RESEARCH

Changes in the level of oxidative stress markers in Indian catfish (*Wallago attu*) infected with *Isoparorchis hypselobagri*

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Abstract

Background: Helminth infection and infestation in fishes are detrimental and have a major effect on fish health and fish production. Among various factors, parasitic infections are known to modulate antioxidant defences in fish. Similar to other aerobic animals, fish are also susceptible to the effect of reactive oxygen species and thus have well established intrinsic and efficient antioxidant defences. 'Oxidative stress markers are an important indicator of the physiological state of the parasite and its host'. Indian catfish,*Wallago attu* is a freshwater fish that serves as the definitive host of the adult piscine trematode *Isoparorchis hypselobagri*. Our two years prevalence data signifies the intensity of the problem revealing a minimum of 5.5% and a maximum of 54% *I. hypselobagri* infection in Indian catfish *W. attu* (unpublished data). The present study aimed to achieve baseline data attributed to changes in some oxidative markers due to parasitic infection.

Results: During the present study, the level of enzyme activities of Catalase (CAT), Glutathione reductase (GR), Glutathione-S-transferase (GST), Glutathione peroxidase (GPx), Superoxide dismutase (SOD) and lipid peroxidation was investigated to explore the pathogenic impact on the fish host. The level of these oxidative stress markers was monitored in the swim bladder, liver, intestine and muscle of the host. We also recorded the enzyme activities in the parasite *I. hypselobagri*. Analysis of data revealed an elevation in GST, SOD, GR, GPx and CAT activity in the infected host tissue as compared to the non-infected fish. Further, we observed presence of GST, SOD, GR and GPx enzymes in the parasite *I. hypselobagri* while CAT did not show any enzyme activity.

Conclusions: Increased level of enzyme activity in liver, muscle and intestine of infected host has been recorded which indicates increased oxidative stress in the host due to parasitic invasion. The presence of antioxidant enzymes in the parasites suggests an active antioxidant defence system to avoid immune responses to long term survival and establishment in their host.

Keywords: *Isoparorchis hypselobagri*, *Wallago attu*, Antioxidant enzymes

1 Background

Isoparorchis hypselobagri (Billet 1898) is a digenetic trematode parasitizing swim bladder of the Indian catfish *Wallago attu* and other important food fishes namely *Channa, Notopterus, Mystus, Mystacembelus* and carps [4, 16, 50] (Rahaman and Manna [42]). Moreover, many

vertebrates like a whale [54], crocodile [8], frog [43], turtle [45], pig [52] and human being [14, 25] have been reported to be infected with *I. hypselobagri*. This species has been recorded from India, Bangladesh, Pakistan, Thailand, Japan, China, Australia and Indonesia [18].The infection makes the fish unsuitable for human consumption since it produces black spot disease or ink spot disease in the muscles and visceral organs, causing mortality of fishes and thus resulting in the great economic loss [16, 29]. Human infection with *I. hypselobagri* has been

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recorded by Chandler and Read [15] which is very common in Manipur State of India due to eating raw swim bladders of fish. Cribb [18] proposed a three-host life cycle of parasite *I. hypselobagri*. Eggs of the parasite do not hatch and must be eaten by a gastropod, within the gastropod, it develops into sporocyst, rediae and cercariae. The cercariae leave the gastropod and are consumed by a second intermediate fish host *Oxygaster bacaila*, *Gagata cenia* and *Gambusia affinis* etc., in which the cercariae develop into metacercariae. The second intermediate host is consumed by a catfish and the metacercariae penetrate the intestine and migrate to the swim bladder and develop into an egg-producing adult [18].

