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EVALUATION OF OXIDATIVE EFFECTS OF CLARITHROMYCIN ON LIVER, KIDNEY AND GILLS OF *OREOCHROMIS NILOTICUS*

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Abstract :
Normally, the Clarithromycin antibiotic is used to treat skin infection and respiratory infections caused by bacteria but recent studies revealed that these antibiotics also initiate nausea, diarrhea, dyspepsia, headache, abdominal pain, angioedema, dizziness, and rash. Further, the antibiotics also produce highly reactive oxygen atom that are dangerous to aquatic life by inducing oxidative stress, cellular disturbance and changing membrane structure, finally. The imbalance between oxidants and anti-oxidents is called oxidative stress. Anti-oxidation mechanism in cells consist of enzymes like glutathione, superoxide dismutase, peroxidase, and catalases that act against oxidative stress as defense system. Assessment of oxidative effects of clarithromycin on various body organs such as; kidney, liver, and gills of *Oreochromis niloticus* were performed in this study. Fingerlings of *O. niloticus* with 1 year of age were used in this experiment and exposed to different doses of clarithromycin. Body organs were taken from fingerlings to examine anti-oxidant enzymes and then compared with control fingerlings. One-Way ANOVA and Tukey's test were performed in statistical analysis. The results showed that the clarithromycin's exposed fish were affected by cardiovascular diseases, anemia along with damaged body organs including kidney, liver, and gills. Further, elevated level of many antioxidant enzymes was also observed such as; superoxide dismutase, peroxidase, and catalase. The physico-chemical parameters such as total hardness, calcium, sodium, magnesium, water pH, temperature of water, dissolved oxygen and carbon dioxide, were monitored on a daily basis. Physico-chemical parameters of experimental groups were compared with control group of fish which shows that concentration of total hardness, calcium, magnesium, carbon dioxide, sodium changes according to the concentration of clarithromycin.While temperature of water and carbon dioxide increased and concentration of oxygen decreased as compare to control group of fish.

Key words: Calithromycin, *Oreochromis niloticus*, Oxidative stress, ROS, Antioxidant, CAT, GPX, SOD

INTRODUCTION

Antibacterial drugs are widely used in both human and veterinary medicines, to control infectious diseases caused by bacteria. These drugs include a variety of bacteriostatic or bactericidal substances, such as chemotherapeutics (artificial antibacterial agents that do not occur in nature) and antibiotics (substances produced by microbes or their artificially manufactured analogs). Animals cannot completely absorb or metabolize antibacterial drugs that have been given to them, and between 30 and 90 percent of the utilized amount is eliminated through urine and feces into the environment because to their overuse, improper dosage, and poor absorption. These drugs are frequently observed in the environment (soil and water) because they are highly soluble in water and resistant to degradation. Due to utilization of manure (animals waste) for crops growth, a lot of veterinary antibacterial drugs end up on agricultural fields that result as a significant source of environmental contamination. Manure can emit antibiotics into the air, which can then permeate into the soil and contaminates surface water. Several classes of anti-bacterial drugs with bactericidal activity, both of human and veterinary medicine are also applied in fish farms to control infections (Romero *et al*., 2011).These days antibiotic use in aquatic environments is of great concern not just due to the risk of the emergence of resistant strains of bacteria, but also due to potential harmful effects on fish and other aquatic life (Zhao *et al*., 2011). In aquaculture, during production cycle therapeutic antibiotics such as florphenicol and oxy-tetracycline are used against antibacterial diseases that may also interact with non-target molecules of fish (Chopra *et al*., 2018).

Clarithromycin (CAM) is one of the prominent macrolide antibiotic, used widely against a variety of bacteria in humans, animals and fish that are difficult to treat with other antibiotics. Like other macrolide antibiotics, clarithromycin binds with 50S ribosomal subunit of bacteria and blocks the formation of protein. In vertebrate groups, CAM produce highly reactive oxygen species in the blood that induces oxidative stress, cellular disturbance and changes the structure of membranes (Wang *et al*., 2004) that is harmful for fish and other aquatic organisms. Accumulation of reactive oxygen species disturb the balance between oxidants and antioxidants ultimately results in oxidative damage to DNA, lipids and proteins. Therefore, oxidative damage is considered as biomarker of oxidative stress that functions as a measure of exposure to stressor (Scandalios, 2005). Oxidative stress cause different types of diseases such as atherosclerosis, inflammatory diseases, degeneration of nervous system, diabetes, atherosclerosis, aging, cardiovascular diseases and cancer (Mayne, 2003). Oxidative stress can be minimized by antioxidants otherwise cellular damages can occur (Berger, 2005). Formation of reactive oxygen compounds like hydroxyl radical, hydrogen peroxide, and superoxide radical that disturb antioxidant defense system and ultimately cause oxidative stress (Volodymyr and Lushchak, 2014). Over production of reactive oxygen species also can change the gene expression, oxidation of protein as well as lipids and change the redox status (Kochhann *et al.*, 2009).

