Studies on Enzymatic Activities by Gastro-Intestinal Helminthic Parasites in Channa Punctata (Snake Headed or Bloch).

J.V Navya Latha & Ramesh K. & Prof.V.Vanithadas
Dept. of Zoology, University College of Science, Osmania University, Hyderabad.

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ABSTRACT: *The aim of this study to biochemically investigate the conventional pathway of carbohydrate metabolism occurs in both in aerobic and anaerobic conditions. In aerobic process glycogen is broken into release energy through a series of reactions. Aerobic segments includes oxidation of pyruvate to acetyl CoA which is utilized in another cycle called citric acid cycle. Such utilization of reduced coenzymes results in ATP synthesis through oxidative phosphorylation (**Martin et al., 1983**).Fish like any other organism utilizes the carbohydrates to meet their energy requirements. The conversion of glycogen to glucose and the related release of energy could be affected by the infections. The gastro-intestinal tract and liver are the most common tissue affected by the parasites. Such intestinal infections might interfere with the absorption of food and might cause disorders of metabolism, liver is the seat of major metabolic activities,and any sort of infection in liver could alter the carbohydrate metabolism and reach the other organs. In anaerobic Glycolysis, through a series of enzymatic reactions, sugars are converted to lactic acid and ATP. Pyruvate which is the end product of glycolysis is converted to acetyl CoA which is oxidized in TCA cycle to release energy in aerobic conditions. Thus both the aerobic and anaerobic segments of metabolism are as important the enzymes that runs the cycles. The objective of present study is to evaluate the effect of internal helminthic parasites on the enzymatic activities like such as SDH, LDH, LPO, SOD and GPx activities of Channa punctata.*

Key Words: : *SDH, LDH, SOD, LPO, GPx, Channa punctata*

Introduction: Parasitism, a phenomenon widespread amongst animals and plants, is a type of symbiotic relationship between organisms of different species. Parasites also commonly show highly specialized adaptations allowing them to exploit host resources. The harm and benefit in parasitic interactions concern the biological fitness of the organisms.Fish is a valuable source of food that is rich in proteins.

Parasites are most important groups of organisms evolved independently in every phylum from protozoa to arthropods and higher phylum like chordates. Fish parasitology is a developing field in the aquatic science (**Muller and Anders 1986**).

All the major groups of animals parasites are found in fish and apparently healthy wild fish often carry heavy parasitic burdens, parasites which undergo a direct life cycle will be important pathogen of cultured fish, whereas parasites which undergo indirect life cycle use fish as an intermediate host **(Barbara D.Petty, DVM).**Several kinds of protozoans and metazoans(such as trematodes, cestodes, nematodes and acanthocephalans) infect fishes and lead a parasitic life as exoparasites and endoparasites.These parasites use the fish for the shelter and food and cause some pathological effects on almost each and every organ. Parasites interfere with nutrition of host disturb metabolism and lesions of alimentary canal and also damage nervous system (**Markov 1946**).

Parasitic infestation will have a harmful influence on growth rate and reproduction of Fishes

Parasites cause inflammation when imbedded and penetrating the mucous, inflammation reactions are accompanied by generation of reactive oxygen species. The oxidative damage is deleterious to the cell and may lead to senescence and cell death (A.H.Siwela, and S.Dube).The parasitic infection which lead to the formation of oxygen free radicles and other reactive oxygen species (ROS) includes oxide radicles (O_2), hydroxyl radicles (OH) and hydrogen peroxide (H_2O_2).

These reactive oxygen species (ROS) induce oxidative damage of macromolecules such as polyunsaturated fatty acids in membrane lipids, essential proteins such as nucleic acids especially DNA.The oxidative damage is deleterious to cell and may lead to senescence and cell death (**Beyer et., al 1996**). However the cell is equipped with several antioxidant defence enzymes which protect the cell from oxidant damage as long as generation of reactive oxygen species do not overwhelm the activities of the antioxidant enzyme activity. These enzymes includes SOD which converts the superoxide radicle (O_2) to hydrogen peroxide (H_2O_2),GPX and CAT which breakdown hydrogen peroxide to water ,DTD which is deemed to protect against lipid

peroxidation by the hydroxyl radical (OH) as well as against oxidation of proteins and DNA (**Ernst and Dallener, 1995**).

***Channa punctatae*(Snake Headed or Bloch):**

The snake headed fish *Channa punctata* is the representative of channiformes order, family Channidae. Habitats large freshwater ponds and tanks found in Bangladesh, Afghanistan, India, Myanmar, Nepal, Pakistan, Srilanka and China by (Talwar and Jhingran 1991).It is a carnivore, voracious and predatory to small fish and fries (Bhuiyan 1964, Rahman 1989and 2005).*Channa punctata is* one of the most delicious and demandable freshwater fishes of theCountry because of its high nutritional value, cheap cost and availability in the local Markets.

