Mansoura Veterinary Medical Journal

MYXOSPORIDIOSIS IN OREOCHROMIS NILOTICUS; MOLECULAR IDENTIFICATION AND OXIDATIVE STRESS BIOMARKERS

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ABSTRACT

Myxosporidiosisis a protozoan parasitic fish disease that causes damages to fish organs and might eventually end with mortalities and deterioration of meat quality of the affected fish. The presence of parasitic infestation is usually accompanied with the production of reactive oxygen and nitrogen species that produces extra damage to host organs and inactivation of antioxidant defense system that increase the intensity of parasitic infestation. Recently, the response of the infested tissue to oxidative stress is varieddepending on the type of parasite; therefore the use of oxidative stress markers as indicator for myxobolus infestation is relevant. In the present study, Oreochromis niloticus was investigated for oxidative stress markers (GST, SOD, NO, H_2O_2 and MDA) in liver, muscle, spleen and gills infested with myxobolus spp. Histopathological examination was performed to clarify the cellular damageassociated withmyxobolus infestation. Results indicated that there was a non-significant change in GST activity in all organs, while SOD activity was significantly decreased in liver and gills after myxobolus infestation. The concentration of nitric oxide, hydrogen peroxide, and lipid peroxidation were significantly increased in (gills and spleen);(liver, spleen and muscle); (liver and muscles), respectively. Intestine and eyes of the affected fish showed a degenerative changes and replacement of the epithelial tissues with myxobolus spores with characteristiceosinophilic granules that has a role in the immunity of host against parasitic infestation.The phylogentic analysis showed that the identified species of myxobolusin the present study was revealed a close relationship as well as a high identity percentage with, Myxoboluskisutchi and similar tissue tropism which highlighted the use of both molecular identification and oxidative stress markers for diagnosis of myxobolus infestation in dead fish when the parasitological examination alone becomes inadequate foraccurate detection of myxobolus infestation.

Key words:Myxosporediosis, oxidative stress biomarkers, O. niloticus

INTRODUCTION

Fish protein is a valuable source of protein that is important for human health distributed in every portion of the world either marine or fresh sources **(Kent et al., 2001)**. *Oreochromisniloticus*is, the most popular fish species, is used extensively in aquaculture for decades to guarantee a perfect source of cheap and high quality protein **(Scholz, 1999)**.

Fish industry worldwide suffered from different diseases problems either environmental or microbial. In Egypt, about 80% of fish diseases are parasitic **(Eissa, 2002).**The phylum Myxozoais a primitive multi-cellular micro-parasite affects wide range of living species (more than 1500 species

parasitizing both fresh and marine water fish) causing mass mortalities and even deteriorated the quality of meat due to the lysis of muscle fibersthat found disapproval to the consumers **(Dudin, 2010; Lom and Dykova, 2006)**. It could extend to internal organs causing severe mortalities **(Abdel-Ghaffar et al., 2008)**. The similarity of several myxobolus species in morphological characters requires further tools for accurate identification of their presence. From this point, **Camus and Griffin, (2010)** suggested the use of molecular identification of small ribosomal subunit for identification of myxobolus infestation as a tool for accurate diagnosis.

It was recorded that environmental pollution with the presence of different pathogenic microorganism are capable of performing oxidative stress through causing imbalances of the ratio between oxidants and antioxidant defense system in body which would eventually result in oxidative damages due to the production of reactive oxygen and nitrogen species that leads to enzyme inactivation and the increase of lipid peroxidation and nitrosative stress in the exposed species **(Banudevi et al., 2006)**. However, the oxidative stress markers against different parasitic disease are not yet established. Furthermore, the need to study the host response after parasitic infestation is an important task to determine the required methods to control parasitic diseases and subsequently resolve the cause of mortalities and then increase fish production **(Rudneva et al., 2010).**From this point, the aim of this study is to establish oxidative stress biomarkers against myxobolus infestation in *Oreochromis niloticus* explaining the cellular damages of this parasite in different organs of the affected fish species for diagnosis of myxobolusinfestation with the aid of Molecular biological study using small ribosomal subunit and the basic parasitological techniques..

MATERIAL AND METHODS

2.1 Fish sampling and parasitological examination:

In the present study, fifty fish samples ofOreochromis niloticus were randomly collected from fish farm located at Kafr El-Sheikh governorates suffering from signs of off-food, dullness, ocular opacity and stunted growth during autumn season of 2014with average body weight (35 ± 1.5). The collected fish were transferred to the laboratory of fish diseases and management, Dept. of internal medicine, Infectious and Fish diseases, Faculty of veterinary medicine, Mansoura University for further parasitological identification. A skin and gill scraping, tissue smears were prepared with a drop of a normal saline (wet mount technique) and examined microscopically and the positive smears were air dried, fixed with absolute methanol and stained with 10 % Giemsa stain for detection of the spores according to**(Melanin et al., 2010)**.