Diseases in fish are one of the major factors which impede the successful development of the fish industry including the safety of fish products [34]. Helminth infection can cause a major effect on the fish trade due to the harmful effect of several helminth species. Many commercially important fish species are infected by helminth parasites, which may have a considerable impact on physiological and biochemical processes in the host organisms and are harmful to their health [49]. Humans get fish borne helminth infection by ingesting raw or undercooked fish containing infective parasitic larvae [51] (Chai and Lee [13]). Parasitic infection may also harm the function, growth, reproduction and survival of its host [46]. Financial losses due to parasitic diseases in the fish sector were estimated at 9.6 billion US dollars per year at the global level [44]. World Health Organization (WHO) has estimated that 'the number of people currently infected with fish-borne trematodes exceeds 18 million, and many more are at risk' [40]. Parasites can affect the fish population by causing mechanical, physiological as well as reproductive damage which may lead to a decline in the stock [30]. Morphological changes including partial necrosis of fin tissue, scale loss, loss of pigmentation, damage to the viscera, especially the gonads, and the abdominal muscles, on fish *Channa punctata* infected with *I. hypselobagri,* has been reported by Mahajan et al., [35]. Reduced feeding behaviour and a high mortality rate have been observed in infected fish. Infection by helminths causes 'oxidative stress in their host [25] which as a result generates reactive oxygen species (ROS) including superoxide anion radicals, hydroxyl radicals, singlet oxygen, hydrogen peroxide and hypochlorous acid [21]. These are mainly produced as a part of the immune defence mechanism against foreign particles [39]. Oxidative stress is described as an imbalance state between pro-oxidants and antioxidants, resulting in higher production of ROS and free radicals which causes detrimental effects [38] on the host. Production of ROS is increased due to parasitic infection, which is responsible for killing or expulsion of parasites from their

hosts and also prevents the establishment of infection [5, 11, 47]. The antioxidant system protects against the damage by scavenging free radicals. The main enzymatic antioxidants are catalase, GPx and SOD while reduced glutathione, vitamin C, vitamin E, β carotene, ceruloplasmin and bilirubin are non-enzymatic factors [21]. Superoxide dismutase converts superoxide radicals into hydrogen peroxide and molecular oxygen, glutathione peroxidase and catalase convert hydrogen peroxide into water. Hence, two toxic species, superoxide radical and hydrogen peroxide are converted into water [53].

The oxidative stress due to *I. hypselobagri* infection in its host and the oxidative stress experienced by the parasites have not been reported earlier. Therefore, the present study aims to estimate the levels of some antioxidant enzymes and lipid peroxidation in the parasite *I. hypselobagri* and the liver, swim bladder, intestine and muscle tissues of the host *Wallago attu*. The intestine has an important function in food digestion and nutrient absorption, however, the intestinal tract is prone to suffering from oxidative damage by the oxidants in foods or the larval stages of helminth parasites migrating through the intestine. The muscle (skeletal muscle) is crucial in maintaining ROS below a threshold level to keep up redox homeostasis. Since, the juvenile worms of *Isoparorchis hypselobagri* penetrate the intestine, liver and swim bladder before transforming into an adult stage, we hypothesise that the migrating juvenile worms cause tissue damage that could result in the down-regulation of antioxidant enzymes.
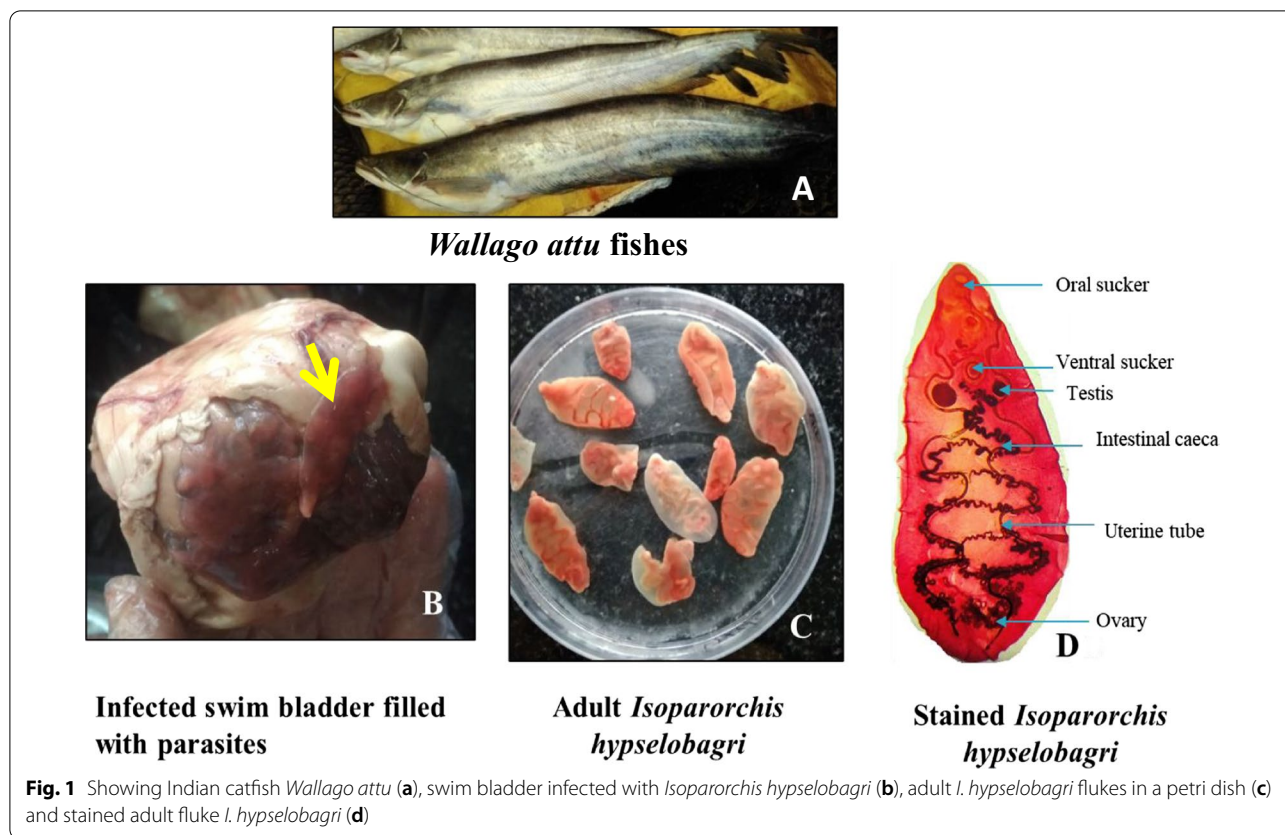
2 Methods

