Int Aquat Res (2022) 14:13-22
https://doi.org/10.22034/IAR.2022.1937373.1179

ORIGINAL RESEARCH

Oxidative stress, serum biochemistry and DNA damage of *Cyprinus carpio communis* naturally infected with helminths

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Received: 10 August 2021 / Accepted: 22 January 2022 / Published online: 28 January 2022

Abstract Helminth parasites cause severe physiological disturbances and pathological conditions in fish due to generation of different free radicals or reactive oxygen species. These reactive oxygen species cause extensive damage to antioxidant status of fish which is an important and sophisticated defense in fish against the reactive oxygen species. Present study was conducted to analyze the effect of some helminth parasites on antioxidant markers, some biochemical parameters and DNA in the tissues of *Cyprinus carpio communis* collected from Sukhnag stream. Fish specimens only with *Bothriocephalus acheilognathi* (n=10), with *Pomphorhynchus kashmirensis* infection (n=10) and uninfected (n=10) were selected for carrying out the present work. Estimation of antioxidant markers (GSH, glutathione peroxidase, glutathione reductase, glutathione-s-transferase, superoxide dismutase and catalase), lipid peroxidation (LPO), protein content and DNA damage in muscle, intestine, liver and gill tissues was carried out. Serum biochemical constituents like alanine aminotransferase (ALT), aspartate aminotransaminase (AST), cholesterol and triglycerides were also monitored in parasitized and non-parasitized fishes. Analysis of data revealed the decline in GSH content in muscle, intestine and liver whereas increase in enzymatic antioxidants and lipid peroxidation (LPO) in muscle, intestine and liver was noticed in parasitized fishes in comparison to non-parasitized fishes. Significant elevation also observed in ALT, AST, cholesterol and triglycerides in parasitized fishes. DNA degradation was observed in intestinal tissues in parasitized fishes with respect to intestinal DNA of uninfected ones. This study concluded that helminth parasites have a significant impact in fish tissues by inducing oxidative stress which modifies the antioxidant activities, biochemical constituents and damages DNA. The parameters outlined in the present study may be employed as tools in monitoring the health status of fish in culture practices.

Keywords Lipid peroxidation . Antioxidant . Glutathione . Sukhnag . Biochemical constituents

Introduction

Parasitic infection has deleterious effects on fish health due to the generation of reactive oxygen species like superoxide radical, nitric oxide (NO) and hydrogen peroxide (Dimri et al. 2010). During parasite invasion, macrophages and neutrophils form free radicals or reactive oxygen species in order to facilitate the removal of invaded microorganisms and are highly toxic to invaded parasites. Under oxidative stress that is when imbalance between oxidants and radical scavenging systems occur (Almeida et al. 2009), these chemically unstable compounds, react with the polyunsaturated fatty acid (PUFA) of cellular or sub-cellular

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membranes of the fish and leads to disintegration of membrane lipids called lipid peroxidation (Stumbo et al. 2012; Mordvinov et al. 2021). Due to increased level of ROS the structure of proteins, lipids, nucleic acids, etc. gets manipulated, leads to severe diseases and ultimately to cell death (Gaschler et al. 2017). The DNA damage due to free radical generation is an important risk factor for carcinogenesis and malignancies (Aldini et al. 2010). Evidences showed that the parasites can also induce changes in cholesterol in the blood serum, liver enzyme activities like serum aspartate transaminase (AST), alanine transaminase (ALT) and alkaline phosphatase (ALP).

In order to protect the cells from these reactive oxygen species fish are recruited with effective defense mechanisms called antioxidant system that destabilize the reactive oxygen species by terminating their chain reaction and protect various cells from oxidative damage (Varoni et al. 2016). The antioxidant defense system consists of enzymatic (Drogee 2002) and non-enzymatic antioxidants which terminate the chain reaction, scavenge free radicals and thus clear the damage of the cell. Non enzymatic antioxidant includes GSH that is called the master antioxidant of the cell (Gupta 2015) and reacts directly with free radicals. Enzymatic antioxidant includes superoxide dismutase that inactivates the superoxide ion by transforming it into hydrogen peroxide (H_2O_2) and molecular oxygen (McCord et al. 2005). Catalase quickly converts hydrogen peroxide into oxygen (O_2) and water (H_2O). Glutathione peroxidase converts lipid hydroperoxides into corresponding alcohols and free hydrogen peroxide to water in presence of glutathione (GSH) that acts as an electron donor. Glutathione reductase (GR) plays an important role in scavenging hydroxyl radicals, singlet oxygen, and various electrophiles. Glutathione-S- transferase (GST) an important antioxidant enzyme eliminates various by-products generated from oxidative reactions (Tjalkens et al. 1998).

