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Study of Ultrastructural Appraisal, Haemato-biochemical Profiling and Elemental Mapping in Liver of *Heteropneustes fossilis* (Bloch, 1794) Naturally Infected with Cestode Parasite

Raghuveer Kumar Gupta [a], Raghvendra Niranjan [a] and Malabika Sikdar [b++*]

[a] Fish Parasitology Lab., Department of Zoology, Dr. Harisingh Gour University, Sagar, India.
[b] Department of Zoology, Dr. Harisingh Gour University, Sagar, India.

Authors' contributions

This work was carried out in collaboration among all authors. Author RKG conceptualized the research work, wrote original draft, performed the methodology and did formal analysis. Authors RKG and RN reviewed and edited the manuscript and author MS reviewed and edited, did visualization and supervised the study. All authors read and approved the final manuscript.

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[++] Associate Professor;
**Corresponding author: Email: msikdar@dhsgsu.edu.in;*

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ABSTRACT

The pathological deformities and systemic inflammatory response induced by infection is considered to be an important feature of the pathophysiology of cestode parasite. In this study, it was aimed to determine histopathology, oxidative status and pathological markers for infection as well as check health status. A sample of thirty *Heteropneustes fossilis (H. fossilis)* fish was used for this purpose, of which fifteen were healthy and fifteen were naturally infected with cestode parasite. After dissection cestode parasite infection was confirmed during the examination of fish. Infected liver tissue was tested for histopathological deformities, oxidative stress parameter malondialdehyde (MDA) and antioxidants i.e., superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), glutathione reductase (GR) and glutathione content (GSH). We used blood serum for estimation of pathological injury markers i.e., Aspartate amino transferase (AST), alanine amino transferase (ALT), acid phosphatase (ACP) and alkaline phosphatase (ALP). For health checkup was perform elemental mapping, hematological analysis and some nutrition related biochemical. We found histopathological changes and Oxidative stress i.e., MDA, SOD, CAT and GPx were increased but GR and GSH was decreased in infected liver of *H. fossilis*. Pathological markers i.e., AST, ALT, ACP and ALP were also increased which are indicate that liver are infected due to cestode parasite. During health check-up we found that, most of minerals was decreased except sodium (Na) in EDS mapping and white blood cells (WBCs) were increased but level of red blood cells (RBCs) decreased. Serum protein (g/dL) was decreased but glucose was (mg/dL) in infected *H. fossilis.* It can be stated that, histological injuries, oxidative stress and Pathological damage markers may increase in infected tissue which sing of infection as well as nutritional status decreased in naturally infected fishes with cestode parasite.

Keywords: Cestode parasite; H. fossilis; pathological injury; oxidative stress; elemental mapping; health status.

1. INTRODUCTION

Fish plays a vital role in a balanced diet because it's an excellent source of protein and minerals. High-quality protein found in fish, is needed for the synthesis of hormones and enzymes as well as for the maintenance and repair of tissues. In addition to being highly digestible, fish protein provides all the essential amino acids that the body requires especially high in omega-3 fatty acids [1]. These fats are essential for heart health, brain function, and lowering body inflammation. Fish is a great source of nutrients like iodine, potassium, sodium which is crucial for thyroid function and selenium, which functions as an antioxidant and shields cells from harm [2]. Fish also contains minerals, such as iron and zinc.

Aquaculture is one of the possible foods producing industries which provide quality fish production to world expending population with generates income and the employment [3]. An essential first step in raising fish production to satisfy the rising demand is species diversification in aquaculture [4]. India has been supplying the world with high-quality fish output in recent years. India is the world's second-largest producer of aquaculture and the third-largest producer of fish therefor 8% of fish produced in the world. In 2022–2023, 16.24 million tons of fish was produced. In which 4.12 million tons come from marine fish and 12.12 million tons came from aquaculture in fresh water. Indians use many types of fishes for freshwater aquaculture. In which mostly use of catfishes i.e., *Pangasius pangasius. Clarius batracus, H. fossilis, Clarius gariepinus. H. fossilis* is the subject of this article because it has successfully diversified aquaculture species, as seen by its increasing popularity throughout the India. Mostly find in India, Sri Lanka, Thailand, Myanmar, Bangladesh, Pakistan, Nepal and Bhutan. *H. fossilis*, also known as fossil cat or Asian stinging catfish. These fishes of also called ‘singhi’ in Indian local language and some south state ‘tarru’. It is found mainly in ponds, swamps, and marshes, but sometimes occurs in muddy rivers [5]. It can tolerate slightly brackish water. It is omnivorous. This species breeds in confined waters during the monsoon, but can breed in ponds, derelict ponds, and ditches when sufficient rainwater accumulates. It is in great demand due to its alleged medicinal value. This species grows to a total length of 30 cm (12 inch), and is an important fish of local commercial fisheries [6]. It is also farmed and found in the aquarium trade. In

India, *H. fossilis* is available in local markets and is well-liked for its test. Fish markets frequently sell it live or fresh. It is Considered a wholesome food item, *H. fossilis* is high in protein, minerals and some other nutrition [4,5]. Tradition medical systems also have the belief that it possesses therapeutic qualities [7]. With a variety of programs and efforts, state and federal governments in India have been encouraging aquaculture, particularly *H. fossilis* (singhi) cultivation, in an effort to boost food security and assist regional farmers [6,7].

