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Research Article
Glutathione-S-transferase, Superoxide Dismutase (GST, SOD) Levels, Protein Content and Lipid Peroxidation in *Schizothorax plagiostomus* under the Infection of *Pomphorhynchus* in Nallah Sukhnag of Kashmir Valley

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Abstract

Background and Objective: Presence of parasites in fishes has been reported to severely damage the status of antioxidants in them. Among helminth parasites, *Pomphorhyncus* an acanthocephalan parasite is commonly found in *Schizothorax* fish of Kashmir and is causing a considerable damage to fish health. The aim of this study was to investigate the antioxidant status in muscle, intestine and liver tissues of *Schizothorax plagiostomus* parasitized by *Pomphorhyncus*. **Materials and Methods:** For this, 9 fish specimens collected from Nallah Sukhnag (Budgam) were found to be infected only with *Pomphorhyncus*, an acanthocephalan parasite. Fishes having the infection of *Pomphorhynchus* and the same number of uninfected ones were then analyzed for enzymatic antioxidants. Lipid peroxidation and protein content was also assayed for both infected and uninfected fishes. Statistically the whole data were represented as mean and standard deviation. **Results:** Results showed the reduction of glutathione-s-transferase (GST), superoxide dismutase (SOD), protein content while increase in lipid peroxidation in infected muscle, intestine and liver tissues as compared to respective organs of the uninfected fishes. **Conclusion:** This study concluded that parasitic infections induce oxidative stress.

Key words: Peroxidation, antioxidant, *Pomphorhyncus*, *Schizothorax plagiostomus*

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Infection of parasites considerably worsens the condition of fish and their protective mechanisms. Parasitic infection is known to generate reactive oxygen species (ROS). These reactive oxygen species include superoxides, hydroxide radicals, oxides of nitrogen and glutathione peroxide. They were mainly produced as a part of the immune defense against foreign microorganisms. Antioxidant system provides protection against the damage caused by the ROS[1]. Antioxidant defense system consists of enzymatic and non-enzymatic antioxidants which had been found to scavenge free radicals and reactive oxygen species (ROS). The enzymatic antioxidants mainly involved specific enzymes superoxide dismutase (SOD), catalase (CAT), glutathione-S-transferase, glutathione peroxide (GPX) and glutathione reductase (GR). Decline in antioxidants enhanced the formation of ROS[2] that results in a condition termed as oxidative stress.

Fishing is an important economical source in Kashmir due to the abundance of fresh water reservoirs and perennial rivers. The major fish fauna present in the fresh water bodies of Kashmir comprises of mainly indigenous carp (*Schizothorax* spp.) and exotic carp (*Cyprinus carpio*). Indigenous carp or *Schizothorax* sp. was represented by *S. esocinus*, *S. labiatus*, *S. curvifrons*, *S. punctatus*, *S. plagiostomus*, *S. micropogon, etc.* Oxygen depletion in the water bodies due to eutrophication has deteriorated the water quality over the years that hamper the growth of *Schizothorax* species which is a highly sensitive fish. Besides, oxygen depletion, presence of parasites in fish is a major factor in declining the production of *Schizothorax* fish. Among different types of parasites, *Pomphorhynchus* (acanthocephalan) is a common parasite localized in the intestine that caused considerable damage in *Schizothorax* fish. After the analysis of all available information on negative influence of the above-mentioned parasite on the host fish organism it seemed interesting to study the influence of this parasite on antioxidant enzymes.

MATERIALS AND METHODS

Collection and identification of fish hosts: The fish hosts were collected at different sites from Nallah Sukhnag with the help of a local fisherman from Oct, 2014-Sep, 2015. The fishes were brought alive or fresh to the Parasitology Research Laboratory Department of Zoology, University of Kashmir. The fish hosts examined during the present study was *Schizothorax plagiostomus* Heckel, 1838.

The fish hosts were first dissected and examined for helminth parasites. For this, alimentary canal of fish was stretched in normal saline and carefully opened using needles. In this study, 9 fishes were found to be infected only with *Pomphorhyncus*, an acanthocephalan parasite. These *Pomphorhyncus* infected and 9 samples of uninfected fishes were then analyzed for antioxidants. Organs of infected fishes like muscle, intestine and liver tissues were removed and homogenized in a homogenizing buffer (50 mM Tris-HCl, 1.15% KCl, pH 7.4) by using Teflon homogenizer. The homogenate was centrifuged at 10,000 rpm for 10 min. The supernatant was collected and stored at 4°C for further analysis.