2.1 Collection of samples
Indian catfish *W. attu*, (Fig. 1a) were purchased from the local fish market and brought to the laboratory. Mature *I. hypselobagri* (Fig.1c, d) were collected from the infected swim bladder (Fig. b) and washed several times with 50 mM phosphate buffer solution, pH 7.4 to remove host-related debris. The liver, intestine, muscle and swim bladder of both infected and non-infected fishes were collected to determine the level of antioxidant enzymes.

2.2 Homogenate preparation
Tissue samples were subjected to 10% (w/v) homogenization in chilled phosphate-buffered saline, (pH7.0) using pestle and mortar. The homogenate was centrifuged at $10,000 \times g$ for 10 min at 4 °C; then the supernatant was collected and stored at -20 °C till further use.

2.3 Protein estimation
The protein concentration was estimated by the Bradford dye-binding method (1976) as modified by Spector [48] using bovine serum albumin (BSA) as standard.

Fig. 1 Showing Indian catfish *Wallago attu* (**a**), swim bladder infected with *Isoparorchis hypselobagri* (**b**), adult *I. hypselobagri* flukes in a petri dish (**c**) and stained adult fluke *I. hypselobagri* (**d**)

Coomassie Brilliant Blue G-250 dye was prepared by dissolving 0.01% (w/v) in 5% Ethanol (v/v) and 10% Orthophosphoric acid (v/v). The optical density was recorded at 595 nm on a UV spectrophotometer.

2.4 Estimation of glutathione-S-transferase (GST)
GST enzyme activity was assayed spectrophotometrically at 340 nm following the method of Habig et al. [28]. A total of 3.0 ml reaction mixture contained 50 μl of sample, 2.640 ml of 0.1 M phosphate buffer (pH,6.5), 0.3 ml of 1 mM reduced glutathione and 10 μl of 1 mM 1 chloro-2,4 dinitrobenzene (CDNB). The enzyme activity was expressed as nmol/mg protein/min (molar extinction coefficient $= 9.6 \times 10^3$ M/cm).

2.5 Estimation of superoxide dismutase (SOD)
SOD enzyme activity was assayed spectrophotometrically at 420 nm by the method of Marklund and Marklund [36]. A total of 3.0 ml reaction mixture contained 50 μl of the sample, 2.85 ml of 50 mM Tris cacodylate buffer and 0.1 ml of 0.2 mM pyrogallol. One unit of enzyme activity is defined as the amount of enzyme required to cause 50% inhibition of the rate of pyrogallol auto-oxidation. The enzyme activity was expressed as units/mg protein/min.