Since last two decades aquaculture has become fast growing sector of world by continuous production of different types of edibe fish to fulfill world food demand. *Oreochromis niloticus or Nile tilapia* is an important food fish in Pakistan. It lives in fresh or brakish water and is an omnivorous column feeder that feeds on algae as well as planktons. All over the globe, this fish is contributing 43% of total fish production (Ibge, 2013) that is expected to be doubled till year 2030 (Armugam*et al*., 2023). Many bacterial species like *Aeromonas*, *Flavobacterium,* P*seudomonas fluorescens, Streptococcus* and *Enterococcus* etc. are the cause of high mortality in *O. niloticus* (Moraes and Martins, 2004) resulting in severe economic losses. Therefore, current study is designed to determine the effect of clarithromycin on liver, kidneys and gills of *O. niloticus* and also to report its oxidative damage.

MATERIALS AND METHODS

Sample collection and acclimatization

During current study, one year old fingerlings of *O. niloticus* of similar length and weight were purchased from fish seed nursery Faisalabad, Punjab. All samples were physically checked for any injury or damage and were placed in 250L glass aquarium with a maintained water temperature and

Oxygen concentration. Samples were transported to the cell and molecular biochemistry laboratory University of Agriculture Faisalabad. All samples were kept in glass aquariums for acclimatization and were properly fed two times a day with pelleted food for 7 days on normal laboratory conditions. Clarithromycin antibiotic purchased from medical store of Faisalabad. Fingerlings were divided into three groups 'C' controlled or untreated group, 'T_1' treated group 1, and 'T_2' treated group 2, with fifteen fish in each group.The minimum sub lethal dose of the antibiotic for maximum survival of fingerlings (> 72 hours) was accessed by continuous exposure of fifteen samples with 10mg/L, 15mg/L and 20mg/L of clarithromycin, respectively. It was observed that survival rate was decreased with increase in antibiotic concentration> 30mg/L for 24 hours, however the survival rate of fingerlings increased to more than 3 days on lower dose up to 20mg/L. Therefore, the minimum concentration 20mg/L of clarithromycin was selected as the optimum dosage for further experiments. Fingerlings of *O. niloticus* were exposed to different physiological doses of clarithromycin and constant exposure to air under laboratory conditions by static system of water. After exposure of clarithromycin the organs of fish such as liver, kidneys and gills were separated and measured the antioxidant activity. Then, every test was performed for each concentration treatments as well as activity of antioxidant in the selected organs (Liver, kidneys and gills) and was compared to control group. Physicochemical parameters were measured on a daily basis through (A.P.H.A, 1998) to determine the effects of physicochemical parameters on activity of enzyme. A normal range of dissolve oxygen was kept constant. Clarithromycin exposed fingerlings of *O. niloticus*were scarified as well as their liver, kidney, and gills separated and then save at -40C for measurement of oxidative stress.

Sample preparation

Antioxidant enzyme's activity was analyzed through various body organs of fish such as gills, kidney and liver. First of all, the collected organs were rinsed in phosphate buffer(pH 6.5, 0.2M) then samples were blended in cold buffer (1:4 W/V) for homogenization. After homogenization, samples were centrifuged at 10,000 rpm and -4^0C for 15 minutes and analyzed to estimate the antioxidant enzymes SOD, POD, and CAT.

MEASUREMENT OF OXIDATIVE STRESS

Oxidative stress was determined by measuring antioxidant enzyme assay.

Superoxide dismutase

Superoxide dismutase solution contained 0.222gm methionine, 15mL distilled water, 0.015gm NBT in 17.5mL of distilled water, 0.0375mL Triton-X in 17.5 mL of distilled water, 0.0132gm riboflavin in 17.5mL of distilled water and 0.2 M buffer. Test tubes with reaction mixture were exposed to UV lamp for 15 min before the addition of Riboflavin. Spectrophotometer was used to detect absorbance of solution at 560nm. Simple, the concentration of enzymes that inhibit NBT photo-reduction by 50% is known as one unit of SOD activity(Bradford, 1976).

Glutathione Peroxidase (GPX):

A kinetic colorimetric assay was configuring the activity of glutathione peroxidase that uses an enzyme glutathione reductase for the measurement of activity indirectly through a coupled reaction (Paglia and Valentine, 1967). Peroxidase reaction solution contain 0.1mL of enzyme extract, 40mM H2O2, 50mM phosphate buffer (pH 5), and 20mM guaiacol. Further, the changes in absorbance at 30nm of reaction solutionwere measure after each 20sec with spectrophotometer.

Catalase

Activities of catalase were measured using the method of (Chance and Maehly, 1955).Total 50mM phosphate buffer (with pH 7), 8mM H_2O_2 and 0.1mL enzymes extract are present in CAT reaction solution. First of all enzymes extract was added to initiate the reaction. Absorbance modification of reaction solution was detected for each 20 sec at 240nmwith spectrophotometer.

MESUREMENT OF PHYSICO-CHEMICAL PARAMETERS

Physicochemical parameters such as temperature, carbon dioxide, dissolved oxygen, calcium, PH, magnesium, total hardness, and total ammonia were observed of each control and treatment groups on a daily basis through (A.P.H.A, 1998) for determine the effects of physicochemical parameters on activity of enzyme. A normal range of dissolve oxygen was kept constant by using air pumps and fitted water capillary system.