These fishes are often infected by parasites, which decrease the rate of growth as well as the reproduction rate of the host fishes, resulting in deteriorating the food value of Fishes and economic loss to the culturists. It will be mostly infected with nematodes, cestode like proteocephalus and acanthocephalans.These fishes are known as "Zeal fish".

METHODS AND METHODOLOGY:

The fish's *Channa punctata* were brought to the laboratory from local fish market Hyderabad. External examination was made by observing the colour of fish. Fish were sacrificed and dissected. Dissected fish were examined with naked eye or magnifying glass, observing the colour of gills, liver, spleen, intestine and kidney. The tissues were carefully examined for the presence of the parasites.

ISOLATION OF HELMINTH PARASITES:

The helminth parasites were detected macroscopically and microscopically. They were separated and counted. The parasites were isolated and identified as *Pallisentis punctatin*. The fish without parasites were considered as normal fish and fish with parasite was considered as infected fish. The muscle near intestine was collected for enzyme assays.

PARASITE INFESTATION:

Among observed parasites*(Pallisentis punctatin*), protozoa and monogenean were very common on the gill skin and fin of the host fish. They were found alive and active in gill filaments. They strongly attached with the gill skin or fin base. Crustacean was mostly abundant to be attached on the fin and skin. On the other hand digenean fluke and nematodes were found from intestine. Infestation was studied from the recorded parasites of host species, which indicated the general parasitological condition of host fish. In the present study, infestations were studied in snake-head, *Channa punctatus*. The mean, standard deviation and standard error were studied the parasite was mainly found on different organs like gill, skin, fin, intestine and stomach of the host.

RESULTS:

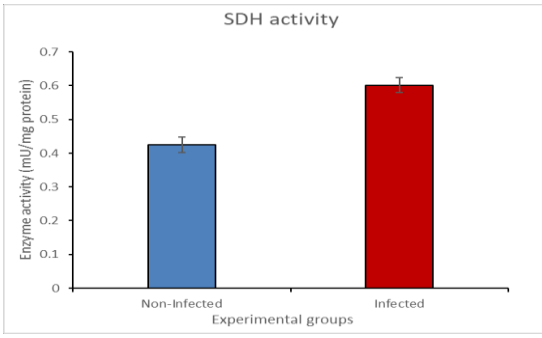
Statistical analysis: The whole data were fed into Microsoft Excel 2013, a computer programme SPSS The data were represented as mean of replicates followed by standard deviation i.e. mean ±standard deviation (SD).

TABLE-01:

Succinate dehydrogenase (SDH) level (micromoles formazan/g protein /hour formed in normal infected muscle tissue.

	NORMAL FISH	INFECTED FISH
MEAN	0.425	0.602
+/-SD	0.055	0.053
SE	0.022	0.022

All the changes are statistically (p<0.001) significant



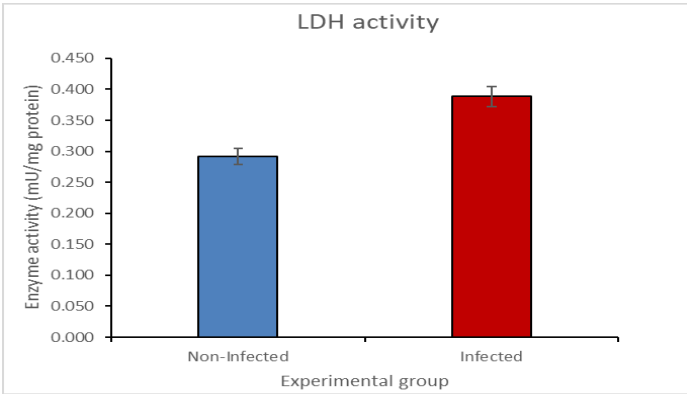
Infected fish muscle showed a marginally higher level of Succinate dehydrogenase activity than the uninfected (Normal) fish.

TABLE.02:

Lactate Dehydrogenase (LDH) Level (Micromoles Formazan/G Protein /Hour Formed In Normal Infected Muscle Tissue.