2.2 Oxidative stress biomarkers:

Fish were dissected for collection of gills, muscle, liver and spleen for oxidative stress biomarkers determination. A negative control group (apparently healthy fish) was collected for normalization of the studied parameters. The collected tissues were washed thoroughly with normal saline (0.9%) and immediately homogenized in ice-cold sterile phosphate buffer saline (PH, 7.4) with pellet pestles homogenizing device supplied from (Sigma Aldrich, USA). The homogenized samples were centrifuged at 3000rpm for 30 minutes at 4ºC, and the collected supernatant was stored at - 80ºC for the determination of oxidative stress biomarkers as follow:

2.2.1 Glutathione S transferase(GST) activitydetermination:

Glutathione -S-transfease was determined according to the method of **Habig et al., (1974)** where an aliquot of the homogenized sample

was reacted with Dichloronitrobenzene to form mercaptouric acid which was detected by using (Jenway 6305, UK) spectrophotometer at 340 nm.

2.2.2 Superoxide dismutase (SOD) activity determination:

The activity of superoxide dismutase was determined using the technique of **Nishikimi et al., (1972**) through the inhibition of phenazinemethosulphate by the tissue content of SOD which would subsequently reducednitrobluetetrazoliem dye to violet color that was spectrophotometrically determined at 540 nm using (Jenway 6305, UK).

2.2.3Nitric oxide concentration (NO) determination:

The determination of concentration of nitric oxide was determined through formation of bright red color after the reaction of nitric oxide with sulphanilamide and 1-naphthyl ethylenediamine**(Montgomery and Dymock, 1961).**

2.2.4Hydrogen peroxide (H₂O₂) concentration determination:

Hydrogen peroxide reacted with 3,5-dichloro-2-hydroxybenzensulfonic (DHBS) acid and 4-aminophenazone in the presence of horseradish peroxidase to form Quinoneimine Dye that appeared as red color where its intensity was measured spectrophotometerically at 510 nmaccording to**Fossati and *Prencipe*(1982).**

2.2.5 Malondialdehyde (MDA) concentration determination:

Malondialdehydewas measured according to the method of **Yoshioka et al., (1982).** The aliquot of total homogenate was shaken with 2.5 ml of TCA 20% in 10 ml centrifuge tube. To the mixture, 1 ml of 0.67%TBA was added, shaken and warmed for 30 minutes in boiling water bath followed by

rapid cooling. Then 4 ml of Iso- butanol were added. Then after shaking the mixture, it was centrifuged at 3000 rpm for 10 minutes. The resultant upper layer was taken into a separate tube and the MDA content was determined from its absorbance at 532 nm.

2.3 Histopathological examination:

The affected organs were dissected out, washed with normal saline (0.9%) and fixed in 10 % neutral buffered formalin for histopathological examination. The paraffin embedded sections was cut at 5 μm stained with Meyer's hematoxylin and eosin **(Gridley, 1960)** for further investigation with Leica light microscope.

2.4 Molecular identification of the detected Myxobolus sp.

All fish samples suffered from corneal opacity were collected for molecular identification. Tissue samples were fixed on ethanol for further DNA extraction procedures. DNA extraction was performed using phenol/chloroform method according to **Sambrooketal., (1989).** DNA concentration was measured with nano spectrophotometer (Implen, USA) and DNA integrity was checked using gel electrophoresis (Hybaid, UK).The polymerase chain reaction was carried out on a 25 □l reaction using TaKaRamastermix with the following primer pairs MyxoF: GATAACCGTGGGAAATCTAGAG and MyxoR: CGTGTTGAGTCAAATTAAGCC. The PCR protocol was as followed: Initial denaturation (95°c for 5 minutes), that followed by 40 cycles of denaturation step (95 for one minute), annealing step (58 °c for one minute), extension step (72 °c for 2 minutes) with a final extension step (72 °c for 9 minutes). The PCR product was visualized using gel electrophoresis (agarose 1.5% with 20□l ethidium bromide), and thenthe collected fragment of gel-containing PCR product was purified using (GeneJET Gel Extraction Kit - Thermo Fisher Scientific), and sequenced using

sequencer device (*Applied Biosystems* 3130 Genetic Analyzer)in both direction with MyxoFand MyxoR primers.The phylogenic analysis of the sequenced *Myxobolus species* of the present study was performed using (mega5.2 software) (**Tamura et al., 2011**) to determine its' position among the previously recorded myxoblous species that listed in table (1). Neighbour joining method was used for constructing phylogentic analysis where 1000 replicates were used for bootstrap analysis.

Statistical analysis:

The result of oxidative stress markers was statistically analyzed using SPSS software V. 20 using a P-value with a significant level at 0.05. The use of Independent T-test was performed between control and myxobolus infested species to compare means.

RESULTS

4.1 Parasitological results:

The detected myxobolus species was morphologically similar to *Myxobolus niloticus fahmii 1971*. The spores were rounded to drop shapewith a blunt posterior end and tapered anterior end. The spore measurement was as follow:12±0.5 μm (11–13) in length and 6±0.4 (7–8) in width. The equal-sized polar capsules were pear shaped and exceeded half of the spore length, measuring 7±0.3 μm (6–7) in length×3±0.4 (3–4) in width. The sporoplasm located near posterior end of the detected spores (Fig. 1).

4.2 Oxidative stress biomarker

There was a non-significant change in GST activity in all the examined organs (Fig. 2), whileSOD activity was significantly (P < 0.05) decreased in liver and gills after myxobolus infestation (Fig. 3). The concentration of nitric oxide, hydrogen

peroxide, and lipid peroxidation were significantly (P < 0.05)increased in (gills and spleen), (liver, spleen and muscle), (liver and muscles) respectively in organs suffering from myxobolus infestationwhen compared with control group (Fig. 4, 5, 6).

