Activity of total protein in stomach.

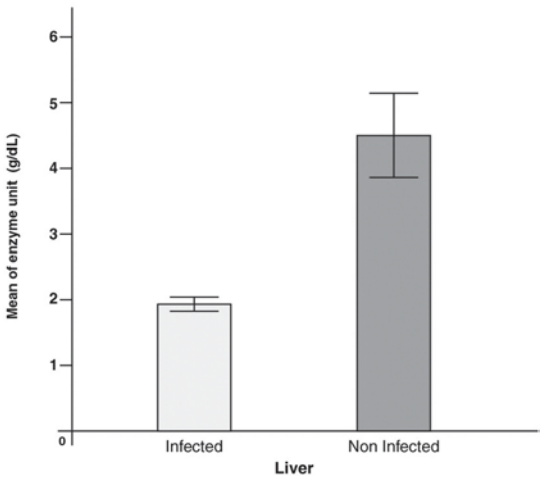

Fig. 4: Activity of total protein in liver.

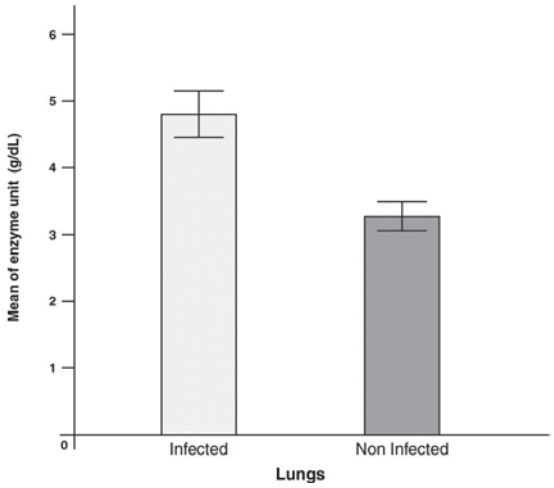

Fig. 5: Activity of total protein in lungs.

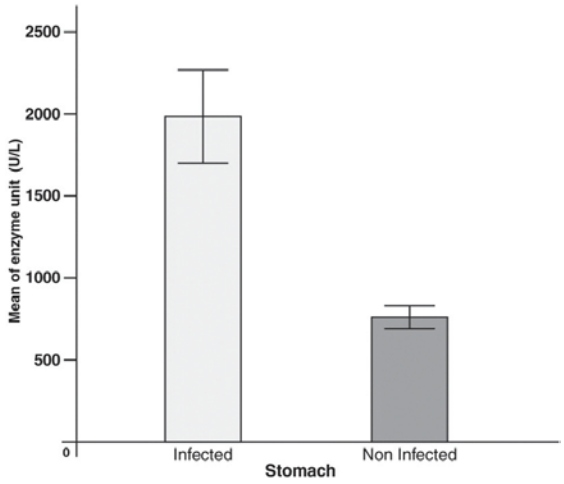

Fig. 8: Activity of lipase in stomach.

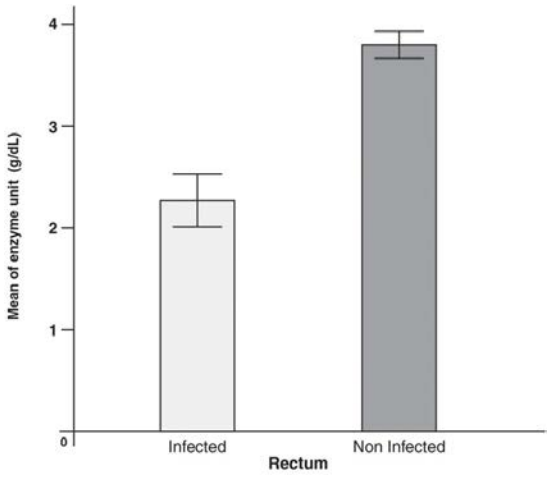

Fig. 6: Activity of total protein in rectum.

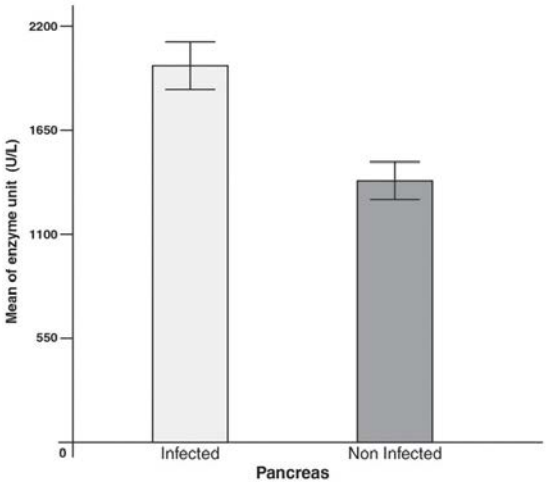

Fig. 9: Activity of lipase in pancreas.