2.6 Estimation of glutathione peroxidase (GPx)
Glutathione peroxidase enzyme activity was assayed spectrophotometrically at 340 nm by the method of Flohé's and Günzler [26]. The reaction mixture (1.0 ml) contained 500 μl of 0.1 M potassium phosphate buffer (pH7.0) containing 1 mM EDTA, 100 μl of 10 mM GSH (prepared in water), 100 μl of 1.5 mM NADPH (prepared in 0.1% NaHCO_{3)}, 100 μl of enzyme sample and 100 μl substrate (H_2O_2). The enzyme activity is expressed as nmol/mg protein/ml.

2.7 Estimation of glutathione reductase (GR)
Glutathione reductase enzyme activity was assayed spectrophotometrically at 340 nm by the method of Carlberg and Mannervik [12]. The reaction mixture (1.0 ml) contained 500 μl of 0.2 M potassium phosphate buffer (pH 7.0) containing 2 mM EDTA, 50 μl of 2 mM NADPH (prepared in 10 mM Tris HCl pH 7.0), 50 μl of 20 mM GSSG, 350 μl distilled water. The reaction was initiated by adding 50 μl of the sample. The enzyme activity is expressed as nmol/mg protein/min.

2.8 Estimation of catalase (CAT)
Catalase activity was assayed spectrophotometrically at 240 nm by the method of Aebi [1]. A total of 3.0 ml

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reaction mixture contained 2.85 ml of 50 mM potassium phosphate buffer (pH 7) and 0.05 ml sample. The reaction was initiated by adding 0.1 ml of 30 mM H_2O_2. One unit of enzyme activity is defined as the amount of enzyme needed for degradation of 1 μm of H_2O_2 per min. The enzyme activity is expressed as units/mg protein/min.

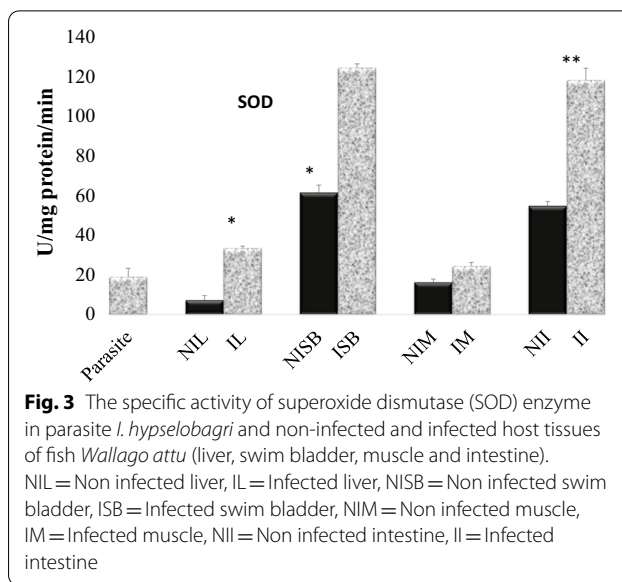### 2.9 Estimation of lipid peroxidation (LPO)

Lipid peroxidation was assessed by determining MDA (a thiobarbituric acid reactive species: TBARS) spectrophotometrically following the method of Beuge and Aust [11]. The reaction mixture contained 1 ml of sample and 2 ml of TBA-TCA-HCl reagent (0.37% thiobarbituric acid, 0.25 N hydrochloric acid and 15% trichloroacetic acid), placed in boiling water bath for 15 min, cooled at room temperature and centrifuged for 10 min. The pink chromogen formed by the MDA-TBA complex was detected at 535 nm. The level of lipid peroxidation was expressed in nmoles of malondialdehyde (MDA) formed/ mg protein.