4.3 Histopathological examination

From all the examined organs, the pathological changes were obvious in eyeand intestine of the affected fish as shown in plate 1 as follow:Severe pathological lesions were noticed in the eyes, in peri- orbital tissue, multiple spores of myxobolus parasites were noticed. The spores were aggregated in the form of cyst- like lesion containing different developmental stages of the parasite (A). On the periphery of the cyst, developmental stages of myxobolus were noticed, while in the centre, the characteristic spores occupied the whole cyst. The individual spore appeared with two polar bodies and sporoplasm(B).

Necrosis and desquamation of the intestinal epithelium was a common picture. In the lumen, large number of desquamated epithelium was noticed together with different stages of protozoa seems to be the stages of myxobolus parasite species (C). Many eosinophilic granular cells were noticed along the intestinal tissues especially in the submucosa(D).

4.4 Molecular identification and phylogenetic analysis:

In figure 7, the phylogentic analysis of the current study revealed the presence of three clades of Myxobolus species that was classified according to tissue tropism into branchial tropism clade, neurotropism clade and muscular tropism clade. The identified species of myxobolus in the present study was showing a close relationship to the *Myxoboluskisutchi* have a neurotropism.
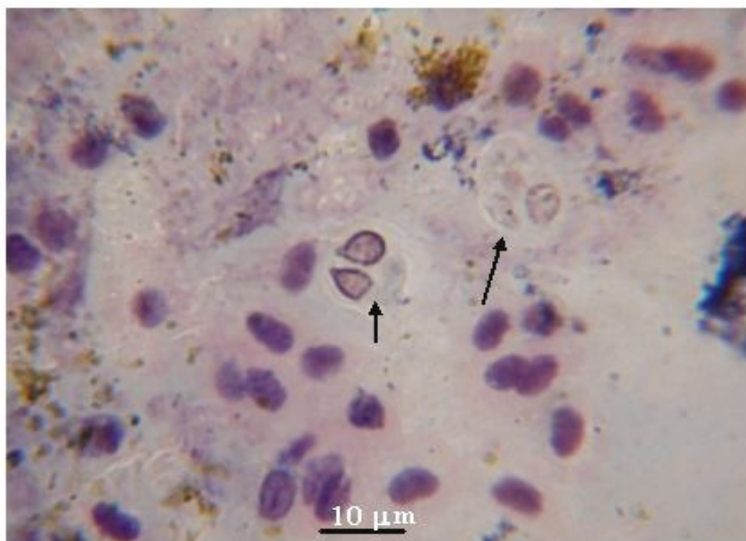
Fig 1: Myxosporidae spores taken fromis the gill filaments of *Oreochromisniloticus* stained with Giemsa stain (arrows) (100X)
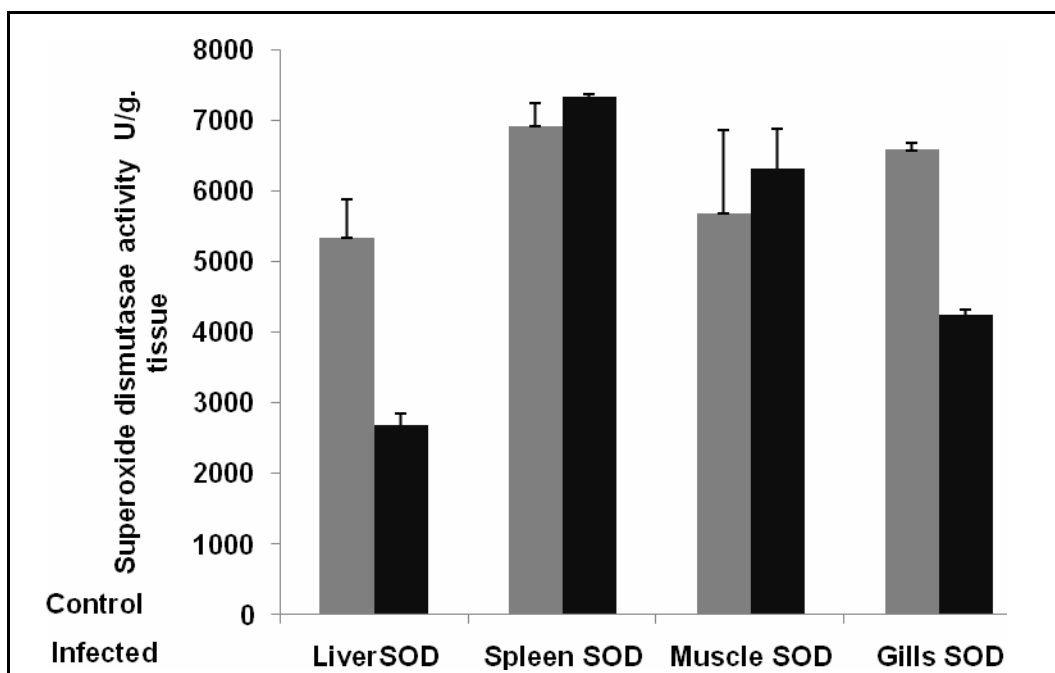