In *H. fossilis* forming, the contaminated water quality, disease outbreaks, and market competitiveness are all obstacles [8]. On the other hand, *H. fossilis* is quite hardy, thus it can withstand some of these difficulties. Nonetheless, parasite disease is the most obstacle to *H. fossilis* cultivation [9]. Mostly, *H. fossilis* infected by helminth parasites. Depending on the type of parasite and the infection's location, fish infections can result in different kinds of tissue damage [10]. Helminths are class of parasite that clings to fish gills, intestine, liver or skin can inflict mechanical harm by eating on the tissues of their hosts or just by being there [7,10]. This can result in tissue erosion, irritation, and inflammation [10]. Fish that have hemorrhage (bleeding) in their tissues due to parasites, such as some worms and protozoa, may become weaker and more prone to diseases [11]. A fish's inflammatory response to parasites may result in swelling, redness and tissue damage in the afflicted area. Damage can be caused to internal organs including the liver, kidneys or intestines by parasite infections. Some common helminth parasite diseases found in catfish i.e., heteropolaria and gyrodactylus [12]. In helminth parasite including cestodes, which are highly prevalence to create infection in *H. fossilis* [11]. Infected fish with cestodes, are create in health problems in body. Usually, these parasites have a convoluted life cycle involving several hosts [12,13]. Cestodes frequently infect the gastrointestinal system of fish, where they can harm the fishes and compromise its general health [13]. Fish with cestode infection exhibit symptoms like slowed growth, weight loss, behavioral abnormalities and in extreme situations organ damage [11-13]. Fish health can be evaluated with the help of histopathology and biochemical which reveal details on the extent of injuries, tissue damage, and organ function [13]. Inadequate nutrition can also lead to fish tissue stress. Conversely, the level of contamination present in fish tissue can influence the potential health risks associated with consuming contaminated fish. Eating raw or undercooked fish contaminated with protozoan, cestode, trematode, and nematode parasites can lead to parasitic illnesses in humans [11-14]. These infections can cause allergic reactions, gastrointestinal distress, and in extreme situations intestinal blockage. In the histology of fish organs i.e., intestine, liver, and gills are crucial to the pathological alterations brought on by endogenous and exogenous as well as lead to stress responses [12-14].

Numerous research conducted in the past have evaluated the histopathological alteration, oxidative stress response and pathological injury markers may contribute to understanding the host response to pathogenic invasion [15,16]. On the other hand, hematological and some biochemicals parameters, which is revel the nutrition status in fish body. But there is no study explaining the health status of parasitic infection caused by cestode in the *H. fossilis*. In this study, we revealed how cestode parasitic infections are creating alterations in health of freshwater catfish i.e., *H. fossilis*. For doing the same, we have done histopathology, biochemical, hematological and mapping of minerals in infected and non-infected fish tissue, which are important to evaluate the impact of parasites on fish tissue by identifying early cell damage and indicating fish health. Next, we have estimated enzymatic and non-enzymatic antioxidant levels against oxidative stress. Hematological parameters, mineral content and biochemical constitute an important tool that reveals the health state of fish.

2. MATERIALS AND METHODS

2.1 Collection of Fishes and Parasite

In the spring season between January 2023 - December 2023, we have collated fifteen *H. fossilis* fishes naturally infected with cestode parasite and fifteen non-infected *H. fossilis* fishes at local fishpond of Shahdol district, M.P., (India). The weight was both sex between 150-200 gram. All the fishes were carefully packed in aerated polythene beg and keep the laboratory, Department of Zoology, Dr. Harisingh Gour University, Sagar. During fish collection, Infected fishes examine according to external symptoms i.e., skin lesion, opaque eyes, lethargy and excessive mucus production of body surface and gills. Identification of fish on the basis of morphological characters according to Jhingran [17]. For parasites, firstly we examined surface of

gills after this dissect the fish and examine carefully all body organs of fish. We found parasites mostly presented in liver followed by intestine, stomach and body cavity in fish body. After the collected of parasites keep in saline water (0.9% NACL) and immediately transfer for fixation according to Marcogliese [18]. After 24-hour prepare slide and watch under stereo zoom microscope. Parasite identified by morphological characters according to Yamaguti [19].

2.2 Histopathological Finding

During the examine of parasites in fish body, if found parasites after this collated the liver tissue and immediately transfer to fixative for 24 h according to Margolis and Carleton [20,21]. After 24 h was washed the tissue and kept in ascending alcohol series and fix in paraffin wax. Section cut of wax embedded tissue by microtomy (3-5 µm) and prepare hematoxylin-eosin (HE) stanning slide and watch under EVOS light microscope according to Marcogliese and Margolis [18,20].

2.3 Scanning Electron Microscope (SEM)

No-infected and infected liver tissue of *H. fossilis* were fixed with 2.5% glutaraldehyde, then fixed in 1% osmium tetroxide. After this tissue were dehydrated with ethanol descending series and dried. Finaly tissue was mounted with gold sputter-coated and examined using scanning electron microscope (JSM-6380 LA SEM) at 0.3–30 kV according to Amann and Drüeke [22,23].

2.4 Oxidative Stress and Antioxidant Analysis

The level of oxidative stress and amount of antioxidant enzymes were estimated in the liver of *H. fossilis* which were infected and those that were not. Firstly, followed the process of formation of tissue homogenates. The homogenate was produced with saline water. liver was homogenized in 1: 4 with saline water (0.9 % NACL) and supernatant was collated. After these processes of centrifugation with 3000 rpm were perform for 10 minutes and preserved the supernatant at 4°C for further study [24].

MDA level was assayed given method by Niehaus and Samuelson [25]. MDA is a biomarker of lipid peroxidation. Firstly, 1.3 ml of potassium chloride (KCL) buffer (pH 7.4) and 1.5 ml of Ethylene-diamine-tetra acetic acid (EDTA) buffer was added with 0.2 ml of tissue homogenate. Thereafter mixer was heated for 10 minutes in boiling water. After this cooling, 1 mol/l NaOH and 3 ml of pyridine–butanol (3:1) were added and measuring the absorbance at 548 nm. The MDA unit was expressed as (mM/100g protein).

SOD activity in homogenate supernatant was determined by method of Kakkar and Viswanathan [26]. Firstly, 10 µl of supernatant was added with 970 µl of buffer (1 mM EDTA (pH 8.2) and 100 mM Tris-HCl) and heated. After some time, again added 20 µl of 13 mM pyrogallol with mixture and absorbance was recorded at 480nm. The expression of value was (U/mg protein).

Estimated the level of CAT by calorimetrically at 620 nm described by Maehly and Chance [27]. Briefly, 1.0 ml of 0.01 M phosphate buffer (pH 7.0) and 0.1 ml of Plasma was added in 1.5 ml of homogenate supernatant and boiling with water for 10 minutes. After this cooling, 0.4 ml of 2 M hydrogen peroxide (H2O2) and 2.0 ml of dichromate-acetic acid reagent was added. Reeded the absorbance level and value expressed as (IU/mg protein).

GPx activity were perform according to method given by Rotruck at. al [28]. Briefly, the (1.0 ml) supernatant was mixed with 450 µl of 0.1 M potassium phosphate (pH7.0) buffer and 100 µl of 10 mM GSH. After this, added 1 mM EDTA and 100 µl of H_2O_2 in the mixture. The mixture was measured absorbed at 340 nm by spectrophotometer. The GPx value was expressed as (µ gram/mg protein).