2.10 Statistical analysis

Statistical analysis was done by student's t-test followed by Prism 8.0.1.244. All the values are mean of three independent replicates ± SEM (Standard error mean). Values of $p < 0.05$, $p < 0.01$, $p < 0.001$ and $p < 0.0001$ were considered to be significant.
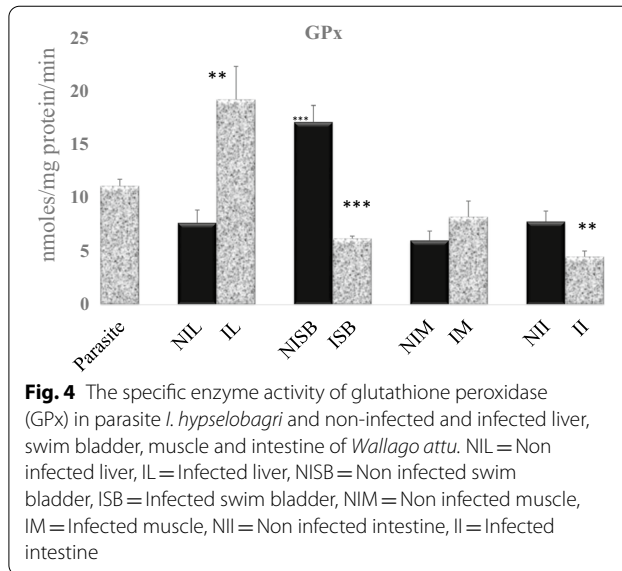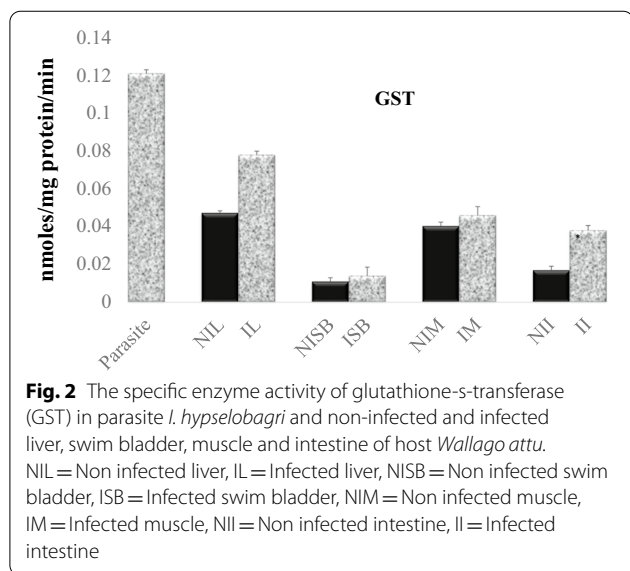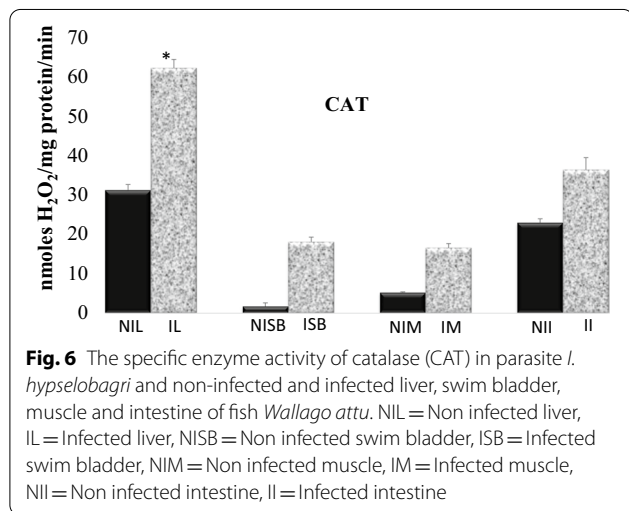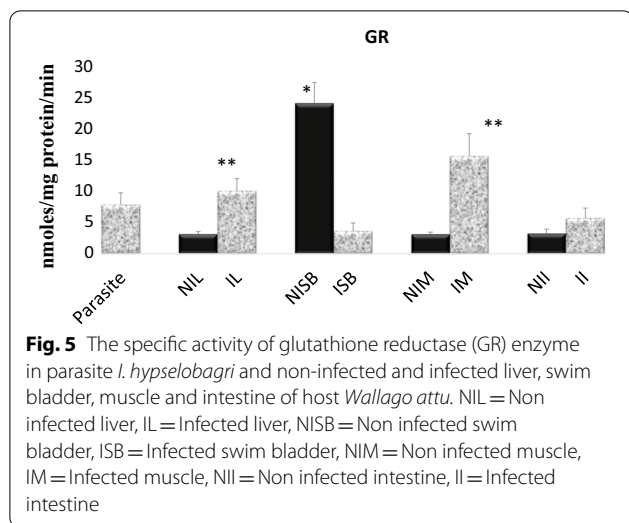
3 Results