In Jammu and Kashmir (India), lot of work has been carried out on helminth parasites of fish and it has been revealed that helminth parasite is one of the major factor for depleting the fish population in the water bodies of Kashmir (Chishti et al. 2006). Scanning of pertinent literature revealed that no work has been carried out regarding the impact of helminth parasites on antioxidant status, biochemical parameters and DNA in *Cyprinus carpio communis* in Kashmir. Hence a comprehensive work was undertaken to study the effect of helminths on the antioxidant status, biochemical parameters and DNA in *Cyprinus carpio communis* of Sukhnag stream (Budgam).

Materials and methods

The study was conducted on *Cyprinus carpio communis* collected from Sukhnag stream in Jammu and Kashmir for three years from 2017- 2019. Sukhnag, is a torrential stream and one of the important tributary of River Jhelum. It is among the five major inflows of the Wular Lake (Ramsar site) (Bhat et al. 2014) and flows through Budgam district. It has its origin both from spring 'Sukhnag spring' (Sokha Nag; the spring of solace) and high altitude glacier called Damdam in Damsar near Tossa Maidan.

Ethical considerations

The research protocol was approved by the Institutional Animal Ethics Committee, Department of Pharmaceutical Sciences, University of Kashmir and an approval certificate was issued with Approval no. F(IAEC-Approval/KU/2018/118). A similar approval was obtained from the Department of Fisheries, Jammu and Kashmir with approval no DOF/Tech/2017/8799-8801.

Fish collection

Fish specimens identified as *Cyprinus carpio communis,* Linnaeus, 1758 were collected from Sukhnag stream and brought alive to the Parasitological Research Laboratory, University of Kashmir by using small containers containing water. Fish hosts were identified using the keys given by Skeleton (2001). *Cyprinus carpio communis* is commonly called as Scale carp and locally as "Punjab gaad" Its body is elongated with comparatively small head and convex dorsal portion. For serum biochemical analysis careful collection of blood was done via venipuncture of the caudal vein using 2 ml syringes from the collected fishes. Blood samples were collected in tubes and subjected to centrifugation at 3000 rpm for 10 minutes in order to get serum. Serum obtained was stored at -20º C until analyzed (Kundu et al. 2016).

Examination of the fish for ectoparasites and endoparasites

The fishes were first killed and then external body surfaces like opercula, mouth cavity and the fins were carefully scanned for helminth parasites. The gills were removed in petridishes containing normal saline (0.7% Nacl, Cable 1958). The fishes were dissected for internal examination and the body cavities as well as visceral organs were scanned thoroughly for the presence of helminth parasites. Identification of helminth parasites was carried out by the keys given by Yamaguti (1971) and Hoffman (1999). Helminth parasites recovered from fish were identified as *Bothriocephalus acheilognathi* Yamaguti, 1934 (cestode) and *Pomphorhynchus kashmirensis* Kaw, 1950 (acanthocephalan). The permanent slides of collected specimens of helminths were deposited in the "Zoological Museum", Department of Zoology, University of Kashmir, India. Acquisition numbers: ZoKU-Hel/04202 and ZoKU-Hel/04602 were assigned for *Bothriocephalus acheilognathi* Yamaguti, 1934 *and Pomphorhynchus kashmirensis* Kaw, 1950, respectively.

Antioxidant analysis

For analyzing the antioxidant status, both infected and healthy fishes were dissected. Organs such as intestine, muscle, liver and gill tissues were removed from the dissected fishes, immediately washed with ice-cold 0.9% saline solution to remove blood, weighed and homogenized in a homogenizing buffer (50 mM Tris-HCl, 1.15% KCl, pH 7.4) by using homogenizer. The homogenate was centrifuged at 10,000 rpm for 10 minutes. The sediment was discarded and the supernatant was collected and stored at 4ºC. The resulting supernatant fraction was used to determine antioxidant activities as well as lipid peroxidation.

Estimation of non-enzymatic antioxidant assay (GSH)

The activity of GSH was assayed by the method of Moren et al. (1979). Glutathione content or GSH was calculated using molar extension coefficient of 13,100.
Estimation of Lipid peroxidation: Lipid peroxidation was assessed by the formation of thiobarbituric acid reactive substances using the method of Nichans et al. (1968). The absorbance of the clear supernatant obtained on centrifugation was measured against reference blank at 535 nm.