Glutathione-S-transferase activity: For GST analysis, 1.67 mL of sodium phosphate buffer (0.1 M p.H 6.5) was added to the test tube containing 0.2 mL of 1 mM GSH, 0.025 mL of 1 mM CDNB and 0.1 mL of homogenate in a total volume of 2 mL. The change in absorbance was recorded at 340 nm and the enzyme activity was calculated as n moles of CDNB conjugates formed min^{-1} mg^{-1} protein using molar extinction coefficient of 9.6×10^3 M^{-1} cm^{-1} [3].

Superoxide dismutase activity: This analysis was carried out by adding 1 mL of 50 mM sodium carbonate to 0.5 mL of homogenate and mixed. After mixing, 0.4 mL of 25 µM NBT and 0.2 mL of 0.1mM EDTA were added to the mixture. The reaction was initiated by addition of 0.4 mL of 1 mM hydroxylamine-hydrochloride. The change in absorbance was recorded at 560 nm. The control was simultaneously run without tissue homogenate. Units of SOD activity was expressed as the amount of enzyme required to inhibit the reduction of NBT by 50%[4].

Protein estimation: Protein estimation was carried out by folin phenol reagent method[5], a highly sensitive method and can detect proteins as low as 5 µL mL^{-1}.

Lipid peroxidation: This was assessed by the formation of thiobarbituric acid reactive subastances. Reaction mixture consisted of 0.1 mL of homogenate and 2 mL of TBA-TCA-HCl reagent (0.37% thiobarbituric acid, 0.25N HCl and 15% TCA) placed in boiling water for 15 min, cooled at room temperature and centrifuged for 10 min. The absorbance of the clear supernatant was measured against reference blank at 535 nm[6].

Statistical analysis: The whole data were fed into Microsoft Excel 2010, a computer programme (SPSS 11.5 for windows).

The data were represented as mean of replicates followed by standard deviation i.e. mean±standard deviation (SD).

RESULTS

The effect of acanthocephalan parasite, *Pomphorhynchus* on the level of glutathione-S-transferase (GST), superoxide dismutase (SOD) and protein content in muscle, intestine and liver homogenates in *Schizothorax plagiostomus* in comparison to uninfected fish of *S. plagiostomus* is given below.

Glutathione-S-transferase levels (nmol CDNB conjugates min^{-1} mg^{-1} protein): GST activities decreased in muscle (0.0428±0.2368), intestine (0.1322±0.0941) and liver (0.1073±0.0855) tissues of infected fish as compared to uninfected fish organs (0.0502±0.0474, 0.1808±0.1684 and 0.1128±0.1077), respectively.

Superoxide dismutase levels (the amount of enzyme required to inhibit the reduction of NBT by 50%): The SOD activities observed in the muscle (0.006±0.0019), intestine (0.0518±0.0407) and liver (0.0298±0.0030) tissues were lower in infected fish as compared to respective organs (0.0174±0.0202, 0.0774±0.0474 and 0.0363±0.0286) of the uninfected fish.

Protein content (mg%): Presence of infection in the fish due to *Pomphorhyncus* caused decrease in protein content in muscle (99.2183±32.7825), intestine (101.439±22.822) and liver (104.013±23.8382) as compared to uninfected fish organs (111.762±20.6572, 106.915±22.748 and 108.6483±28.1577), respectively.

Lipid peroxidation (n moles of TBARS formed h^{-1} g^{-1} tissue): Lipid peroxidation levels in muscle, intestine and liver tissues of both infected and uninfected fish are given in Fig. 1. Increased levels of lipid peroxidation were observed in muscle (29.80667±5.50564), intestine (57.60833±16.8891) and liver tissues (61.99±26.7154) of infected fish as compared to un-infected fish organs (6.28±0.0223, 24.20±.298 and 26.67±1.117), respectively.

DISCUSSION

During the present study, fishes infected with *Pomphorhyncus* showed the decline in antioxidants that is SOD, GST activities and protein content, but elevation in lipid peroxidation in muscle, intestine and liver tissues as compared to uninfected fishes.