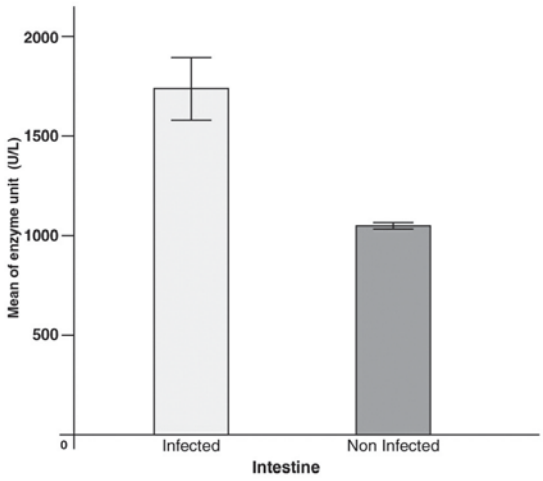

Fig. 7: Activity of lipase in intestine.

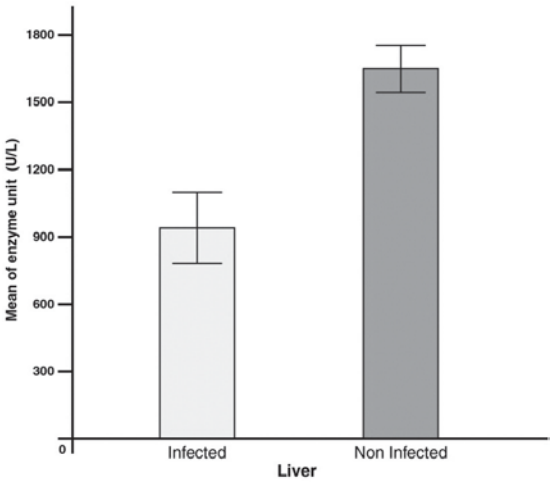

Fig. 10: Activity of lipase in liver.

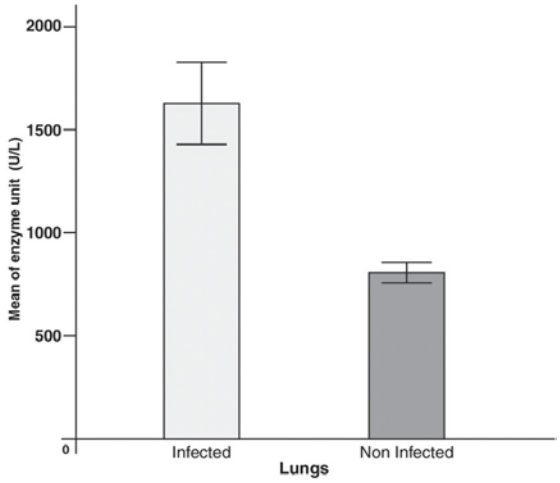

Fig. 11. Activity of lipase in lungs.

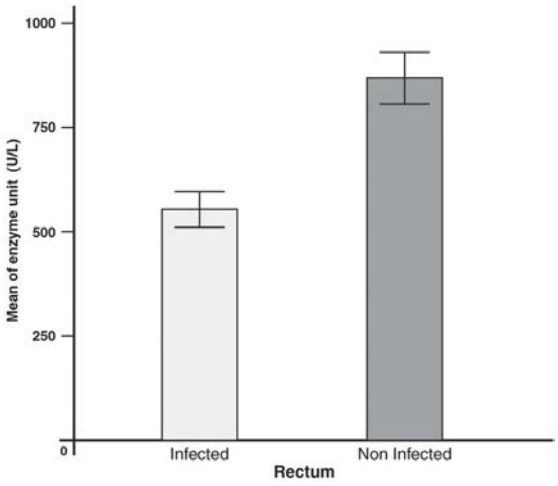

Fig. 12. Activity of lipase in rectum.