GR activity was measured according to Elia et. al [29]. Firstly, (1.0 ml) homogenate supernatant was heated with 500 µl of 0.2 M potassium phosphate (pH 7.0) buffer and 2 mM EDTA. After cooling, added 50 µl of 2 mM nicotinamide adenine dinucleotide phosphate hydrogen (NADPH) and the mixture was measured absorbance at 340 nm. The GR value was expressed as (n Mole/mg protein).

GSH level estimate use the method by Mannervik [30]. In briefly, 150 µl supernatant was centrifuged at 1500 rpm form 10 minutes. Thereafter 1.5 ml of 10 % tricarboxylic acid cycle (TCA) and 3 ml of 0.2 M phosphate buffer (pH 8.0) was mixed in the supernatant and treated with 0.5 ml of Ellman's reagent. Absorbance was measured at 412 nm. Represented the value as n Mole/100-gram protein).

2.5 pathological Injury Marker

Some serum pathological marker enzyme i.e., Aspartate amino transferase (AST), alanine amino transferase (ALT), acid phosphatase (ACP) and alkaline phosphatase (ALP) was assayed according to method given by Bergmeyer at. al and Reitman [31,32]. The value was expressed as (U/L).

2.6 SEM/EDS for Mineral Analysis

For elemental mapping, use EDAX (Energy-dispersive X-ray spectroscopy) equipped with SEM. We estimated the elemental composition of the non-infected and infected liver tissue with EDS. This technique was conducted at Center for advance research (CAR), Dr. Harisingh Gour University, Sagar, (M.P.) India. Prepared sample placed in the chamber of SEM and maintain in low-vacuum at 21 °C and 55–60 % humidity was used to analyze. Elemental analysis was carried out in the scanned area using EDS detector method given by Amann and Drüeke [22, 23]. Backscattered and Secondary electron images were recorded for elemental mapping on the cross-section of infected and non-infected liver tissue.

2.7 Serum Biochemical Analysis

Prior to sacrifice, *H. fossilis* were given anesthesia and immediately removed blood in their body and kept in ice box. Thereafter, according to Niehaus and Samuelson [25] blood was centrifuged at 1000 rpm for 10 minutes and supernatant was collated for further serum analysis. Results were recorded by spectrophotometer. Estimation of glucose (mg/dL) level was done method developed by Cooper and McDaniel [33]. According to Lowry et al. [34] protein was estimated and value expressed as (g/dL). Albumin globulin and A/G ratio were measured according to Lowry et al. [34] and value expressed as (g/dL) and (%).

2.8 Hematological Parameters

We have followed standard protocols for estimation of hematological parameter. According to [15] was Used Neubauer's haemo-cytometer for counting of white blood cells (WBC) and red blood cells (RBC). The value was represented (106x/mm3) and (103x/mm3) respectively. For hemoglobin (Hb) estimation was used Sahli's acid haemitin method as described by [16] and value expressed as (g/dL). Packed cell volume (PCV) was estimated micro-hematocrit method given by [17] and value was expressed as (%). Another parameter of hematology i.e., mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) were assayed using formulae given by Hesser [35]. Value expressed given below.

$$\text{MCV (femtoliters)} = \frac{\text{PCV}}{\text{RBC}} \text{X } 10$$

$$\text{MCH (femtogram)} = \frac{\text{Hb}}{\text{RBC}} \text{X } 10$$

$$\text{MCHC (\%)} = \frac{\text{Hb in mg blood}}{\text{RBC}} \text{X } 10$$

2.9 Statical Analysis

Statical Data was analyzed according to Nash et. al [36]. All values are reported with three replicates. Bars represent Mean ± SD (Standard deviation) with value are statistically significant at P value <0.05 (Independent T-Test) by prism software version (9.1) followed ^{ns} (P value >0.05), *(P value <0.05), **(P value< 0.01) and ***(P value <0.001).

3. RESULTS

3.1 Clinical Examination and Parasitic Finding

Naturally infected *H. fossilis* showed abnormal swimming behavior, respiratory distress symptoms of asphyxia. A clinical examination of *H. fossilis* observe body secreting excess mucus and skin abrasion in ventral side of body skin that progress to skin ulcer (Fig. 1a). During the external examine of *H. fossilis* found clinical deformities in gills i.e., Pale gills, densely attached to each other, excess mucus production and the color of gills was whitish (Fig. 1b). After that, dissected the fish and examine the all-body parts found that, serosanguinous fluid in abdominal body cavity. We observe, the numbers of parasite were different body region i.e., intestine, liver, abdominal cavity and surface of gill epithelium. In which mostly parasites were found on liver or nearby liver. In (Fig. 1c) showing the image of liver with excessive mucus production and disrupted the surface of liver. We have collated the parasite which are found in body of fish and further examine. After that, we were done slide preparation and imaging. We examined these parasites are cestode on the

basis of morphological characters according to Yamaguti [19]. In (Fig. 1d) showing rostellum with hooks in anterior portion of parasite. Another image of posterior portion of parasite also called proglottids (Fig. 1e). In both images showing segmented body which are fundamental character of cestode parasite.

3.2 Histopathological Finding in Liver

In previous research we found that many organs were damage in fish due to parasitological infestation in fishes. Therefore, in this article we examined the liver tissue of *H. fossilis* infested with cestode parasite. We found that, in liver cells damaged due to cestode infestation (Fig. 2). We noticed cestode parasite is disrupt the many hepatocytic cells and create lesion (Fig. 2b). When we observed in different area of infected liver tissue found that many hepatic cells are disappeared and bust the vacuole (Fig. 2d). Thereafter observed again in different side of cells seen numbers of cytoplasmic granule are distributed in cells (Fig. 2f) compare to non-infected which was indicating cells are infested with cestode parasite.

3.3 Scanning Electron Photomicrograph in Liver

For again confirmation of histological deformities in liver of *H. fossilis* due to cestode infestation compared the non-infected and infected liver tissue by scanning electron microscope (Fig. 3). We found hydropic swelling in hepatocytes of liver (Fig. 3b) due to cestode infestation. Liver cells is showing mitochondrial granular hepatocytes are lofted and large size of vacuoles (Fig. 3d) as compare to normal liver cells. We observed that, in infected liver sinusoids are disrupted and accumulation of numerous dark granules in hepatocytic cells with confirming that liver cells are injured due to cestode parasite (Fig. 3f).