Significant reduction of GST activities was also observed in buffaloes (*Bubalus bubalis*) infested by psoroptic mange when compared to controls[7]. In some studies like in African catfish, *Clarias gariepinus*, experimentally challenged with *Escherichia coli* and *Vibrio fischeri*, increase in GST levels was observed that did not support the present work[8]. Significant decrease was also found in hepatic SOD of *Catla catla* infected with microsporidia[9] and in infected black sea whiting *Merlangius merlangus euxinus* (gadidae) as compared to control ones[10] Decline in SOD activity may be due to the presence of increasing amount of reactive oxygen and hydroxyl radicals that were known to inactivate the chemical structure of SOD and ultimately resulted into loss of enzyme activity[11]. But increase in superoxide dismutase was observed in *Salmo trutta trutta* with ulcerative dermal necrosis plus infection of *Saprolegnia* fungus[12] and in goats affected with *peste des petites* ruminants (PPR) that could be due to

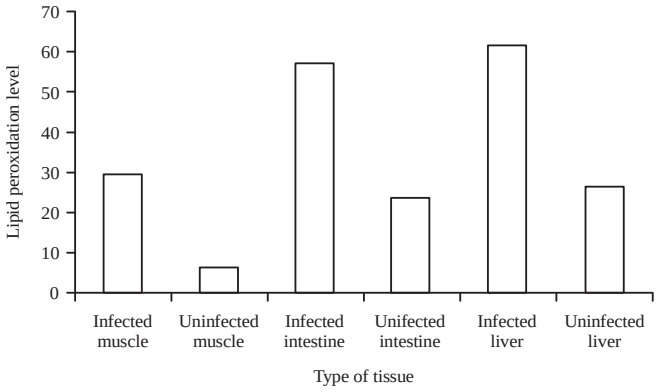

Fig. 1: Lipid peroxidation (n moles of TBARS formed h^{-1} g^{-1} tissue) in muscle, intestine and liver tissues of infected fish in comparison to respective organs of uninfected fish

its active involvement in neutralizing the effect of free radicles[13]. The present results of reduction in protein level are in accordance with the study performed in Koshar Fish, *Epinephelus summana* infected with helminth parastes[14]. Increase in protein content that could be due to expression of new proteins were noticed in fish *Catla catla* infected with microsporidial infection[9] and in orphan children infected with intestinal parasites as compared to un infected ones that are not in favour of our study[15].

Elevated levels of MDA/TBARS/lipid peroxidation were noticed in all the organs in the present study which is in accordance with the study performed in dairy cows naturally infected with the lung worm *Dictyocaulus viviparous*[16]. Lipid peroxidation and reduction in SOD level was also reported in the liver of sheep infected with *Dicrocoelium dendriticum*[17] and in goldfishes parasitized by *Dactylogyrus* spp. when compared with control values[18]. MDA levels were significantly elevated in infected domesticated Ostriches by gastrointestinal helminth parasites (cestodes and nematodes) compared to uninfected birds[19]. The high content of lipid peroxidation and low antioxidant activities, were reported in breams (*Abramis brama)* infected with plerocercoids of *Ligula intestinalis*[20]. This study suggested that infection of endoparasites is among the major causes of oxidative stress[20,21].

Reduction in antioxidant status in the infected fish in this study suggests that the *Pomphorhynchus* is capable of inhibiting the enzyme production as parasites are known to affect the metabolism of infected fish by increasing free radical and peroxide processes which ultimately results in modulating the host antioxidant status[22].

CONCLUSION

Based on the above findings, it is concluded that the antioxidant defense system of the fish serves as a biomarker of oxidant exposure and may be beneficial in assessing the risk of oxidative damage associated with consumption of such fish by humans.

SIGNIFICANCE STATEMENTS

The present study discovers the possible effects of *Pomphorhynchus* on antioxidants activities in fish which is a sophisticated system of defense in them against free radicals or reactive oxygen species (ROS) formed under the effect of parasites as ROS is an important cause for reducing their production by causing DNA damage, protein damage, lipid peroxidation and other diseases. Also this study demonstrates that parasite presence develop tissue specific adaptive responses to neutralize the effect of ROS. Thus according to present findings the antioxidant status can act as a potential biomarker for early warning of adverse effects of parasites on fish health.