Fig. 2: Glutathione S transferase activity (U/g) in liver, spleen, muscle and gills of O.niloticus. The values in this graph is plotted as mean ± standard error of mean (P<0.05).
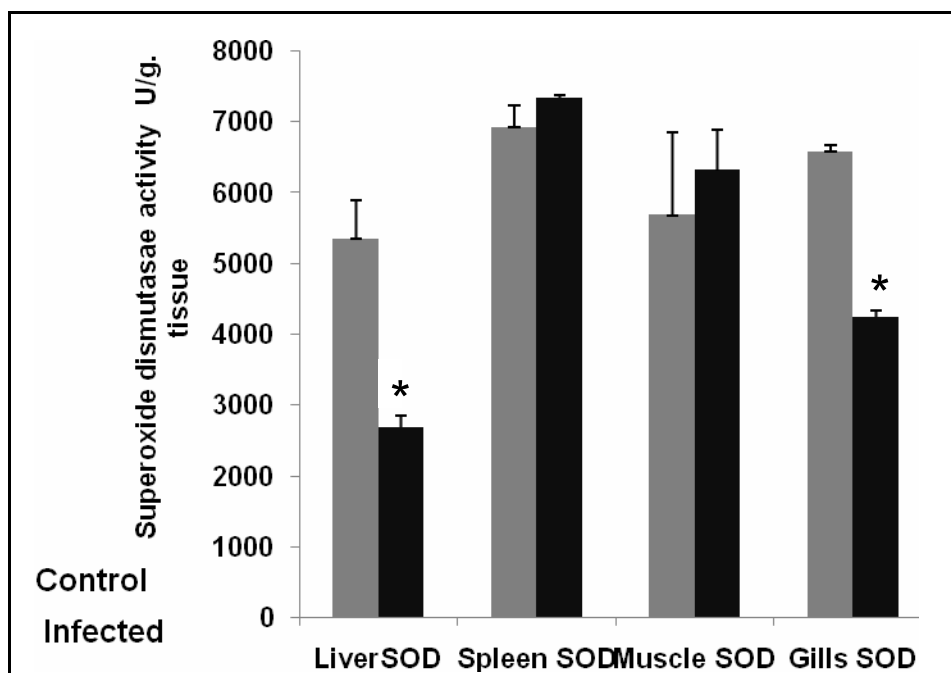
Fig 3: Superoxide dismutase activity (U/g) in liver, spleen, muscle and gills of O.niloticus. The values in this graph are plotted as mean ± standard error of mean. The mean values that highlighted with* means that there is a significant difference between the control and the infected fish(P<0.05).

Fig.4: Nitric oxide concentration (mg/g) in liver, spleen, muscle and gills of O.niloticus. The values in this graph are plotted as mean ± standard error of mean. The mean values that highlighted with* means that there is a significant difference between the control and the infected fish(P<0.05).
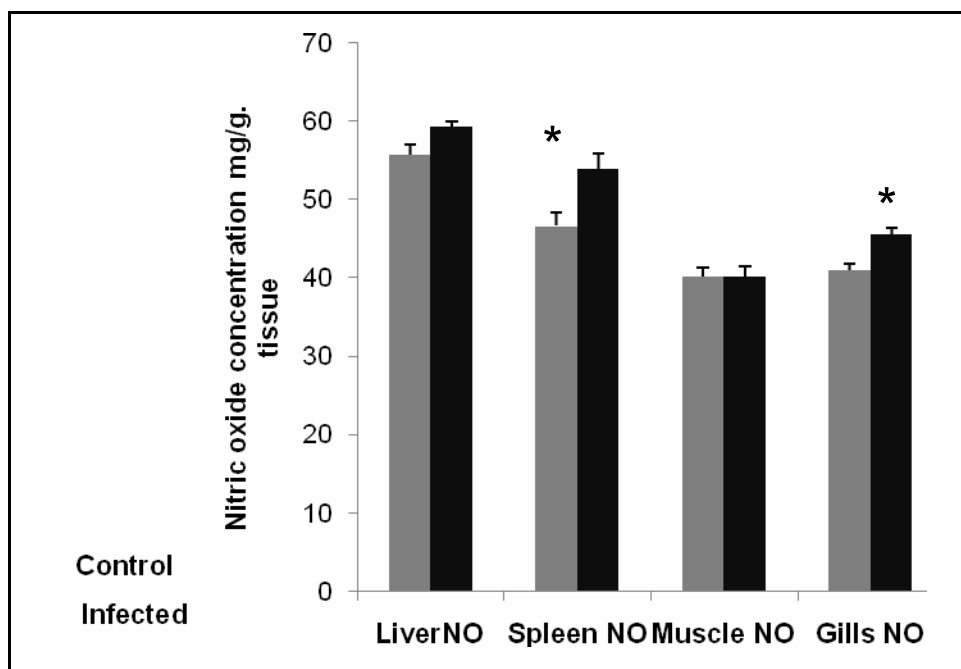
Fig. 5: Hydrogen peroxide concentration (mg/g) in liver, spleen, muscle and gills of *O.niloticus*. The values in this graph are plotted as mean ± standard error of mean. The mean values that highlighted with* means that there is a significant difference between the control and the infected fish(P<0.05).
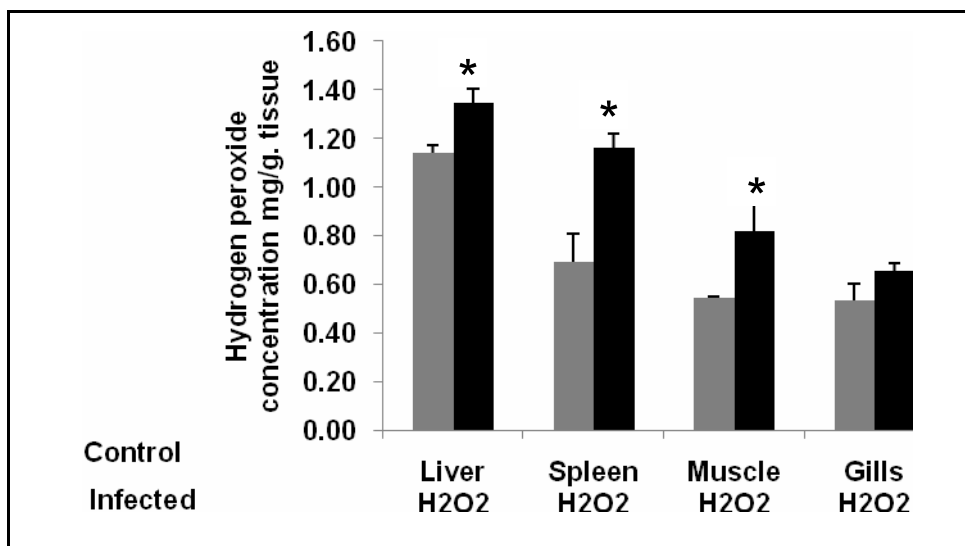