Estimation of enzymatic antioxidant assay

Estimation of Glutathione Peroxidase activity: GPX activity in the tissue homogenates was estimated by using the method of Sharma et al. (2001). The activity of GPX was determined spectrophotometrically at 340 nm by measuring NADPH oxidation. The enzyme activity was calculated as n moles of NADPH oxidized/min/mg of protein using the molar extinction coefficient of 6.22×10^3 M^{-1} cm^{-1}.

Estimation of Glutathione Reductase activity: GR activity was estimated by using the method of Sharma et al. (2001). Absorbance of the mixture that is the oxidation of NADPH was recorded spectrophotometrically at 340 nm. The enzyme activity was calculated as n moles NADPH oxidized/min/mg of protein using molar extinction coefficient of 6.22×10^3 M^{-1} cm^{-1}.

Estimation of Glutathione-S-Transferase activity: Activity of GST was assessed by the method of Haque et al. (2003). The change in absorbance was recorded at 340 nm and the enzyme activity was calculated as n moles of CDNB conjugates formed/min/mg protein using molar extinction coefficient of 9.6×10^3 M^{-1} cm^{-1}.

Estimation of Superoxide dismutase activity: SOD activity was carried out according to the method described by Beauchamp et al. (1971). Units of SOD activity were expressed as the amount of enzyme required to inhibit the reduction of NBT by 50%.

Estimation of Catalase activity: CAT activity was assayed by the method of Claiborne (1985). Change in absorbance is recorded spectrophotometrically at 240 nm. Then CAT activity is calculated in terms of nm of H_2O_2 consumed/minute/mg protein.

Estimation of Protein: Protein concentration was estimated by Lowery method (1951). In this method first standard BSA solution was prepared by dissolving 50 mg of BSA in 100ml distilled water.

Biochemical analysis

Serum of both infected and uninfected fish of *S. plagiostomus* stored at -20° C were analyzed for biochemical parameters like ALT, AST, cholesterol and triglycerides by using commercially available kits of ERBA.
a) Alanine aminotransferase (ALT): Analyzed by ERBA kit (Cat. No.120207).
b) Aspartate aminotransaminase (AST): Analyzed by ERBA kit (Cat. No. 120204).
c) Cholesterol: Analyzed by ERBA kit (Cat. No. 120194).
d) Triglycerides: Analyzed by ERBA kit (Cat. No. 120211).

DNA analysis

DNA extraction: DNA was extracted from fish samples (muscle, intestine, liver and gills) of healthy as well as infected fishes using the method of Biase et al. (2002) with certain modifications. DNA extraction from muscle, intestine, liver and gill tissues was done by using 0.5 ml lysis buffer and incubated at 55° C for several hours that resulted in complete digestion. Then one volume of isopropanol was added to the lysate and the samples were mixed or swirled untill precipitation was complete (about 10-20 minutes) (viscosity completely gone). The DNA was recovered from the solution by lifting the aggregated precipitate with the help of disposable yellow tip. Excess liquid was dabbed off and the DNA was dispersed in a prelabbled eppendorf tube, depending on the size of the precipitate, containing 20- 500ul to 10 mM Tris HCl, 0.1 mM EDTA, pH 7.5. For complete dissolution of the DNA several hours of agitation at 37°C or 55°C was required. DNA was then analyzed by submarine electrophoresis.

Submarine electrophoresis

For carrying out submarine electrophoresis first 1% Agarose gel was prepared. In submarine electrophoresis, 10 µLs sample and 10 µLs bromophenol blue were loaded in separate wells. 1X TAE was used as loading buffer. Then the gel was allowed to run for 30 minutes at 100 volt and exposed to UV light. Photographs were taken for confirming the DNA quality. Presence of highly resolved high molecular weight band indicates good quality DNA whereas presence of a smeared band is an indication of DNA degradation.

Statistical analysis

Data were recorded, entered and fed into Microsoft Excel work sheet and then analyzed by Minitab Version 13 and SPSS Version 16. The data were represented as mean of ten independent experiments presented as mean± standard deviation. One way ANOVA with Tukey's test was used to test the significant differences in antioxidant and biochemical parameters of helminth infected fishes in comparison to uninfected ones which were considered to be significant when the p-value obtained was < 0.05 (using a significance level of 5%).