Comparison of mean of enzyme unit g/ dL of total protein activity in lungs in noninfected and helminth infected samples are shown in Fig. 5. Mean of enzyme unit g/dL of total protein activity in lungs was found significantly different in infected and noninfected samples ($t = 1.85$, $p < 0.05$). Comparison of mean of enzyme g/dL of total protein activity in rectum in the normal and infected samples is shown in Fig. 6. Mean of enzyme unit g/dL of total protein activity in rectum was found significantly different between infected and noninfected samples ($t = 1.89$, $p < 0.05$). Activity of lipase: Comparison of mean of enzyme unit U/L lipase activity in intestine in infected and noninfected samples are shown in Fig. 7. Mean of enzyme unit U/L lipase activity in intestine was found significantly different as against to the noninfected and infected samples ($t = 1.94$, $p < 0.05$). Comparison of mean of enzyme unit U/L lipase activity in stomach to the noninfected and infected samples is shown in shown in Fig. 8. Mean of enzyme unit U/L lipase activity in stomach was found significantly different between infected and noninfected samples ($t = 1.85$, $p < 0.05$). Comparison of mean of enzyme unit U/L lipase activity in pancreas in infected and noninfected are shown in Fig. 9. Mean of enzyme unit U/L lipase activity in pancreas was found significantly different in infected and noninfected samples ($t = 1.81$, $p < 0.05$). Comparison of mean of enzyme unit U/L lipase activity in liver as compared to the infected and noninfected samples is shown in Fig. 10. Mean of enzyme unit U/L lipase activity in liver was found significantly different as compared to infected and noninfected samples ($t = 1.78$, $p < 0.05$). Comparison of mean of enzyme unit U/L lipase activity in lungs infected and noninfected samples are shown in Fig. 11. Mean of enzyme unit U/L lipase activity in lungs were found significantly different between infected and noninfected samples ($t = 1.81$, $p < 0.05$). Comparison of mean of enzyme unit U/L lipase activity in rectum in the infected and normal samples is shown in Fig. 12. Mean of enzyme unit U/L lipase activity in rectum significantly different between infected and noninfected samples ($t = 1.85$, $p < 0.05$).

DISCUSSION

Rattus norvegicus was naturally infected with cestodes, nematodes, and cysticercus larva. In the present study the physiological changes in *Rattus norvegicus*, were examined. Yu *et al.* (2009) studied the Th17: A new participant in gut dysfunction in mice disease spread with *Trichinella spiralis* mediators of inflammation. Six infected and six noninfected samples selected the physiological changes (total protein and lipase) in *Rattus norvegicus*, were examined. Goodchild and Vilar-Alvarez (1962) reported *Hymenolepis diminuta* in surgically altered hosts. The *Rattus norvegicus* is infected with cestodes, nematodes, and cysticercus larva of cestodes. In this study, it was observed helminth infection affected the total protein level in different organs (intestine, stomach, pancreas, liver, lungs, and rectum) of infected *Rattus norvegicus* as compared to the noninfected *Rattus norvegicus*. Suzuki *et al.* (2008) reported the effectors' reaction for the gastrointestinal nematode parasites, *Trichinella spiralis* expulsion in rats. In some organs total protein level was found higher level and in some organs, it was found lower level. The result showed helminth infections evaluated the total protein level in the intestine, stomach, and lungs, and decreased the total protein level in the pancreas, liver, and rectum were observed.

Webb and Xue (1998) reported a comparison between spatial and temporal concentrations of choline in the gut contents of the small intestine of noninfected and infected rats with *Hymenolepis diminuta*. The brown

rats were infected with cestodes, nematodes, and cysticercus larva of cestodes. Suzuki *et al.* (2008) reported the effectors' reaction for the gastrointestinal nematodes parasites. The infection has been found to cause alteration in lipase enzyme in infected *Rattus norvegicus* as compared to the noninfected *Rattus norvegicus*. Ruff and Read (1973) studied the inhibition of pancreatic lipase by *Hymenolepis diminuta*. In this study, it was observed helminth infection produced different effects on lipase enzymes in different organs (intestine, stomach, pancreas, liver, lungs, and rectum). *Trichinella spiralis* expulsion in rats. Lagapa *et al.* (2008) reported an immunohistochemical analysis of cellular proliferation in small intestinal hyperplasia of rats with hepatic *Strobilocercus fasciocularis* disease spread by parasites. Also worked on helminth infection for the immune system as Coakley *et al.* (2016), Zaph *et al.* (2014), and Zvinorova *et al.* (2016). Lipase activity was found higher level in some organs while in some organs it was found at a lower level.

CONCLUSION

It was observed that helminth infection increased the activity of lipase in the intestine, stomach, pancreas, and lungs; while the decreased activity of lipase was observed in the liver and rectum.

CONFLICT OF INTEREST

None declared

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