3.4 Oxidative Stress and Antioxidant Status

We have observed the level of oxidative stress in infected liver of *H. fossilis* as well as antioxidant status (Fig. 4). We found that, Level of MDA of non-infected liver was 0.138 ± 0.05 (m M/100g) and infected was 0.188 ± 0.008 (m M/100g) which are significantly ($P < 0.007$) increases showing in (Fig. 4a). Estimation of enzymatic antioxidant i.e., SOD, CAT, GPx and GR level of infected tissue was 7.65 ± 0.64 (U/mg prot.), 18.76 ± 1 (IU/mg prot.), 12, 22.04 ± 1.0 (µg./mg prot.) and 8.95 ± 0.07 (n mole/mg prot.). On the other hand, in non-infected liver tissue showing value of SOD, CAT, GPx and GR was 4.12 ± 0.22, 14.03 ± 1.05, 18.98 ± 0.5 and 8.95 ± 0.07. Therefor SOD, CAT and GPx were significantly increased ($P < 0.008$), ($P < 0.006$) and ($P < 0.006$) but GR level was significantly (0.006) decreased (Fig. 4b, c, d, e). Another non-enzymatic antioxidant GSH level of non-infected liver was 1353.54 ± 56.5 (n Mole/100g prot.), while in infected liver was 830.32 ± 36.5 which are significantly ($P < 0.002$) decreased (Fig. 4f).

3.5 Pathological Injury Marker

We have estimated pathological injury marker of infected and non-infected liver of *H. fossilis* (Fig. 5). We observed that, in non-infected liver tissue was level of AST, ALT, ACP and ALP are 41.03 ± 5.5, 18.00 ± 3.0, 28.66 ± 7.37 and 36.66 ± 5.03 (U/L) respectively and level of infected liver tissue was 64.33 ± 7.09, 41.66 ± 5.5, 43.66 ± 3.05 and. 62.33 ± 6.02 (U/L) respectively. Therefore, we found that, level of ALT ($P < 0.002$) and ALP ($P < 0.004$) was more significant as compare with AST ($P < 0.01$) and ACP ($P < 0.03$).

3.6 Elemental mapping with SEM/EDS

Image showing the Surface topography of non-infected and infected liver of H. fossilis assessed by SEM in the (Fig. 6a, 7a). As well as elemental mapping images of Na, Ca, Mg, Zn, Fe and K are shown in (Fig. 6a-f, 7a-f) by EDS. We found that, composition of mineral content of non-infected and infected liver of *H. fossilis* was significantly different as above mention in (Fig. 6,7). In the element composition of the non-infected liver of *H. fossilis*, Ca was the predominant element followed by Na and Mg. The Ca concentration was significantly lower ($P < 0.008$) in the infected (23.08 ± 1.61%) (Fig. 7b.VI) than non-infected (30.15 ± 1.91%) (Fig. 6b.VI). And the Mg concentration was no significantly lower ($P < 0.09$) (Fig. 7b. IV) in the infected (19.05 ± 1.49%) than non-infected (22.23 ± 2.05%) (Fig. 6b. IV). For Na, significant difference was found between non-infected and infected liver tissue of *H. fossilis*. Na concentration was significantly higher ($P < 0.005$) (Fig. 7b.I) in the infected (44.83 ± 0.60%) than non-infected (32.82 ± 1.95%) (Fig. 6b.I). Other elements i.e., Zn, Fe and K found as very trace elements as compare to Na, Ca and Mg (Fig. 6b, 7b.). The element Zn was present at a concentration in non-infected liver of *H. fossilis* (16.69 ± 0.71%) (Fig. 6b, 7b), but was found to

significantly (P < 0.001) decrease in infected liver (9.49 ± 1.24%) (Fig. 7b. II). Other than this, Fe and K concentration of non-infected liver was (1.55 ± 0.12%) (Fig. 6b. II) and (0.53 ± 0.02%) (Fig. 6b.III) respectively. But in infected liver concentration of Fe was (0.16 ± 0.01%) (Fig. 7b.V) and K was (00) (Fig. 7b.III). Therefor Fe and K was significantly lower (P < 0.01) and (P < 0.01) as compare to non-infected liver of *H. fossilis*.

Table 1. Showing elemental weight of non-infected and infected *H. fossilis*.

Elements Name	Series	No-Infected Mean ±SD	Infected Mean ± SD
1. Na (%)	K-Series	32.82 ± 1.95	44.83 ± 0.60
2. Ca (%)	K-Series	30.15 ± 1.91	23.08 ± 1.61
3. Mg (%)	K-Series	22.23 ± 2.05	19.05 ± 1.49
4. Zn (%)	L-Series	16.69 ± 0.71	9.49 ± 1.29
5. Fe (%)	K-Series	1.55 ± 0.12	0.16 ± 0.01
6. K (%)	K-Series	0.53 ± 0.02	00

*Note: In this table showing elemental weight % (E.Wt.) of non-infected and infected partially cross section of liver of H. fossilis. Table are represented with SEM/EDS analysis. All values are representing Mean ± SD with value is statistically significant at P value < 0.05 (Independent T-Test) by prism software version (9.1) followed ^{ns} (P > 0.05) *(P value < 0.05), **(P value < 0.01) and ***(P value < 0.001). Value is reported with three replicates*

Table 2. Showing hematological parameters of non-infected and infected *H. fossilis*

		Non-infected		Infected		
S. No.	**Parameters**	**Range**	**Mean ± SD**	**Range**	**Mean ± SD**	**P Value**
1.	**RBCs**(x10^6/mm$^{3)}$	3.98 - 4.89	4.53 ± 0.4	2.47 - 3.63	3.11 ± 0.5	0.03*
2.	**WBCs**(x10^3/mm$^{3)}$	38.66 - 43.59	41.72 ± 2.6	59.42 - 64.56	62.64 ± 2.8	0.008***
3.	**Hb** (g/dL)	9.02 - 11.02	10.1 ± 1.0	6.03 - 7.06	6.38 ± 0.5	0.005**
4.	**PCV** (%)	36 - 40	37.66 ± 2.08	26 - 32	27.00 ± 4.5	0.02*
5.	**MCV** (fL)	83.02 - 96.88	89.18 ± 7.05	98.02 - 104.02	101.67 ± 3.2	0.04*
6.	**MCH** (fg)	32.66 - 34.72	33.4 6 ± 1.01	41.02 - 43.22	42.10 ± 1.08	0.006***
7.	**MCHC** (%)	33.72 - 39.02	36.58 ± 2.6	23.44 - 26.02	24.51 ± 1.3	0.002**