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REFERENCES

1. Mohankumar, K. and P. Ramasamy, 2006. White spot syndrome virus infection decreases the activity of antioxidant enzymes in *Fenneropenaeus indicus*. Virus Res., 115: 69-75.
2. Halliwell, B. and J.M.C. Gutteridge, 2007. Free Radicals in Biology and Medicine. 4th Edn., Clarendon Press, Oxford, UK., ISBN-13: 9780198568698, Pages: 888.
3. Haque, R., B. Bin-Hafeez, S. Parvez, S. Pandey, I. Sayeed, M. Ali and S. Raisuddin, 2003. Aqueous extract of walnut (*Juglans regia* L.) protects mice against cyclophosphamide induced biochemical toxicity. Hum. Exp. Toxicol., 22: 473-480.
4. Beauchamp, C. and I. Fridovich, 1971. Superoxide dismutase: Improved assays and an assay applicable to acrylamide gels. Anal. Biochem., 44: 276-287.
5. Lowry, O.H., N.J. Rosebrough, A.L. Farr and R.J. Randall, 1951. Protein measurement with the folin phenol reagent. J. Biol. Chem., 193: 265-275.
6. Niehaus, Jr. W.G. and B. Samuelsson, 1968. Formation of malonaldehyde from phospholipid arachidonate during microsomal lipid peroxidation. Eur. J. Biochem., 6: 126-130.
7. Radwan, M.E.I., S.E. Reham and A.E.M. Mohamed, 2017. Biochemical changes investigated by psoroptic mange infestation in buffaloes. J. Med. Med. Sci., 8: 20-24.
8. Adeyemi, J.A., 2014. Oxidative stress and antioxidant enzymes activities in the African catfish, *Clarias gariepinus*, experimentally challenged with *Escherichia coli* and *Vibrio fischeri*. Fish Physiol. Biochem., 40: 347-354.
9. Dhiraj, C. and B. Sujata, 2014. The histopathological and ultrastructural analysis of microsporidian infection in catla (*Catla catla*) and their effects on antioxidant enzymes and protein expression. Int. J. Adv. Res., 2: 608-624.
10. Skuratovskaya, E.N., V.M. Yurakhno and A.V. Zavyalov, 2013. The influence of parasitic infection on the black sea whiting, *Merlangius merlangus euxinus* (Gadidae), morphophysiological and biochemical parameters. Vestnik Zoologii, 47: 309-317.

11. Rameshthangam, P. and P. Ramasamy, 2006. Antioxidant and membrane bound enzymes activity in WSSV-infected *Penaeus monodon* Fabricius. Aquaculture, 254: 32-39.
12. Kurhalyuk, N., H. Tkachenko and K. Pałczynska, 2009. Antioxidant enzymes profile in the brown trout (*Salmo trutta trutta*) with ulcerative dermal necrosis. Bull. Vet. Inst. Pulawy, 53: 813-818.
13. Kataria, A.K. and N. Kataria, 2012. Evaluation of oxidative stress in sheep affected with peste des petits ruminants. J. Stress Physiol. Biochem., 8: 72-77.
14. Hassan, A.H., N.A. Al-Zanbagi and E.A. Al-Nabati, 2015. Biochemical changes in muscles and liver in relation to Helminth infection of Koshar fish, *Epinephelus summana* in Jeddah, Saudi Arabia. Am.-Eurasian J. Agric. Environ. Sci., 15: 2064-2068.
15. Mahittikorn, A., R. Prasertbun, H. Mori and S. Popruk, 2014. Antioxidant enzyme activity among orphans infected with intestinal parasites in Pathum Thani province, Thailand. Southeast Asian J. Trop. Med. Public Health, 45: 1252-1263.
16. Da Silva, A.D., A.S. da Silva, M.D. Baldissera, C.I. Schwertz and N.B. Bottari *et al*., 2017. Oxidative stress in dairy cows naturally infected with the lungworm *Dictyocaulus viviparus* (Nematoda: Trichostrongyloidea). J. Helminthol., 91: 462-469.
17. Bahrami, S., M.H.R. Jalali and A. Jafari, 2015. Evaluation of hepatic antioxidant changes in ovine dicrocoeliosis. J. Parasitic Dis., 39: 766-769.
18. Mozhdeganloo, Z. and M. Heidarpour, 2014. Oxidative stress in the gill tissues of goldfishes (*Carassius auratus*) parasitized by *Dactylogyrus* spp. J. Parasitic Dis., 38: 269-272.
19. Siwela, A.H., L.R. Motsi and S. Dube, 2013. Alteration of some hepatic enzyme activities by gastrointestinal helminth parasites in domesticated ostriches. Adv. Biores., 4: 145-150.
20. Mikrjakov, V.R. and N.I. Silkina, 2006. Characteristic of the index of peroxidation lipids in the system a parasite-host illustrated by *Ligula intestinalis* L. (Cestoda, Pseudophyllidea)-*Abramis brama* (L.). Biol. Vnut. Vod., 4: 63-66.
21. Deger, S., Y. Deger, A. Ertekin, A. Gul, K. Bicek and N. Ozdal, 2008. [Determination of the status of lipid peroxidation and antioxidants in cattle infected with *Dictyocaulus viviparous*]. Turk. Parazitol. Derg., 32: 234-237, (In Turkish).
22. Skuratovskaya, E.N. and A.V. Zavyalov, 2008. The influence of parasite infestation on the state of antioxidant enzyme system in whiting (*Merlangus merlangus euxinus*) blood. Vet. Med., 90: 394-398.