Figure 6: Malondialdehyde concentration (mg/g) in liver, spleen, muscle and gills of O.niloticus. The values in this graph are plotted as mean ± standard error of mean. The mean values that highlighted with* means that there is a significant difference between the control and the infected fish(P<0.05).
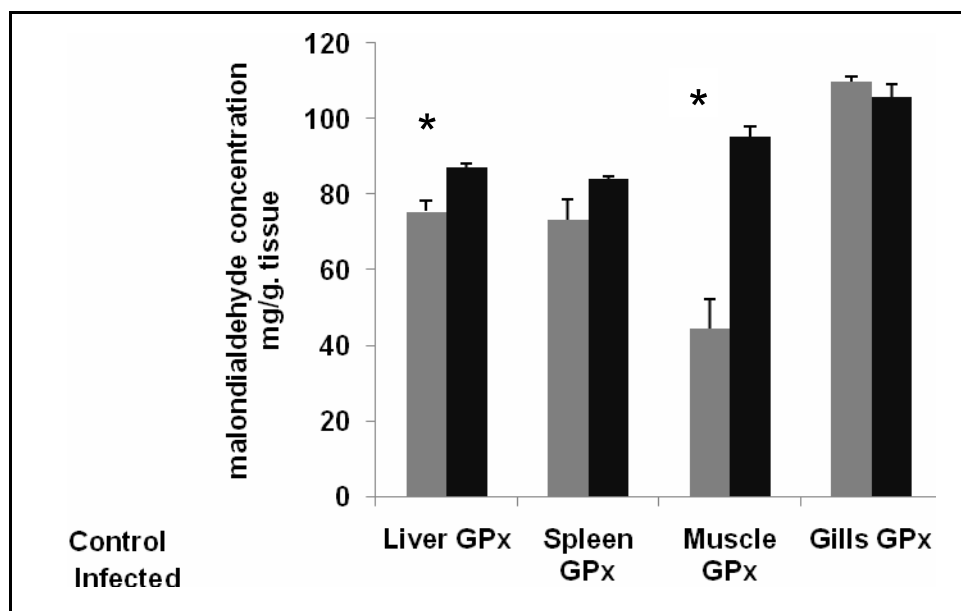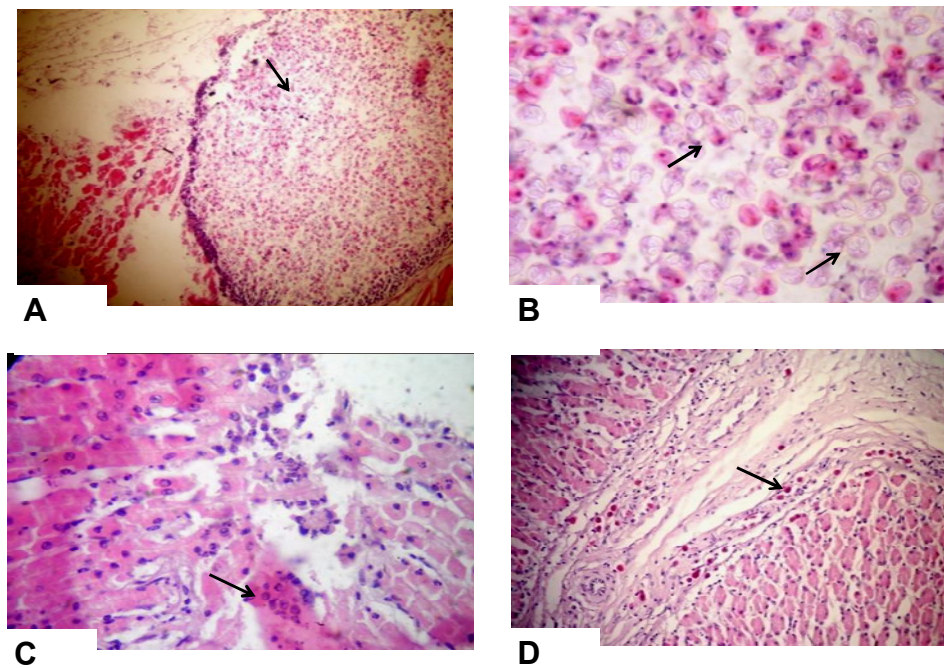
Plate (1) Histopathological section of Oreochromis niloticus tissues infested with myxobolus