*Note: RBC, red blood cells; WBCs, white blood cells; Hb, haemoglobin; PCV, packed cell volume; MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration. All values are representing Mean ± SD with value is statistically significant at P value < 0.05 (Independent T-Test) by prism software version (9.1) followed ^{ns} (P > 0.05) *(P value < 0.05), **(P value < 0.01) and ***(P value < 0.001). Value is reported with three replicates.*

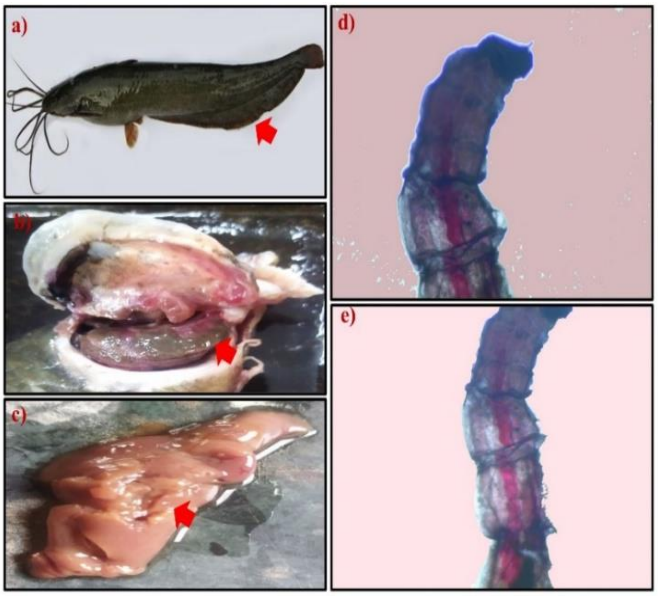

Fig. 1. Showing Clinical sign and postmortern examination of naturally infected *H. fossilis* as well as parasite finding. a) Showing image of infested *H. fossilis* fish collected from Shahdol district. b) Showing photo of contaminated gills of *H. fossilis* during external examination c) Click the photo of infected liver by digital camera showing excessive mucus. d) Anterior region of cestode parasite. e) Posterior region of cestode parasite

Table 3. Showing serum biochemical of non-infected and infected *H. fossilis*.

		Non-infected		Infected		P Value
S. No.	**Parameters**	**Range**	**Mean ± SD**	**Range**	**Mean ± SD**	
1.	**Glucose** (mg/dL)	136 - 143	139.00 ± 3.6	152 - 172	157.33 ± 12.85	0.07^{ns}
2.	**Protein** (g/dL)	4.8 - 5.3	5.26 ± 0.4	2.7 - 3.9	3.43 ± 0.6	0.01^{*}
3.	**Albumin** (g/dL)	2.93 - 3.02	2.97 ± 0.04	1.32 -1.98	1.68 ± 0.3	0.002^{**}
4.	**Globulin** (g/dL)	2.01 - 2.16	2.38 ± 0.5	0.98 - 1.76	1.68 ± 0.3	0.1^{ns}
5.	**A/G ratio**	2.53-2.76	2.62 ± 1.2	1.49-1.98	1.73 ± 0.2	0.005^{**}

*Note: In this table estimated level of glucose (mg/dL), protein (g/dL), albumin (g/dL), globulin (g/dL) and A/G (%) ratio. All values are representing Mean ± SD with value is statistically significant at P value < 0.05 (Independent T-Test) by prism software version (9.1) followed ^{ns} (P > 0.05) *(P value < 0.05), **(P value < 0.01) and ***(P value < 0.001). Value is reported with three replicates*

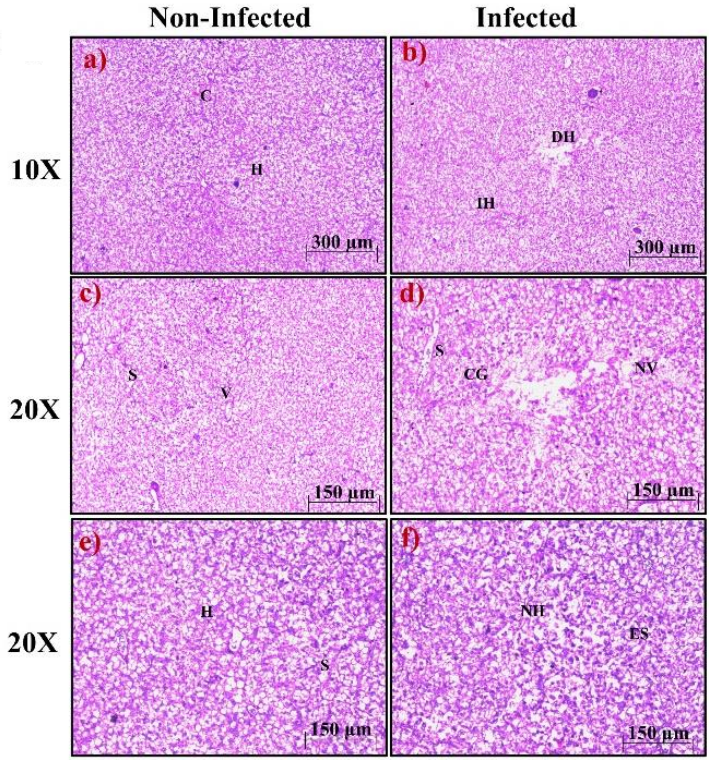

Fig. 2. Photomicrograph of non-infected and infected liver cells of *H. fossilis*. a) Showing normal hepatocytes cells (H) as well sinusoids with cytoplasmic granules (C) in liver (10X). b) Image showing irregular size and shape of hepatic cells (IH) and disappeared hepatic cells (DH) (10X). c) Showing image of storage vacuole (V) and sinusoids (S) (20X). d) Image showing necrosis of storage vacuoles (NV), disrupt the sinusoids (S) and disperse the cytoplasmic granules (CG) (20X). e) image showing some other area of non-infected liver i.e., proper shape of hepatic cells (H) and arrange linear fashion of sinusoids (S) (20X). f) showing necrosis of hepatic cells (NH) and excessive production of cytoplasmic granules between cells (ES) (20X)