Results

An overall comparison of enzyme activities in the infected and uninfected muscle, intestine, liver and gill tissues of *C. carpio communis* revealed a unique pattern in their order of activities. Results showed the higher activities of GSH, CAT, SOD and protein content in intestinal tissues followed by muscle, then liver and lowest in gills of uninfected fish. Activities of GPX, GR and GST were maximum in intestinal tissues, followed by liver, then muscle and lowest in gill tissues of uninfected fish. LPO levels were higher in gill tissues followed by liver, then muscle and lowest in intestinal tissues of uninfected fish (Table 1). In general, we observed that the *C. carpio communis* infected with helminth parasites demonstrated an elevation in the level of the antioxidant enzymes as compared to uninfected fish. However results revealed the content of GSH and protein declined in infected fish when compared with uninfected ones. Observations reported significant decline ($P < 0.05$) in GSH content of muscle, intestine, liver but not in gill tissues of *C. carpio communis* infected with *B. acheilognathi* (47.48%, 65.22%, 59.79%, 7.01%) and with *P. kashmirensis* (41.9%, 53.47%, 47.42%, 1.75%) respectively in comparison to GSH content of uninfected fish. Significant increases ($p < 0.05$) in lipid peroxidation (LPO) of fish with *B. acheilognathi* in muscle, intestine, liver were

Table 1 Effect of helminths on antioxidant parameters in *Cyprinus carpio communis*

Antioxidant parameters	Organs	Uninfected Fish	Fish infected with *B.acheilognathi*	Fish infected with *P.kashmirensis*
GSH (nmoles of GSH conjugated/gram of tissue)	Muscle	0.377±0.026^a	0.198±0.002^b	0.219±0.018^c
	Intestine	0.877±0.013^a	0.305±0.011^b	0.408±0.012^c
	Liver	0.097±0.003^a	0.039±0.003^b	0.051±0.008^b
	Gills	0.057±0.005^a	0.053±0.002^a	0.056±0.006^a
LPO (nmoles of MDA formed/h/gm tissue)	Muscle	0.479±0.033^a	1.14±0.051^b	0.92±0.028^c
	Intestine	0.272±0.031^a	0.76±0.023^b	0.709±0.085^c
	Liver	0.538±0.017^a	1.492±0.039^b	1.146±0.023^c
	Gills	0.568±0.036^a	0.560±0.048^a	0.565±0.048^a
GPX (nmoles of NADPH oxidized/min/mg protein)	Muscle	0.806±0.02^a	1.109 ±0.027^b	1.12 ±0.025^b
	Intestine	2.33 ±0.04^a	3.385 ±0.027^b	3.579±0.038^c
	Liver	0.942±0.014^a	1.567 ±0.021^b	1.814 ±0.016^c
	Gills	0.386±0.028^a	0.389 ±0.019^a	0.396 ±0.025^a
GR (nmoles of NADPH oxidized/min/mg protein)	Muscle	1.844 ±0.036^a	2.535±0.025^b	2.602±0.057^c
	Intestine	4.01±0.057^a	5.755±0.024^b	6.15±0.064^c
	Liver	2±0.048^a	3.124±0.018^b	4.069±0.082^c
	Gills	0.96±0.042^a	0.97±0.042^a	0.98±0.090^a
GST (nmoles of CDNB conjugates/min/mg protein)	Muscle	0.163±0.016^a	0.358±0.018^b	0.589±0.028^c
	Intestine	0.922±0.015^a	2.16±0.022^b	3.82±0.01^c
	Liver	0.22±0.014^a	0.71±0.014^b	0.98±0.015^c
	Gills	0.055±0.017^a	0.059±0.017^a	0.060±0.010^a
SOD (the amount of enzyme required to inhibit the reduction of NBT by 50%)	Muscle	0.483±0.034^a	0.942±0.031^b	1.008±0.015^c
	Intestine	1.578±0.047^a	3.68±0.016^b	3.977±0.022^c
	Liver	0.338±0.018^a	1.025±0.026^b	1.097±0.037^b
	Gills	0.196±0.038^a	0.197±0.040^a	0.199±0.025^a
CAT (nmoles of H_2O_2 consumed /min/mg protein)	Muscle	0.129±0.004^a	0.328±0.007^b	0.424±0.009^c
	Intestine	0.194±0.002^a	0.542±0.006^b	0.775±0.002^c
	Liver	0.098±0.001^a	0.282±0.003^b	0.327±0.002^c
	Gills	0.031±0.002^a	0.034±0.003^a	0.036±0.001^a
Protein content (mg%)	Muscle	329±19.2^a	267.2±12.2^b	328±12.9^a
	Intestine	336.2±12.8^a	71.3±7.4^b	184±10.8^c
	Liver	264.63±13.04^a	185.33±10.38^b	207.57±9.43^c
	Gills	256.5±5.4^a	255.02±5.9^a	255.06±5.5^a