During the present study, we observed both qualitative and quantitative differences in the activity of antioxidant enzymes (Figs. 2, 3, 4, 5, 6). An overall comparison of enzyme activities in the infected and non-infected

Fig. 3 The specific activity of superoxide dismutase (SOD) enzyme in parasite *I. hypselobagri* and non-infected and infected host tissues of fish *Wallago attu* (liver, swim bladder, muscle and intestine). NIL = Non infected liver, IL = Infected liver, NISB = Non infected swim bladder, ISB = Infected swim bladder, NIM = Non infected muscle, IM = Infected muscle, NII = Non infected intestine, II = Infected intestine

liver, swim bladder, muscle and intestine of Indian catfish *Wallago attu* revealed a unique pattern in their order of activity. We recorded the higher enzyme activity of SOD followed by Catalase, GPx, GR and GST in the swim bladder, muscle and intestine, while in the liver tissues, Catalase revealed higher activity followed by GPx, SOD and GST enzymes (Figs. 2, 3, 4, 5, 6).In general, we observed that the fishes infected with *I. hypselobagri* demonstrated an elevation in the level of the antioxidant enzyme as compared to non-infected fish.

A higher level of GST was noticed in the liver and intestine of infected fish but there was no significant increase in muscle and swim bladder of the host
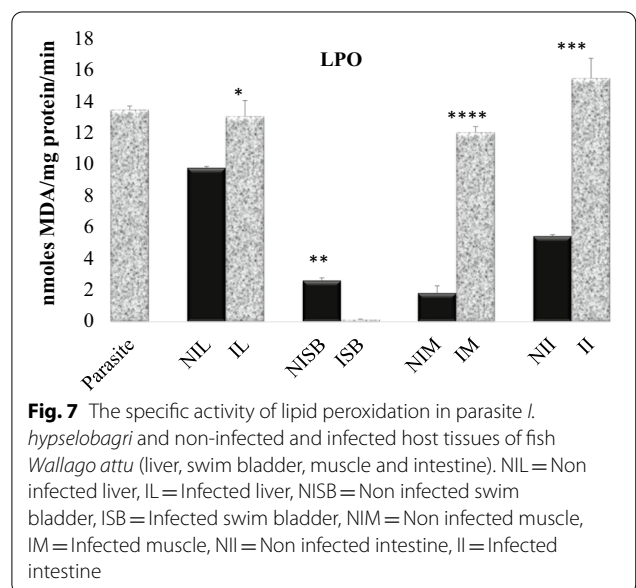
Fig. 2 The specific enzyme activity of glutathione-s-transferase (GST) in parasite *I. hypselobagri* and non-infected and infected liver, swim bladder, muscle and intestine of host *Wallago attu*. NIL = Non infected liver, IL = Infected liver, NISB = Non infected swim bladder, ISB = Infected swim bladder, NIM = Non infected muscle, IM = Infected muscle, NII = Non infected intestine, II = Infected intestine

Fig. 4 The specific enzyme activity of glutathione peroxidase (GPx) in parasite *I. hypselobagri* and non-infected and infected liver, swim bladder, muscle and intestine of *Wallago attu*. NIL = Non infected liver, IL = Infected liver, NISB = Non infected swim bladder, ISB = Infected swim bladder, NIM = Non infected muscle, IM = Infected muscle, NII = Non infected intestine, II = Infected intestine

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Fig. 5 The specific activity of glutathione reductase (GR) enzyme in parasite *I. hypselobagri* and non-infected and infected liver, swim bladder, muscle and intestine of host *Wallago attu*. NIL = Non infected liver, IL = Infected liver, NISB = Non infected swim bladder, ISB = Infected swim bladder, NIM = Non infected muscle, IM = Infected muscle, NII = Non infected intestine, II = Infected intestine

Fig. 6 The specific enzyme activity of catalase (CAT) in parasite *I. hypselobagri* and non-infected and infected liver, swim bladder, muscle and intestine of fish *Wallago attu*. NIL = Non infected liver, IL = Infected liver, NISB = Non infected swim bladder, ISB = Infected swim bladder, NIM = Non infected muscle, IM = Infected muscle, NII = Non infected intestine, II = Infected intestine