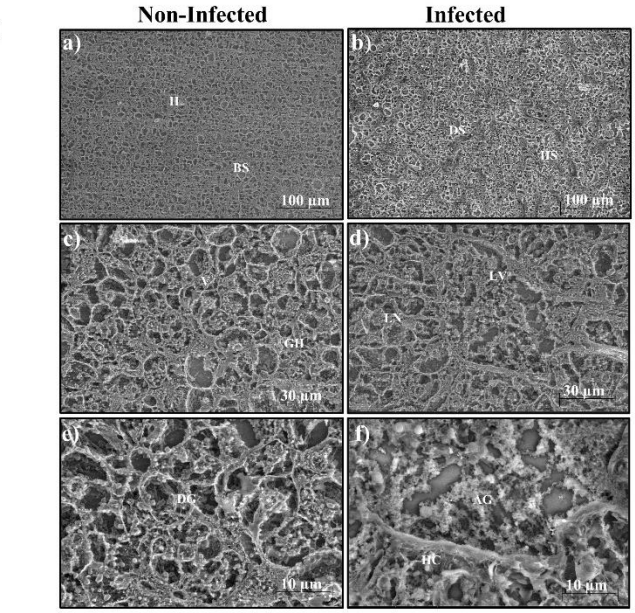

Fig. 3. Scanning electron topographic showing image of liver cells of *H. fossilis*. a) image of non-infected liver showing normal hepatocytic cells (H) and blood sinusoids (BS). b) image of non-infected liver showing dilation of sinusoid with blood congestion (DS) and hydropic swelling of hepatocytes (HS). c) image showing normal size of vacuole (V) and proper arrangement mitochondrial granular hepatocytes (GH). d) in infected liver showing large size of vacuoles (LV) and large number of mitochondrial granular hepatocytes are lofted (LN). e) A slight accumulation of dark granules is showing in some hepatocyte cells (DG). f) showing extensive hydropic swelling of many hepatocytes (HC) and Accumulation of numerous dark granules (AG) were also seen in many hepatocytes

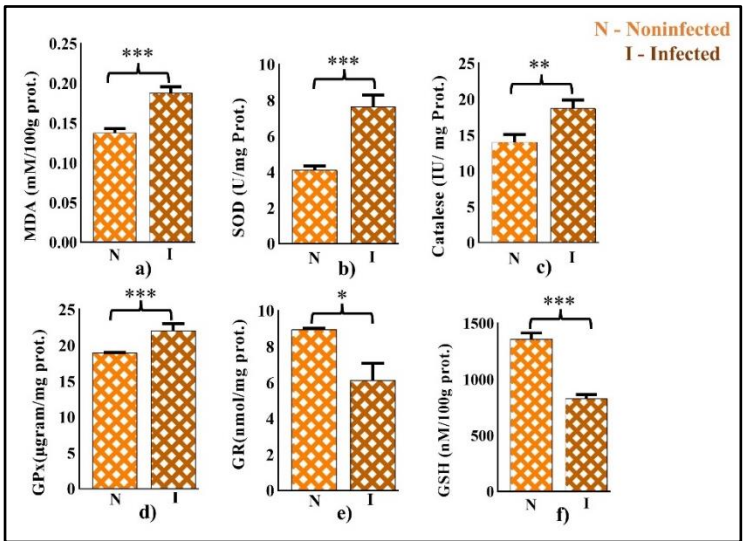

Fig. 4. Graph showing for oxidative stress MDA level and antioxidant level i.e., SOD, CAT, GPx, GR and GSH of non-infected and infected liver of *H. fossilis*. All values are reported with three replicates. Bars represent Mean ± SD (Standard deviation) with value are statistically significant at P value <0.05 (Independent T-Test) by prism software version (9.1) followed ^{ns} (P value >0.05), *(P value <0.05), **(P value< 0.01) and *(P value <0.001)**

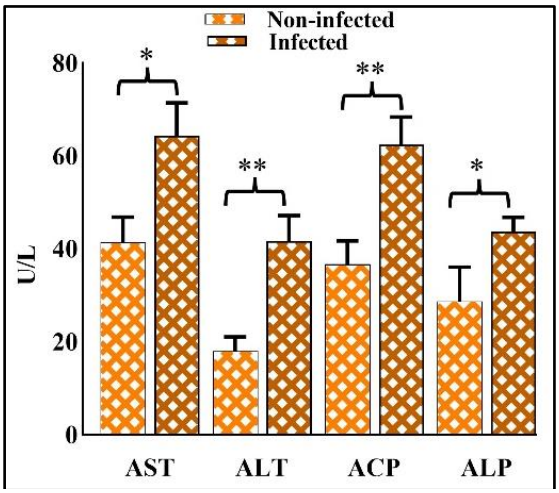

Fig. 5. Graph showing for pathological injury marker i.e., Aspartate amino transferase (AST), alanine amino transferase (ALT), acid phosphatase (ACP) and alkaline phosphatase (ALP). All values are reported with three replicates. Bars represent Mean ± SD (Standard deviation) with value are statistically significant at P value <0.05 (Independent T-Test) by prism software version (9.1) followed ^{ns} (P value >0.05), *(P value <0.05), **(P value< 0.01) and *(P value <0.001)**

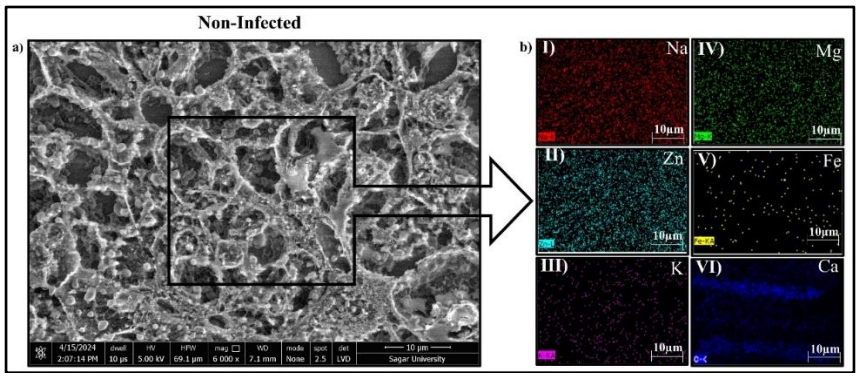

Fig. 6. SEM Image of partially cross section of non-infected liver of *H. fossilis*. a) image showing area of multi-element EDS mapping. b) SEM-EDS digital images of elements Na, Mg, Zn, Fe, K and Ca