Data are presented as Mean ± SD. Values with different superscripts $^{a, b, c}$ within each row are significantly different as determined by Tukeys test ($P < 0.05$)

137.99%, 179.41%, 177.32%, and with *P. kashmirensis* were 92.06% , 159.66%, 113%, whereas gills of infected fish showed non-significant increase ($P > 0.05$) by 1.4% and 0.5% respectively in comparison to LPO levels of uninfected fish. Elevation in GPX activity ($P < 0.05$) in muscle, intestine, liver were 37.59%, 45.27%, 66.34%, in fish infected with *B. acheilognathi* whereas non-significant ($P > 0.05$) elevation as 0.7% was in gills. Similar trend was reported in GPX activities of fish with *P. kashmirensis* (38.95%, 53.60%, 92.56% and 2.5% in muscle, intestine, liver and gill tissues respectively). Significant elevation ($P < 0.05$) in GR activities in muscle, intestine, liver in fish infected with *B. acheilognathi* were 37.47%, 43.51%, 56.2%, but gills showed non-significant ($P > 0.05$) elevation in GR activities (1.04%). Fish with *P. kashmirensis* showed GR activity significantly ($P < 0.05$) increased in muscle (41.10%), intestine (53.36%) and liver (103.45%) whereas showed non-significant ($p > 0.05$) elevation in gill tissues (2.08%) in comparison to uninfected fish.

Significant increase ($P < 0.05$) in GST activity in muscle (119.63%, 261.3%), intestine (134.27%, 314.31%), liver (222.72%, 345.45%) and non-significant increase as 7.27% and 9.09% in gill tissues infected with *B. acheilognathi* and with *P. kashmirensis* were recorded as compared to uninfected fish. Significant

Table 2 Effect of helminths on various biochemical parameters in *C. carpio communis*

Biochemical parameters	Uninfected fish	Fish Infected with *B. acheilognathi*	Fish Infected with *P. kashmirensis*
ALT U/L	30.74±17.8^{a}	64.78±10.6^{b}	54.24±13.01^{c}
AST U/L	246.5±22.2^{a}	514.7±21.5^{b}	375.3±23.5^{c}
Cholesterol mg/dl	164.8±19.5^{a}	292.2±18.2^{b}	223.7±13.0^{c}
Triglycerides mg/dl	77.2±17.37^{a}	115.95±20.56^{b}	104.04±16.3^{c}

Values with different superscripts a,b,c within each row are significantly different as determined by Tukey's test (P <0.05)

increase (P <0.05) in SOD activities in muscle, intestine, liver in fish infected with *B. acheilognathi* were 95.03%, 133.20%, 203.25%, respectively and non-significant (P >0.05) increase in gill tissues was 0.51%. Fish with *P. kashmirensis* showed significant increase (P <0.05) in SOD activities in muscle (108.69%), intestine (152.02%), liver (224.55%), and non-significant (P >0.05) increase in gill tissues (1.5%). Significant elevation (p <0.05) in catalase activity in muscle, intestine, liver but non-significant (P >0.05) in gill tissues of fish infected with *B. acheilognathi* were 154.26%, 179.38%, 187.75% 9.67%. Similar trend in catalase activities were recorded in fish with *P. kashmirensis* (muscle: 228.68%, intestine: 299.48%, liver: 233.67% and gills:10.12%). Significant decline were reported (P <0.05) in protein content in muscle (18.78%), intestine (78.79%) and liver (29.96%) in fish infected with *B. acheilognathi* whereas in gills non-significant decline (P >0.05) (0.57%) was found. Fish with infection of *P. kashmirensis* showed non-significant decline of protein content in muscle (0.3%) and gill tissues (0.56%) (P >0.05), however significant decline (P <0.05) were in intestine (45.27%) and liver tissues (21.56%) (Table 1).

In addition to antioxidant analysis in the tissues of infected and uninfected *C. carpio communis* we also analyzed the effect of helminth parasites on biochemical parameters like alanine aminotransferase (ALT), aspartate aminotransferase (AST), cholesterol and triglycerides in *C. carpio communis* (Table 2). Observations noticed the biochemical parameters significantly increased in infected fish as compared to uninfected fish. Significant percentage increase (P <0.05) reported in serum biochemical components like ALT (alanine aminotransferase), AST (aspartate aminotransferase), cholesterol and triglycerides levels were 110.73%, 108.80%, 77.30%, 50.19% in fish infected with *B. acheilognathi* and 76.44%, 52.25%, 35.74%, 34.76% in fish with *P. kashmirensis* infection in comparison to uninfected fishes.