| Symbol	Plate 1
A	Eye showing cyst- like formation filled with large number of the spores in the peri- orbital tissue (H&E stain.x40)
B	Eye showing the spores with characteristic polar bodies and sporoplasm (H&E stain.x100)
C	Intestine showing necrosis and desquamation of epithelial lining and different stages of myxobolus development (H&E stain.x40)
D	Intestine showing large number of eosinophilic granular cells (EGC) in submucosa (H&E stain.x40)
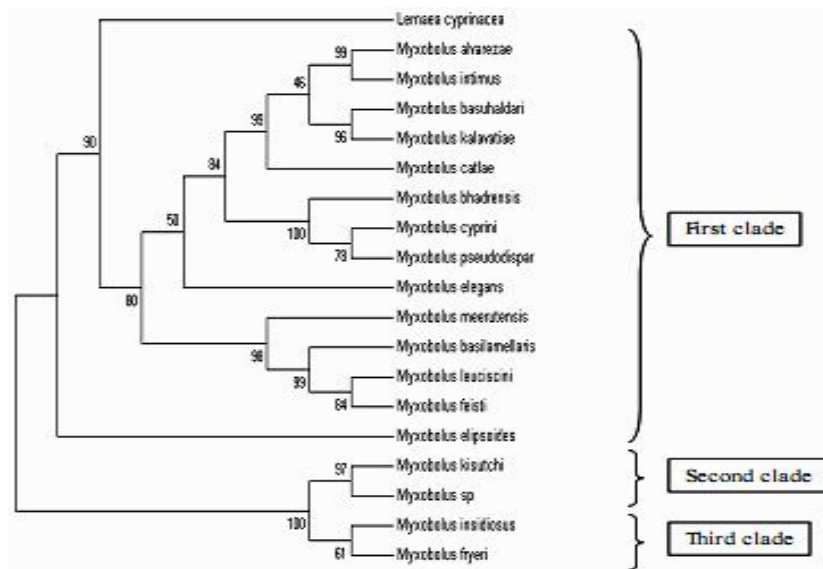
Figure 7: Phylogentic tree analysis using neighbor joining method (boot strap= 1000). The selected sequence was based on the percent of identity of the identified sequence of Myxobolus species with the others located species in genbank. *Lernaeacyprinacea was* named as an out group for the phylogentic tree.

Table (1): List of myxobolus species that is used for phylogentic analysis

Species	**Accession number**	*Fish species*	**Tissue tropism**
Myxoboluskisutchi	EF431919	*Coho salmon*	Brain
Myxobolusinsidiosus	EU346377	*Coho salmon*	skeletal muscle
Myxobolusfryeri	EU346372	*Cutthroat trout*	skeletal muscle
Myxoboluselipsoides	AF085178	*Rutilusrutilus*	Gills
Myxobolusalvarezae	FJ716097	*Aspiusaspius*	Gills filament
Myxoboluscyprini	AF380140.	*Cyprinuscarpio*	-
Myxobolusleuciscini	DQ439811	*Chub*	Interbranchial filament
Myxobolusbasilamellaris	AF507971	*Cyprinuscarpio*	-
Myxoboluspseudodispar	EF466088	*Tubifex*	Gills
Myxobolusmeerutensis	KM029977	*Labeorohita*	Gills lamellae
Myxobolusbasuhaldari	KM029975	*Labeorohita*	Gills lamellae
Myxoboluskalavatiae	KM029973	*Cirrhinuscirrhosus*	Gills lamellae
Myxobolusbhadrensis	KM029972	*Catlacatla*	Muscle
Myxoboluscatlae	KM029967	*Cirrhinuscirrhosus*	Gills lamellae
Myxobolusfeisti	EU598804	*Rutilusrutilus*	Gills filament cartilage
Myxobolusintimus	JF311899	*Aspiusaspius*	Capillaries of gill lamellae
Myxoboluselegans	JN252485	*Leuciscusidus*	Branchial artery
Myxobolus spp.	This study	*Oreochromis niloticus*	Retina of eye
Lernaeacyprinacea	KP235363.1 (out group)	Plecoglossusaltivelis	skin

DISCUSSION

In general, when parasites invaded the host vital organs it is either destroy the tissue or utilize the nutrient in all tissue organs of fish which eventually ended with the massive death as recorded in the case history of the present study. Recently, the most advanced techniques used for detection of the destructive effect of fish parasites is mainly depend on the fact that the presence of parasite in aquatic organisms would result in a change biochemical and normal histological structure of the affected organ showing a certain cellular response that differs from an organ to another. This fact stimulated the idea of studying different biological markers to evaluate the condition of infected hosts (**Vasilyeva et al., 2010**).