(Fig. 2). Elevation in the level of GST by 49.6, 15, 13 and 76.36% in infected liver, swim bladder, muscle and intestine ($p < 0.05$) were recorded as compared to non-infected fish (Fig. 2). It can be surmised that the higher GST activity helps to re-establish the balance between pro-oxidative damage and antioxidants to ease ROS induced oxidative damage. Figure 3, shows a significant elevation in the enzyme activity of SOD in the liver ($p < 0.05$), intestine ($p < 0.01$), muscle and swim bladder ($p < 0.05$) of the infected host which is an indication of increased oxidative stress due to parasitic infection. Further, we observed a significant increase in the level of GPx activity in the liver (86%) ($p < 0.01$) and muscle (31%) while the level decreased significantly in the infected intestine ($p < 0.01$) and swim bladder ($p < 0.001$) respectively (Fig. 4) indicating that this enzyme failed

to influence the expulsion of the parasite. We also observed increased Glutathione reductase (GR) activity in the liver, muscle and intestine of infected fish while the activity decreased in the swim bladder (Fig. 5). GR activity in infected liver ($p < 0.01$), muscle ($p < 0.01$) and intestine increased by 3.1, 5 and 1.75 fold respectively while the activity decreased significantly ($p < 0.01$) by 6.6 fold in the swim bladder (Fig. 5), in comparison to non-infected fish. Figure 6, showed that the enzyme activity of CAT in the infected liver ($p < 0.05$), swim bladder, muscle ($p < 0.05$) and intestine increased by 2, 10 and three fold respectively, as compared to the non-infected host. In the present study, we also observed increased lipid peroxidation activity in the liver, intestine and muscle of the infected host whereas a considerable decrease in the enzyme activity was observed in the swim bladder of infected fish (Fig. 7). We found a significant elevation in the level of lipid peroxidation by 1.3, 2.8 and 6.6 fold in the infected liver ($p < 0.05$), intestine ($p < 0.001$) and muscle ($p < 0.001$), respectively whereas considerable decrease by 18.7 fold in swim bladder ($p < 0.01$) was noticed (Fig. 7).

In addition to examining antioxidant enzyme activities in the tissues of infected and non-infected *W. attu*, we also performed antioxidant enzyme assays in the parasite *I. hypselobagri* which infects the swim bladder of the fish under investigation. Analysis of the result revealed that the parasite possesses an active antioxidant system showing a higher amount of enzyme activity for SOD followed by GPx, GR and GST (Figs. 2–6). Catalase was also examined in the parasite but it did not show any enzyme activity.

Fig. 7 The specific activity of lipid peroxidation in parasite *I. hypselobagri* and non-infected and infected host tissues of fish *Wallago attu* (liver, swim bladder, muscle and intestine). NIL = Non infected liver, IL = Infected liver, NISB = Non infected swim bladder, ISB = Infected swim bladder, NIM = Non infected muscle, IM = Infected muscle, NII = Non infected intestine, II = Infected intestine

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4 Discussion

Antioxidant enzymes represent the first line of defence which has an important role to restrain the damage done by reactive oxygen molecules (ROS) of parasite origin [17], whereas the survival of parasite may rely on its ability to maintain the necessary balance between oxidation and antioxidation [38]. It is well-established fact that the physiological function of the host is altered by high intracellular ROS concentration which weakens the host immune system and becomes more prone to parasitic diseases [39]. While lower levels regulate several physiological mechanisms including cell differentiation, apoptosis and cell proliferation [53].