Effect of helminth infection on DNA of muscle, intestine, liver and gill tissues in *Cyprinus carpio communis*

The effect of *Bothriocephalus acheilognathi* and *Pomphorhynchus kashmirensis* on muscle and intestinal DNA of *Cyprinus carpio communis* collected from Nallah Sukhnag is shown in Fig. 1. Fig. 2 shows the effect of *Bothriocephalus acheilognathi* and *Pomphorhynchus kashmirensis* on DNA in liver and gill tissues of *Cyprinus carpio communis*. The results showed the damage of DNA in intestinal tissues infected with *Bothriocephalus acheilognathi* and *Pomphorhynchus kashmirensis* as DNA is in diffused form in intestinal tissues of infected fish when compared with the intestinal DNA of uninfected fish. DNA in muscle, liver and gills are in the form of intact bands in uninfected as well as in infected fishes that represents no damage in DNA.

Discussion

In current study non-enzymatic antioxidant (GSH) content showed significant decline in muscle, intestine, liver and gill tissues in fishes of *C. carpio communis* infected with *Bothriocephalus acheilognathi* and *Pomphorhynchus kashmirensis* that is strongly supported by Mozhdeganloo et al. (2013) who found the infection of *Dactylogyrus spp.* reduced the level of GSH in the gills of goldfish (*Carassius auratus*). Nabi et al. (2017) also reported decline of GSH content in *S. plagiostomus* infected with *Pomphorhynchus sp.* infection. In current study, elevation has been observed in GPX and GR activities of *C. carpio communis* in muscle, intestine, liver tissues but not in gills that is strongly supported by Al-Olayan et al. (2015) who observed the increase in GPX and GR activities in muscle and liver tissues under the infection of encysted metacercaria of *Diplostomum tilapiae* and *Heterophyes sp.* in *Tilapia zillii.* Significant increase in GPX activity was also observed in liver of *Sarotherodon galilaeus* infected with *Diplostomum tilapiae,*

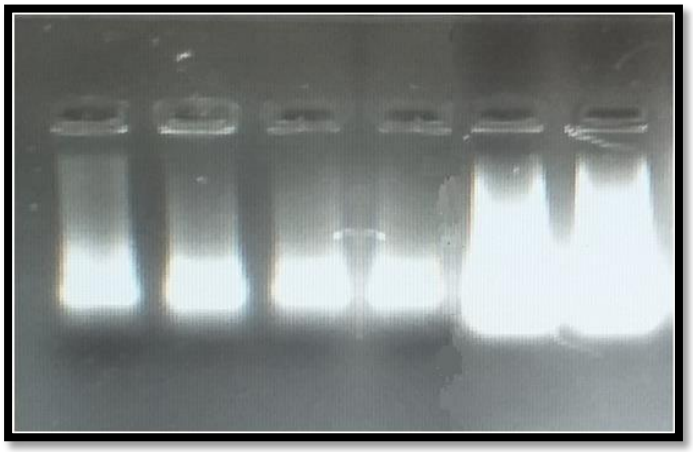

Fig. 1 Shows muscle and intestinal DNA of fish. Lane 1 represents the DNA in muscle of uninfected fish, Lane 2 represents the muscle DNA of fish infected with *Bothriocephalus acheilognathi,* Lane 3 represents the muscle DNA of fish infected with *Pomphorhynchus kashmirensis,* Lane 4 represents the intestinal DNA of uninfected fish, Lane 5 represents the intestinal DNA of fish infected with *Bothriocephalus acheilognathi* and Lane 6 represents the intestinal DNA of fish infected with *Pomphorhynchus kashmirensis.*