	NORMAL FISH	INFECTED FISH
MEAN	0.292	0.388
+/ - SD	0.031	0.040
SE	0.012	0.016

All the changes are significantly (p<0.001) significant



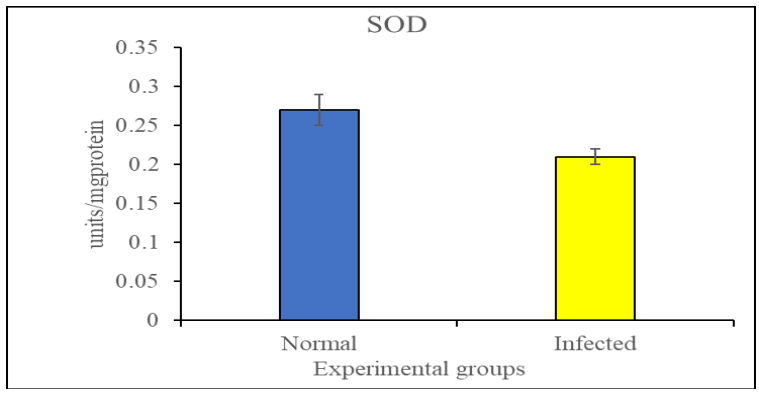
Infected fish muscle showed a marginally higher level of Lactate dehydrogenase activity than the uninfected (Normal) fish.

TABLE.03:

SOD Activity (Units/Mg Protein) in Normal and Infected Muscle Tissue

	NORMAL FISH	INFECTED FISH
MEAN	0.27	0.21
+/- SD	0.04	0.02
SE	0.02	0.01

All the changes are statistically (p<0.01) significant



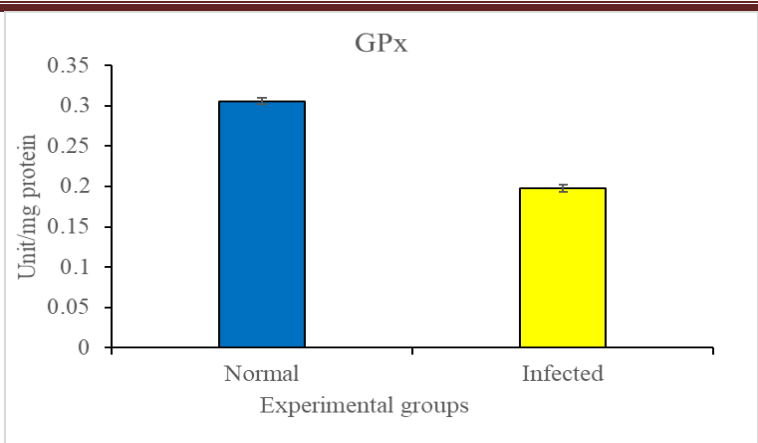
Infected fish muscle showed a decreased level of Sodium dismutase activity than the uninfected (Normal) fish.

TABLE.4:

GPx or GPX Activity (Units/Mg Protein) in Normal and Infected MuscleTissue

	NORMAL FISH	INFECTED FISH
MEAN	0.306	0.198
+/- SD	0.009	0.010
SE	0.004	0.005

The changes are statistically (p<0.001) significant

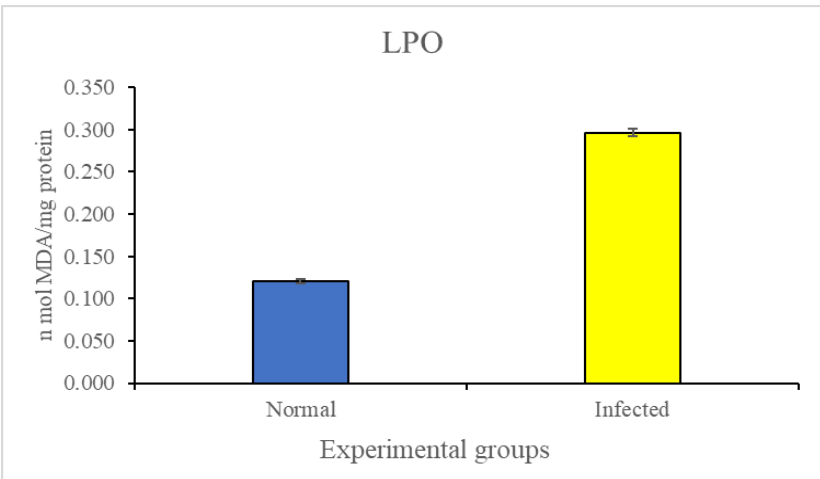


Infected fish muscle showed a decreased level of Glutathione peroxidase than the uninfected (Normal) fish.

TABLE.5: Lipidperoxidation (LPO) activity (nmoles MDA/mg protein) in normal and infected tissues

	NORMAL FISH	INFECTED FISH
MEAN	0.121	0.296
+/- SD	0.007	0.010
SE	0.003	0.005

All the changes are statistically (p<0.001) significant



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Infected fish muscle showed an increase level of Lipid peroxidation than the uninfected (Normal) fish.