The most important result obtained from the present study indicated that myxobolus infestation would associate with normalization of GST activity although GST was considered as an important indicator for hepatic damage in some mammalian species as recorded in mice artificial hydatid infestation (**Gollapudi and Vivekavardhani, 2013**).The non-significant change in the current study may be due to the level of damage in these organs did not stimulate GST enzyme. On the other hand, a study performed by **Frank et al., (2011)** clarified that experimental infection of *Schistocephalus solidus* and *Ligula intestinalis,*cestode parasites, decreased GST activity in three fish species which suggested that the trend of GST activity in wild and induced infection showed a different manner due to increase resistance to oxidative stress.

Liver and gills SOD activity was showing a significant decline by nearly 2 fold after myxobolus infestation which suggested the use of SOD activity in those organs as an effective biomarker for myxosporediosis in *O.niloticus*. It was claimed by several authors that oxidative stress weakens the ability of hepatic and branchial tissues of Nile tilapia in enhancing SOD activity. **Skuratovskayaet al., (2013)** discovered that hepatic SOD activity in Black Sea Whiting *(Merlangiusmerlangus)* showed a significant decrease after experimental infection with myxosporean parasite which was suggested to be due to the production of free radicals from macrophage to defeat the invaded parasites. In fact, the presence of parasite inside living organisms is eventually accompanied with a significant increase in reactive oxygen species production which mainly performed a deleterious effect on anti-oxidant status of living organisms (**Mertinez-Alvarez et al., 2005**). From this point, it could be suggested that hepatic and gills SOD was a biomarker for Myxobolus infection in Nile tilapia of the present study. The levels of nitrosative stress were determined by measuring the concentration of nitric oxide, which is increased in tissues of spleen and gills revealing the sensitivity of both tissues for nitrosative stress performed by myxosporediosis infection. In rainbow trout, the expression levels of nitric oxide synthetase revealed a significant up-regulation after the exposure to *Myxoboluscereberalis*(**Severin et al., 2010**). In sea bream,**Golomazou et al., (2006)** said that*Enteromyxumleei* a Myxosporeanparasitecaused an increase in nitric oxide production in host tissues. The increase in nitric oxide concentration was showing high levels in spleen due to the over

expression of inducible NOS in these tissues in hybrid tilapia **(Wang et al., 2007)**. In the same manner, the elevation of nitric oxide concentration in gills of fish was mainly attributed to the over-expression of neuronal NOS in these tissues which would result in an increase in nitric oxide production **(Hyndman et al., 2006)**.

From the presented results, there was no doubt that parasitic infestation produced huge and different responses from tissues through the production of reactive oxygen species that depresses the anti-oxidant defense system of the tissues. One of the most common free radicals is hydrogen peroxide which was found to be significantly increased after exposure to myxosporediosis infestation. Similar result was also observed in *Plasmodium falciparum* as **Atamna and Ginsburg, (1993)** found an elevation in both catalase and hydrogen peroxide activity and concentration, respectively after *Plasmodium falciparum* infection. The result of this study revealed that the hydrogen peroxide production in liver, spleen and muscle was increased approximately by 1.5 fold which could be used as a biomarker for myxoboliasis infestation in *O.niloticus*

The level of lipid peroxidation was used before as a biomarker for parasitic infestation in case of Dactylogyrus spp. infestation in gold fish **(Mozhdeganloo and Heidarpour, 2014)**,*Raphidascarisacus* in Yellow perch**(Marcogliese et al., 2005)**and *Ornithodiplostomum spp.* in fat head minnows**(Stumbo et al., 2012).** The continuous exposure of fish to multiple stressors executed by parasitism would eventually result in production of oxidative stress that lead to lipid peroxidation and the increase of the concentration of

Malondialdehyde concentration in tissues of fish **(Di Giulio et al., 1989)**.

The cellular damages of organs affected by myxobolus spp. were more specific in intestinal, liver and eye of all affected species of O.niloticus. In intestine, a desquamation of epithelial lining with invasion with easinophilic granular cells was spread in the epithelial lining of intestine. In a study performed by **Molnar, (2002**) in common carp**,** *Myxoboluscyprinicola*invaded lamina propria of small intestine which appeared as a thick walled spherical plasmodia attached to mucosal fold of intestine, while the myxobolus spp. isolated in this study was appeared as elliptical body with different developmental stages scattered in the epithelial lining of intestine. In the same respect, *M. nodulointestinalis* was occupied the muscular layer of *Barbussharpeyi*gut in the form of large plasmodia **(Masoumianet al., 1996)**. It was also described by **Ali et al., (2002)** that *Barbusbynni* was infested by oval shaped *Myxobolusintestinalis* infiltrated the circular muscular layer of the fish gut without the presence of developmental stages in intestine. In general, the localization of Myxobolus Spp. in intestinal epithelium differed from one host to another. In eyes, a cyst containing different developmental stages of myxobolus spp. was found in peri-orbital tissues. Similar result was obtained by **Eissa et al., (2006**), whom found the presence of Myxobolus cyst around the iris of the eye on O. niloticus. In liver, the most prominent pathological lesion was the infiltration of hepatic parenchyma, portal vein, hepatopancreas and blood vessels with eosinophilicgranules which was also observed in stomach and intestinal epithelium in Indian major carpaftermyxosporidea infestation. It was believed that the invasion of eosinophilic

granules in host tissue suggested its importance role in immunity against parasites which revealed the host response in defense against Myxobolus infestation **(Dezfuli et al., 2004)**.