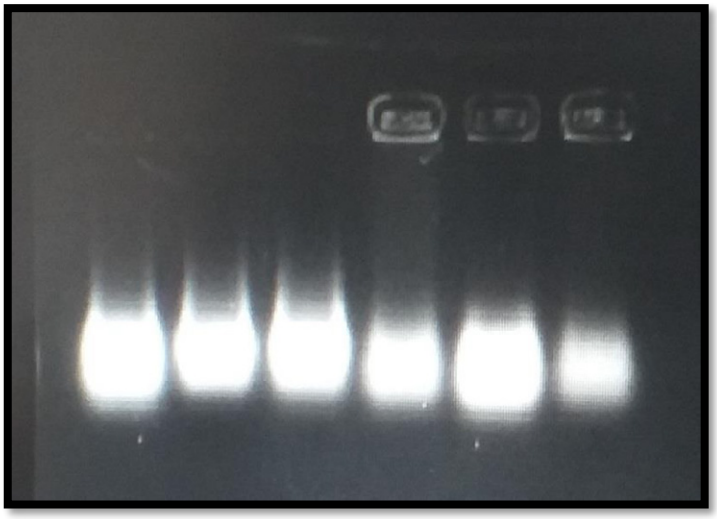

Fig. 2 Shows liver and gill DNA of infected fish. Lane 1 represents liver DNA of uninfected fish, Lane 2 represents liver DNA of fish infected with *Bothriocephalus acheilognathi,* Lane 3 represents liver DNA of fish infected with *Pomphorhynchus kashmirensis,* Lane 4 represents DNA of gills of uninfected fish, Lane 5 represents DNA of gills of fish infected with *Bothriocephalus acheilognathi* and Lane 6 represents DNA of gills in fish infected with *Pomphorhynchus kashmirensis.*

Centrocestus formacenus and *Hetrophyes* sp. Our results showed increase in GST, CAT and SOD activities in infected *C. carpio communis* that is in accordance to Skuratovskaya et al. (2013) who found enhancement in GST, CAT and SOD activities infected with *Merlangius merlangus euxinus* fishes. Our results are also in accordance to Samani et al. (2018) who observed increase in catalase activity in blood of pigeons infected with *Haemoproteus columbae*. Elevated levels of MDA/lipid peroxidation were observed in muscle, intestine and liver in infected *C. carpio communis* in the present study which is in accordance with the study carried out by Azimzadeh (2016) who reported an elevation of MDA in Rainbow Trout (*Oncorhynchus mykiss* Walbaum 1792) with Ichthyophthiriasis. Nabi et al. (2017) also reported lipid peroxidation in *S. plagiostomus* infected with *Pomphorhynchus sp.* infection. David et al. (2005) and Eissa et al. (2014) also noticed the increase in MDA levels in fish infected with parasites which is the measure of lipid peroxidation. Sahreen et al. (2021) alsoreported increase in GPX, GR, GST, SOD, CAT activities and lipid peroxidation in Indian catfish (*Wallago attu*) infected with piscine trematode *Isoparorchis hypselobagri.* According to David et al. (2005) parasite induces oxidative stress (an imbalance between pro-oxidants and non-enzymatic antioxidants) and a higher level of membrane damage in the fish that is lipid peroxidation.

In present study no significant elevation of lipid peroxidation was observed in gills of fishes infected with helminthes that is attributed to antioxidant defense mechanisms which are sufficient to neutralize oxidative stress in these organs (Saluja et al. 1999). Elevated levels of lipid peroxidation in current study might be due to increase in free radical generation that could have damaged the lipid membranes (Eissa et al. 2014). Protein concentration decreased in infected *C. carpio communis* in muscle, intestine and liver tissues as compared to non-parasitized ones in the present investigation which is supported by the study performed by Mustafa (2000) who observed the decrease in protein and also in lipid concentration in muscle, liver and ovaries of Powan (*Coregonus lavaretus*) infected with *Diphyllobothrium spp.* (Cestoda) from Scotland. Nabi et al. (2017) also reported decline in protein content in *S. plagiostomus* with *Pomphorhynchus sp.* infection.

According to Lemly et al. (1984), fishes in order to eliminate parasites from their body require more energy that leads to more oxygen requirement and in turn generates free radicals which are the main cause for oxidative stress. GSH is an important cellular scavenger of free radicals and so an important defense in cells that protect them from the damage caused by these molecules. Decline in thiol level is considered an important cause for modulating the antioxidant activities. Elevation in antioxidant enzyme activities in our results could be attributed to the larger degree of immunity and protection shown by *C. carpio communis* against helminth parasites (Garcia et al. 2011). This increase in antioxidant response observed in present endeavor in the tissues of *C. carpio communis* could be also due to the efficiency of this fish to neutrilize the oxidative stress caused by helminths (Dautremepuits et al. 2003).