SDH, LDH AND OXIDATIVE STRESS MARKERS:

In the SDH, LDH activities and LPO content significantly decreased in non-infected fish compare to the infected fish where as SDH, LDH activities and LPO content are significantly increased in infected fish compare to non-infected fish. SOD and GPx activities significantly increased in non-infected fish compare to infected fish whereas the activities of SOD and GPx significantly decreased in infected fish compare to the non-infected fish.

DISCUSSION:

The present study demonstrated a parasitic induced enzymatic changes in the host fish *Channa punctata* the parasite was localized in the fish digestive tract and was identified as ***pellisentis punctatin*** an acanthocephalan species. This species is known as a parasite of marine and fresh water fish as endoparasite invading intestine. It is characterized with that of spiny headed worm. **Rahman et al., (2017)** reported that two species of ectoparasites and six species of endoparasites were identified from Channa punctata of Belei Beel, Gazipur, Bangladesh.

It was also noticed that most of the parasites were belonged to five groups' viz., cestode, trematode, nematode, acanthocephalan Crustacea.The effect of parasite on the activities on muscle SDH, , SOD, GPx, LPO are shown in the tables and figures from 1 to 5 there was a significant differences in the activities between

infected and non-infected fish SDH, LDH, GPx and LPO were **(p<0.001)** statistically significant and SOD was **(p<0.01**) statistically significant.

SDH and LDH:
SDH oxidizes the metabolite succinate to fumarate, SDH removes electrons from succinate which reduces FAD the reducing the enzyme complex to E-$FADH_2$. The reduced co-enzyme then transfers LDH catalyses the interconversion of pyruvate and lactate with concomint interconversion of NADH and NAD+ Convert the final product of glycolysis to lactate.SDH and LDH activity was found to be more in the infected fish when compare to normal fish such increased in activity in fish was also noticed by P.Anil kumar 2010 in *Labeo rohita* and *Catla catla* and suggested that an increase in the activity supports the high energy requirement of host caused by the parasitic infection. Parasites and physiological stress due to various reasons were reported to increase the energy demand in the host system such energy demands can be met by several means; one which is active formation of ATP.
Increase in the formation of ATP can be interpolated by the increase SDH activity. Gastrointestinal parasites cause marked production losses in farm animals throughout the world (Sykes, 1994) and their control is difficult because of their resistance to anthelminthic drugs **(Prichard, 1994; Waller, 1994)**.Usually in mammals, the parasitic infections lead to the inhibition of antioxidant enzyme activities associated with the production of reactive oxygen species by macrophages at the infected site in order to eliminate the parasite. Therefore parasitic infections were often associated with an oxidative stress in the hosts.
Such response was also noticed in fish by Bello et al who suggested that the fish response to parasitic infection could involve reactive oxygen intermediates and therefore, induced an oxidative stress, but without inducing significant differences in the superoxide dismutase and catalase activities (antioxidant enzymes) between healthy and parasitized fish.
Lipid Peroxidation (LPO): In the present study there is an increased LPO activity ininfectedfishthanto normal fish. Extensive lipid peroxidation in biological membranes causes loss of fluidity, falls in membrane potential, increased permeability to H+ and other ions, and eventual rupture leading to release of cell and organelle contents. Some end products of peroxide fragmentation are also cytotoxic (reviewed by Ester Bauer et al.). A wide variety of techniques has been used to show that lipid peroxidation increases in many diseases.
Behind many of these reports is the unspoken assumption that the disease or toxin causes increased lipid peroxidation, which is then responsible for the toxicity. However, it was established many years ago that disrupted tissues undergo lipid peroxidation more quickly than healthy ones; e.g., lipid peroxides accumulate in a brain homogenate much more reasons for this increased peroxidizabilityof damaged tissues include inactivation of some antioxidants, leakage of antioxidants from the cell. The release of metal ions (especially iron and copper) from storage sites and from metalloproteins hydrolysed by enzymes released from damaged lysosomes increased lipid peroxidation can explain many of the reports of increased lipid peroxidation in disease or toxicology the cell damage and that prevention of peroxidation by antioxidants prevents the cell damage. Measurement of lipid peroxidation may therefore be an excellent marker of tissue damage.
GPx and SOD: In the present study there is decrease in GPX and SOD activity compared to normal fish. One possible explanation for the reduced activity of GPX and SOD is the sensitivity of the enzyme to inactivation after endogenous exposure to potent oxidants as well as aldehydes by-products of lipid peroxidation.

CONCLUSION:
The present study describes the changes in the enzymatic activity on fish *Channa punctata* by gastrointestinal parasite *Pallisentis punctatin*. During the study it was observed that there is an impact of parasite on fish metabolic enzymes (SDH AND LDH) and oxidative stress marker enzymes (LPO, SOD, GPX). This might be due to dependence of parasite on the host for food and shelter. The authors hope that study will promote further parasitological research on C*hanna punctata* and on other cultivated fishes.

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