Liver and intestine tissues of infected fish revealed an elevation in the GST activity whereas no significant increase in GST was recorded in the muscle and swim bladder of the host. In a study by Radovanović et al. [41], higher glutathione-dependent enzyme activities in barbel (*Barbus barbus*) infected with intestinal parasite *Pomphorhynchus laevis* has been observed. Higher antioxidant enzyme activity could be correlated with a greater scale of protection against parasitic infection [27]. Our present study shows a significant elevation of SOD in the liver, intestine, muscle and swim bladder of the infected host. Elevation in the level of SOD in sheep liver infected with *Fasciola* spp. has also been observed by Assady et al. [2], which is an indication of increased oxidative stress due to parasitic infection. Further, in our study, a significant increase in the level of GPx in the infected liver and muscle and a decrease in the infected intestine and swim bladder of *W. attu* has been observed. Increased level of peroxidase activity in the liver due to *F. hepatica* infection has also been observed by Benzer and Ozan [6]. Elevation in the GPx enzyme activity could be suggested as an adaptive change against potential liver injury [21]. The lower level of peroxidase activity in the intestine and swim bladder of infected fish indicated that the GPx enzyme could not compensate for the damage caused by the parasitic invasion. During the present study, infected liver, swim bladder, muscle and intestine revealed a significant increase in CAT enzyme activity. Increased level in the enzyme activity of catalase indicates an accumulation of hydrogen peroxide in infected tissue [27]. Significant elevation of CAT and GST has been reported in the liver and head kidney of *Cyprinus carpio* infected with *Ptychobothryumsp.* [21]. Eissa et al. [23] reported increased SOD, CAT, GR, GPx activities and MDA concentration in liver and muscle tissues of *Tilapia* infected with *Diplostomum* and *Heterophys* sp. Kumar et al. [33] reported induction of antioxidant enzymes (GST, SOD and CAT) in gills and liver tissue of fish *Pangasianodon hypophthalmus* infected with *Thaparocleidus* sp. Our results showed significant elevation in the level of lipid

peroxidation in the infected liver, intestine and muscle whereas a considerable decrease in swim bladder ($p < 0.01$) was noticed. A similar finding has also been reported by Nabi et al. [39] who observed an increased level of LPO in the liver, intestine and muscle of *Schizothorax plagiostomus* infected with *Pomphorhynchus* sp. The enhanced level of LPO protects the parasite from host immune responses. Low level of lipid peroxidation in infected swim bladder may indicate that cells stimulate their maintenance and survival through essential antioxidant defence that upregulate antioxidant proteins resulting in an adaptive stress response [3]. Decreased activity of GR and GPx may be due to protein structure modification leading to change in its function and also responsible for accumulation of peroxides up to toxic level [32].

Analysis of antioxidant enzyme assays in the parasite *I. hypselobagri* under study revealed the presence of an active antioxidant system showing a higher amount of enzyme activity for SOD followed by GPx, GR and GST. Moreover, we observed the absence of catalase activity in the parasite. Our finding is in agreement with the study of Khan et al. [31], who observed that the catalase activity was not present in the somatic extract of the fish infecting parasite, *Clinostomum complanatum* while GST and SOD enzymes have been observed. In parasites, the production of antioxidant enzymes is helpful to avoid immune responses thereby facilitating long term survival in the host. According to Deger et al. [21], the presence of an elevated level of antioxidant enzyme in the host is to provide a cellular protective role to oppose the oxidative stress generated by parasitic invasion. It is reported that parasitic infection can disturb the metabolic activity of its host [10].The antioxidant defence system of the parasite helps to tackle the host's immune responses for successful establishment and long-term survival in the definitive host. Parasites need protection from reactive oxygen species that arise from host phagocytic cells [19] and [22]. Antioxidant molecules of parasite origin represent a faceted shield in drug resistance, as well as in the modulation of the host immune response. Thus, antioxidants are not only a barrier but an essential factor to establish the host–parasite relationship [17]. The increase or decrease in the activity of different antioxidant enzymes in host tissues infected with *I. hypselobagri* during the present study can be linked to modulated metabolic activity in the host owing to parasitic load.

5 Conclusions

It is concluded that infection of parasite *I. hypselobagri* induces oxidative stress which results in the generation of reactive oxygen species in the host *Wallago attu*, with modulation in the level of essential antioxidant enzymes (GST, SOD, GPx, GR, CAT and LPO). Parasitic infection

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induces significant oxidative stress which is evident from the increased antioxidant enzymes and lipid peroxidation causing a pathogenic response in the host. Moreover, the antioxidant defence system of the parasite helps to tackle the host's immune responses for successful establishment and long-term survival in the definitive host.

Availability of data and materials
All data generated or analysed during this study are included in this published article.

Declarations

Ethics approval and consent to participate
Not applicable. Since we purchased dead fishes from the local fish market to collect the parasite *Isoparorchis hypselobagri* (from the swim bladder), needs no approval from the Ethical committee.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

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