Molecular and morphological analysis of the myxobolus identified in the current study indicated that there was an identity by 90% with *Myxoboluskisutchi*after performing Basic Local Alignment Search Tool (blast) for the isolated sequence. The discrimination of every myxobolus and the other was occurred through the site of entry and the basic morphological characters that recently accompanied with molecular identification through 18s subunit **(Ferguson et al., 2008)**. The phylogentic analysis that obtained from neighbor joining analysis revealed the presence of three obvious clades. The first clade was consisted of most of the listed myxobolus species used in the phylogentic analysis which was restricted to gills lamellae and only one species that was restricted only in muscle (*Myxobolusbhadrensis)*. The second clade called aneurotropism clade that consisted of*Myxoboluskisutchi* and the identified sequence of this study. The last clade was named as muscular tropism clade that contained two species *Myxobolusfryeri and M. insidiosus*. The sequenced species of Myxobolus was showing a high identity percentage with *Myxoboluskisutchi.* Myxobolus infestation is characterized by its tissue tropism which separates the different myxobolus infestation into different clades according to the site of invasion. *Myxobolusfryeri and M. insidiosus*infested muscular tissue while*Myxoboluskisutchi* invaded the nervous tissue **(Hogge et al., 2004**). In the current study, the identified species of Myxobolus was isolated from the

eyes of *O.niloticus*in retina of the eye which related to nervous tissues.

The traditional parasitological techniques applied for diagnosis of myxosporideawere always thought as effective tool for diagnosis due to the diversity of spore morphology. However, the discrimination between different species of myxobolus with the aid of spores' morphology was considered a difficult task as several species of myxobolusshared the same spores shape and diameter **(Ferguson et al., 2008).**The use of molecular biological studies in the diagnosis of myxobolus was always the technique of choice especially when 18S ribosomal subunit gene was used for diagnosis because of high diversity of this gene among closely morphological related species of myxobolus**(Kent et al., 2001).** The small ribosomal subunit gene was also used to differentiate between the same species infested different hosts as *M.articus* affected sockeye Salmon in Columbia was showing a different sequence from *M.articus*infestedthe same species in Japan recorded by **Andree et al., (1999)**

CONCLUSION

It could be concluded from the result of this study that superoxide dismutase, Nitric oxide and Malondialdehyde showed an observed elevation which reinforced the fact of use these parameters in biomonitoring of myxobolus infestation in *Orechromisniloticus*, while SOD activity revealed a significant decline due to the production of reactive oxygen species inactivated its action. The histopathological examination revealed an infiltration of intestinal, hepatic and periorbital

region with myxobolus bear shaped plasmodia with different developmental stages accompanied with eosinophilicgranular cells that have an essential role in host immunity. Thephylogentic analysis showed that the identified species of Myxobolus was revealed a low genetic distance as well as a high identity percentage with*Myxoboluskisutchi*which clarified the tissue tropism of myxobolus infestation for each species.

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الملخص العربى
مرض الميكسوسبوريديا فى أسماك البلطى النيلي: التشخيص الجزيئي
واستخدام الدلائل الاكسدة

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مرض الميكسوسبوريديوزس من أمراض الأسماك الطفيلية التي تسبب الأضرار في أجهزة الأسماك ويمكن أن ينتهي في نهاية المطاف مع وفيات وتدهور نوعية اللحوم الأسماك المتضررة. وعادة ما يصاحب وجود الإصابة الطفيلية في إنتاج أنواع الاكسجين والنيتروجين التفاعلي الذي ينتج ضرر اضافي وتعطيل نظام الدفاع المضادة للأكسدة التي تزيد من شدة الإصابة الطفيلية. في الآونة الأخيرة، استجابة الأنسجة المصابة إلى الاكسدة تختلف على حسب نوع من الطفيل. وبالتالي فإن استخدام علامات الإجهاد التأكسدي كمؤشر للإصابة اصبح مهما. في هذه الدراسة، تم تجميع أسماك البلطي النيلي و قياس علامات الإجهاد التأكسدي(GST) ، اكسيد النيتريك، ٌNO فوق اكسيد الهيدروجين و (MDA)في الكبد والعضلات والطحال والخياشيم فى الاسماك المصابة والغير مصابة. تم إجراء فحص الأنسجة لتوضيح الاصابة الخلوية. وأشارت النتائج إلى أن كان هناك تغيير غير ملحوظ في النشاط GST في جميع الأجهزة، في حين انخفض نشاط ٍ(SOD) في الكبد والخياشيم في الاسماك المصابة. وقد زاد تركيز أكسيد النيتريك، بيروكسيد الهيدروجين، وبيروكسيد الدهون بشكل ملحوظ في (الخياشيم والطحال)؛ (الكبد والطحال والعضلات)، (الكبد والعضلات)، على التوالي. أظهر الأمعاء وعيون السمك تأثر التغيرات التنكسية واستبدال الأنسجة الظهارية بجراثيم الميخوط مع حبيبات الايزينوفيل التي لها دور في مناعة ضد الطفيل وأوضح تحليلhالجزيئ أظهرت أن الأنواع التي يتم تحديدها من لل وقد كشفت الدراسة وجود علاقة وثيقة وكذلك نسبة عالية الهوية مع، و الأنسجة مماثل الذي سلط الضوء على استخدام كل من تحديد والاكسدة الواسمات الجزيئية للتشخيص الميخوط الإصابة في السمك الميت عند فحص الطفيليات وحده يصبح عدم كفاية الكشف من الميخوط الإصابة.