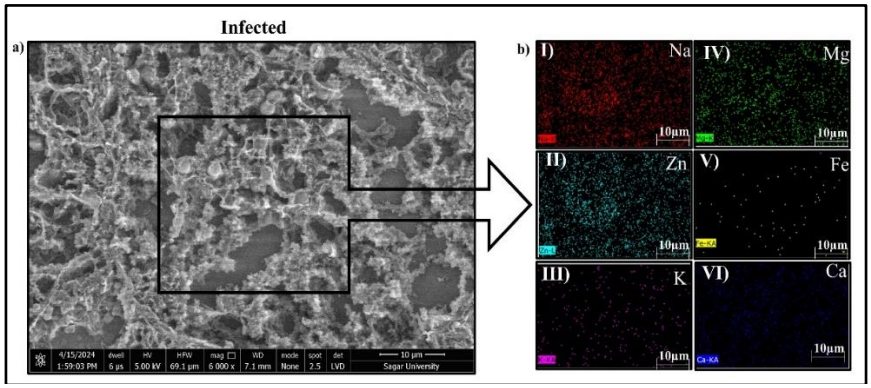

Fig. 7. SEM Image of partially cross section of infected liver of *H. fossilis*. a) image showing area of multi-element EDS mapping. b) SEM-EDS digital images of elements Na, Mg, Zn, Fe, K and Ca

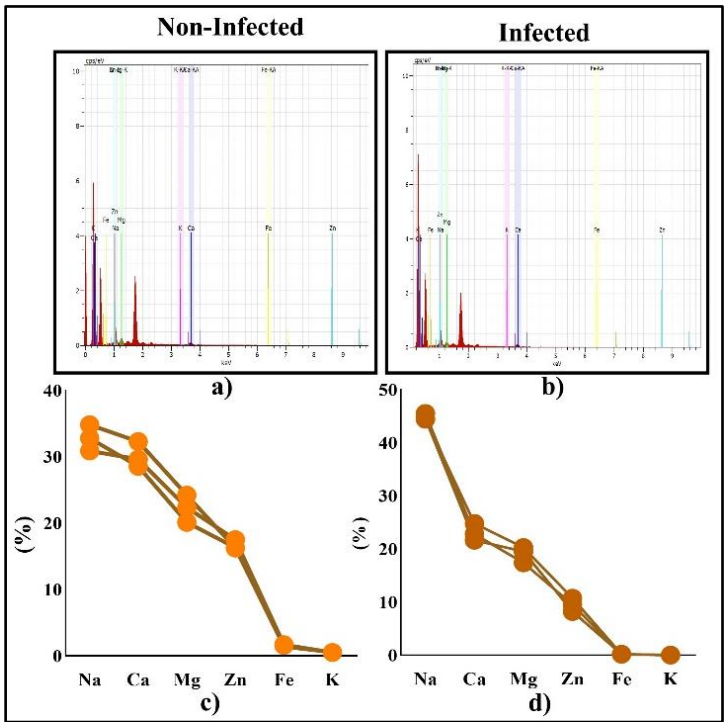

Fig. 8. Showing SEM/EDS graphs for elemental analysis and showing the level of mineral status a) non-infected liver of *H. fossilis* b) infected liver of *H. fossilis.* Graph showing level of minerals in non-infected c) and infected d)

4. DISCUSSION

In aquaculture production, helminth parasites are a problem because they infect fish and impair their general health and growth. Fenbendazole, levamisole, and praziquantel are a few of the drugs used to treat fish helminth infections according to Hoffman [37]. To get rid of the parasites, these substances are frequently added to the fish's food or water. To manage helminth parasites in aquaculture, certain biological agents can be employed, including certain bacteria and fungus given by Smith and Roberts [38]. These substances directly target the parasites and have the potential to decrease their population.

In this article we focused on impact of fish nutrition due to cestode infection. When any types of parasite attack in any aquatic water body mainly in fishes than inter in body with gills and with food reached in intestine mostly. In case of Cestode make the colonies and slowly migrate the different parts of body according to Reavill and Roberts [39]. Cestode have sucker in anterior part of body (Fig. 1). They inject in the tissue of fish and availing the nutrition for survive. Therefor histopathological study is a best way to define cestode infection in tissue and how to create injury in different tissue in body reported by Koyun and Noga [40,41]. In many previous studies have already reported that histopathological deformities due to cestode parasites in freshwaters fishes Smith and Roberts [38]. So, in this article, we performed histology of liver tissue for study of cestode infection on *H. fossilis*. We found that, during examination of infected fish many parasites were situated in nearby liver. We compare the infected liver tissue to non-infected many deformities found (Figs. 2,3). Many areas of infected liver tissue found lack of hepatocyte cells (Fig. 2d) already reported by Koyun and Noga [40,41]. When we observed by scanning electron microscope, we found that, many cytoplasmic granules accumulated in infected liver tissue (Fig 3f). Therefore, this article confirms that, cestode parasite are create histological injuries in *H. fossilis*.

Numerous research works have highlighted the production of oxidative stress in fish bodies with parasite infections as a result of histopathological abnormalities already reported by Irshadullah et. al and Shan et. al [42,43]. When parasite infection coexists, changes in the levels of

enzymatic and non-enzymatic antioxidants which are essential for protection against oxidative stress are commonly seen. The first line of defense, antioxidant enzymes play a critical role in limiting the damage caused by ROS originating from parasites according to Mordvinov et. al [44]. On the other hand, a parasite's ability to sustain the necessary equilibrium between oxidation and antioxidation may be essential to its survival. It is a well-established fact that elevated intracellular ROS concentrations affect the host's physiological function, weakening the host immune system and increasing susceptibility to parasite infections according to Mordvinov et. al and Nabi et. al [44,45].