Among different enzymes elevation in GST, SOD and CAT was more in infected *C. carpio communis* as they are the first line of defense enzymes against ROS production and thus predicted their efficient role in neutralizing the impact of ROS. Maximum percentage increase or fold increase of antioxidant status in infected *C. carpio communis* were observed in intestine and liver tissues in comparison to other organs that might be due to their active involvement in neutralizing the free radicals, generated during helminth infection (Dhiraj et al. 2014). In current study significant percent increase in biochemical parameters like ALT, AST, cholesterol and triglycerides were reported in *C. carpio communis* infected with parasites. Our findings are also supported by Kundu et al. (2015) who found significant increase in ALT, AST and cholesterol in Freshwater Fish *Channa punctatus* infected with Nematode Parasite *Eustrongylides sp.* Rastiannasab et al. (2015) reported elevation in enzymatic activity ALT, AST of Common carp, *Cyprinus carpio* in response to parasites, *Dactylogyrus spp.* and *Gyrodactylus spp.* A similar result was observed by Nnabuchi et al. (2015) in catfishes from Anambra River, Nigeria. Omeji et al. (2018) reported elevation in ALT and AST in selected bagrid species from lower river Benue Nigeria under the infection of nematodes. Increased content of cholesterol and triglycerides is attributed to the increase in degredaion of lipids to overcome the shortage of energy due to inadequate absorption of carbohydrate and glycogen shortage in parasitized fishes. The increased lipolysis has been reported to liberate triglycerides and non-esterified fatty acids which are processed by the liver into acetyl CoA in the tricarboxylic acid cycle for energy production (Al-Attar 2010). Elevation in the activities of ALT and AST in infected fish is an indication of extensive liver damage that has resulted in impaired liver function (Svoboda et al. 2001).

In current study, DNA degradation was found in intestinal tissues of infected *C. carpio communis* when compared with the tissues of *C. carpio communis*. Our results are supported by Bagdonas et al. (2007) who reported DNA damage in erythrocytes of Prussian carp (*Carassius auratus gibelio*) infected with *Aeromonas* and *Pseudomonas* bacteria causing ulcerative disease in fish Our findings are also supported by Kucukkurt et al. (2014) who reported damage in DNA of goats naturally infected with *Babesia ovis*. Helminth parasites have been reported to activate macrophages, mast cells, eosinophils that generates nitric oxide (NO) from L-arginine and is considered as the main cause for DNA damage (Wink et al. 1991).

Conclusion

In current study, rise in lipid peroxidation levels and antioxidant enzymes in the tissues of infected fish was observed which is mainly due to the oxidative stress caus.ed by the helminths. Oxidative stress and increase in lipid peroxidation levels resulted in the decline of GSH content and damaged DNA of infected tissues. This study concluded that study of antioxidant defense system and DNA damage in helminth infected fish is important as it serves as an important marker of oxidant exposure and play a crucial role in assessing the

risk of oxidative damage in humans by the consumption of such fish. The observations of this study provide information regarding the characteristic features of biochemical, and antioxidant changes in fish due to helminth infection, suggesting that antioxidant parameters and serum biochemical studies may be effective in monitoring the effects of helminth infestation in fish; and would be effective in fishery management programs.

List of Abbreviations ALT, Alanine aminotransferase; AST, Aspartate aminotransferase; NBT, Nitro blue tetrazonium chloride; CAT, Catalase; SOD, Superoxide dismutase; GST, Glutathione S transferase; GSH, Glutathione; LPO, lipid peroxidation; DNA, Deoxyribonucleic acid; MDA, Melonylaldehyde; ROS, Reactive oxygen species; GPX, Glutathione peroxidase; GR, Glutathione reductase; HCl, Hydrogen Chloride; NaCl, Sodium Chloride; NADPH, Nicotineamide Adenine Dinucleotide Phosphate; NBT, Nitro Blue Tetrazonium Chloride; CDNB,1-Chloro-2,4-Dinitrobenzene; DTNB, Dithiobisnitrobenzoic Acid, EDTA, Ethylene Diamine Tetra acetic Acid; TBA, Thiobarbituric Acid; TCA, Trichloro Acetic Acid; H_2O_2, Hydrogen peroxide ; UV, Ultravoilet

Competing interests The authors have no conflicts of interest to declare.

Author's Contributions ST and SAG designed and supervised the experiments. SN was involved in collecting the sample from the Sukhnag stream, isolated the parasites from different organs of fish, prepared the host sample for antioxidant, biochemical, DNA assays, prepared the manuscript and carried out the statistical analysis. KB analysed the data.

Acknowledgements The authors are thankful to the HODs, Department of Zoology and Department of Clinical Biochemistry, University of Kashmir for providing the necessary laboratory facilities. Thanks to our colleagues for technical support.

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