Due to GSH depletion ROS buildup and cellular antioxidant defenses may be compromised given by Kolodziejczyk and Halprin [46,47]. In the present article significantly low ($P < 0.05$) level of GSH in infected fish are showed (Fig. 4f). The reason behind that, due to parasitic infection the increased oxidation of GSH into glutathione disulfide (GSSG), which is catalyzed by free radicals, and the usage of GSH by GPx as a co-substrate for the conversion of H_2O_2 to water and oxygen in the diseased liver already reported by Kolodziejczyk and Halprin [46,47]. Increased GPx enzyme activity may be interpreted as an adaptive response to possible hepatic damage according to Rehman et. al and Kolodziejczyk et. al [48,49]. Therefore, the amount of GSH in the liver tissue of *H. fossilis* infected with cestode may have significantly decreased ($P < 0.05$) as a result of the enhanced GPx activity previously reported by Rehman et. al [48]. The GSH level drop would eventually create a barrier to the GPx enzyme's effective function, making this route useless for getting rid of lipid and hydrogen peroxides already reported by Kolodziejczyk and Rehman et. al [46,48]. Decreased activity of GR and protein structure modification can leading to change in its function and can responsible for accumulation of peroxides up to toxic level. Therefore, in present article the GPx level of infected liver was increased (Fig. 4d). Similarly, the concentration of GR was also significantly decreased ($P < 0.05$) (Fig. 4e) as already reported by Kolodziejczyke et. al [46]. As per our current analysis, the liver of the infected *H. fossilis* exhibited a considerably increased level of SOD ($P < 0.05$) according to Deger et. al [50]. There have also been reports of an increased in SOD in the liver of *Clarius batracus*, a different species of freshwater catfish infected with cestodes reported by Khan et. al [51]. This indicates a parasite infection-related increase in oxidative stress. Elevated catalase enzyme activity signifies a build-up of hydrogen peroxide within the contaminated tissue given by Kumar et al [52]. During the current investigation Infected liver demonstrated a significantly increase ($P < 0.05$) in CAT enzyme activity (Fig. 4c). previously reported on the rise of CAT in the liver and head kidney of *Cyprinus Carpio* infected with Ptychobothryumsp by Eissa et. al [53]. The liver and muscle tissues of Tilapia infected with *Diplostomum* and *Heterophys* species showed increased MDA concentration, SOD, CAT, GR, and GPx activities also reported by Garcia et. al [54]. The parasite is shielded from host immunological reactions by the elevated MDA levels. Infected liver with low levels of lipid peroxidation may be a sign that cells are promoting their own survival and upkeep by upregulating antioxidant proteins, which triggers an adaptive stress response reported by Zeghir-Bouteldja et. al [55]. The current findings indicated a significantly higher ($P < 0.05$) level of MDA in the infected liver, respectively. Similarly, an elevated level of MDA was seen in the liver, gut, and muscle of Schizophora phagosomes infected with Pomphorhynchus species also reported by Da Silva et. al [56]. Buffaloes with parasite cyst infections had significantly higher MDA levels in their livers (Fig. 4a), which is indicative of oxidative stress, protein oxidation, and lipid peroxidation processes also proposed by Da Silva et. al and Kutu et. al [56,57]. As with human cestodes, our results imply that oxidative stress increases in the cestode infected liver, which may be the cause of inflammation and tissue damage suggested by Kutu et. al [57].

Numerous researchers have found increased levels of ALT and AST in sheep with cystic echinococcosis [58,59], distomatosis [60] and F. gigantica [61] infection in rabbits and they have linked these findings to increased lipid peroxidation. In the current study display that, due to cestode infection in the liver of *H. fossilis* elevate levels of ALT, AST, ALP, and ACP (Fig. 5). According to Heidarpour et. al (a) [62] elevated levels of these enzymes may be associated with pathogenic responses to the parasite and liver damage brought on by mechanical pressure. In the abomasal tissue of goats infected with *H. contortus*, found higher levels of ALT, AST, ACP, and ALP and associated them with membrane permeability alterations and damage already reported by Heidarpour et. al (b) [63].

Previously many research articles defined that, the mineral status of fishes are imbalance due to

parasitic infection [64,65]. In this article we estimated elemental mapping with SEM/EDS are defined mineral status of *H. fossilis* due to cestode infection. We found in infected liver tissue the level of Ca was significantly decrease ($P < 0.05$) followed by Mg and Zn (Table 1) also previously noticed by Hansen and Illanes [66]. But Na level was significantly increased ($P < 0.05$) due to accumulation of Na in infected cells also Previously reported by Hansen and Lopez [66,67]. A well as some trace elements i.e., Fe and K was also affected.

When the fish are infested with parasites, then they are suffering low nutrition condition already reported by Lith et. al [68]. In this article, significantly decreased ($P < 0.05$) level of RBCs, Hb and significantly increased ($P < 0.05$) WBCs was again confirmed that fish was suffering to infection and nutritional imbalance (Table 2). We have also estimated level of serum protein and glucose. In infected *H. fossilis* significantly increased glucose level due to the cestode infection accelerate glucose cycle already reported by Rautelaa and Rawat [64] and significantly decreased level of protein (Table 3) indicated that, *H. fossilis* was suffering to nutritional deficiency already noticed by Rivadeneyra et al. [65].

5. CONCLUSION

Our research indicates that cestode parasite infection in *H. fossilis* fish may result in histopathological deformity, increased generation of reactive oxygen species (ROS), enhanced lipid peroxidation, and altered levels of different antioxidants and detoxification enzymes, all of which can cause a substantial amount of oxidative stress in the *H. fossilis* fish's infected liver. The hepatocytes are damaged by the cestode parasite as a result of mechanical pressure, which increases the release of liver damage indicators such as AST, ALT, ACP, and ALP. Initial information on health evaluation based on hematological, elemental mapping, and serum biochemical characteristics for *H. fossilis* may be obtained from the data provided in this study. In the end, this study's conclusions will help aquaculture and environmental authorities make decisions in the future about the upbringing and cultivation of fish meant for human consumption.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declares that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during writing or editing of manuscripts.

DATA AVAILABILITY

The corresponding author can provide the data sets created or examined during the current investigation upon reasonable request.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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