STATISTICAL ANALYSIS

One Way ANOVA and Tukey' test were used for statistical analysis. Different levels of variability calculated and statistically significant difference were established if p value were be <0.05 between enzymes of liver, kidney and gills of *O. niloticus*. Data analyzed and graphs were made with ANOVA and Tukeys test by using Bradford software (Jilani*et al.,* 2013). The parameters were monitored for control and two treatment groups for different variance of water such as pH, temperature, oxygen, carbon dioxide, calcium, magnesium, sodium and total hardness(A.P.H.A, 1998).

RESULT

Activity of Superoxide Dismutase in Gills, Liver and kidney of *O. niloticus*

The effect of clarithromycin after five days of exposure and of enzyme assay (superoxide dismutase) has been described in Table 1 and Fig1.

Arithmetic mean ± standard error mean (SEM) (n=15) of superoxide dismutase in liver, kidneys and gills of *O.niloticus* after exposure of one year old fingerlings with clarithromycin for five days showed the extremely significant increase in superoxide dismutase level in liver, kidneys and gills of experimental groups T1 and T2 as compared to the untreated group. It means due to oxidative stress there is an upturn in the level of superoxide dismutase (Maier *et al.,* 2002) in these groups.

Activity of Catalase in Gills, Liver and kidney of *O. niloticus*

Mean ± standard error (SEM) (n=15) of catalase in gills, liver and kidneys of *O.niloticus* after five days exposure of one year old fingerlings with clarithromycin showed the extremely significant rise in catalase level in liver, kidneys and gills of experimental groups T1 and T2 as compared to the untreated group (Table 2, Fig2). Due to oxidative stress,rise in level of kinase (Digiulio *et al.,* 2008) in T1 and T2 groups was observed as compared to the control group.

Activity of Glutathione Peroxidase(GPX) in Gills, Liver and kidney of *O. niloticus*

Arithmetic mean ± standard error mean (SEM) (n=15) of glutathione peroxidase (Gpx)in liver, kidneys and gills of *O.niloticus* after five days exposure of one year old fingerlings with clarithromycin showed the extremely significant increase in Gpx level in liver, kidneys and gills of experimental groups T1 and T2 as compared to the untreated group (Table 3, Fig3). Due to oxidative stress there is arise in the level of Gpx (Damian *et al.,* 2012) in T1 and T2 groups as compared to the control group.

PHYSICO-CHEMISTRY OF CLARITHROMYCIN TEST MEDIA

The physico-chemical parameters were observed on daily basis by using following method of (A.P.H.A, 1998). Such as pH, temperature, carbon dioxide, dissolved oxygen, magnesium, sodium, calcium and total hardness were analyze as a physico-chemical parameters. These parameters were measured as a physico-chemical parameter of clarithromycin test media at different concentrations represented in tables.

The value of Table 4 shows the analysis of variance of test media (A.P.H.A, 1998). Comparison of mean values of control group and experimental groups shows significant increase in the pH, temperature, carbon dioxide and decrease in oxygen after 5-days clarithromycin exposed on test media was found as it compared with the control media .

Values of Table 5 shows the analysis of variance on contents of magnesium, calcium, sodium and total hardness test media (A.P.H.A, 1998). Mean values of control group and experimental groups show significant decrease in the magnesium, calcium and increase in sodium as well as total hardness remained similar after exposure of clarithromycin for 5 days on test media was found as it compared with the control media

DISCUSSION

Assessment of stress responses in O. *niloticus* under antibiotic exposure was done in this research. Antibiotics use reactive oxygen species that causes oxidative damage to cells and tissues (Mohd*et al.,* 2015, Al-Ghanim, 2014, Sharbidre*et al.,* 2011, Isik and Celik, 2008). The response of anti-oxidant system depends on intensity of oxidative stress (Massarsky *et al.,* 2017). In extreme conditions when oxidative stress cause overwhelming of antioxidant system it leads to DNA, protein and lipid damage (Almeida *et al.,* 2019). Glutathione is already known as oxidative stress biomarker and scavenger of reactive oxygen species (Regoli*et al.,* 2011). CAT, POD and SOD are major antioxidants which have ability to maintain antioxidant system at cellular metabolism (shi*et al.,* 2020). Our study reveals that clarithromycin antibiotic exposure increases the level of antioxidant enzymes SOD, POD and CAT due to oxidatvie stress. Similarly, as the exposure of oxytetracycline was increased, the level of SOD was also incresead in *O. niloticus* due to oxidative stress as revealed by work (Limbu *et al.,* 2018). Treatments with antibiotics enrofloxacin and florfenicol also increased the level of CAT in *Ctenopharyngodon idella* (shi*et al.,* 2022). Gills are very first tissues in fish that are exposed to any kind of water contamination (Gallagher and Di Giulio, 1992a, 1992b), whereas liver play a key role in nutrition and xenobiotic metabolism (Ayadi*et al.,* 2015). Further, many other pesticides also cause lipid oxidation in fish, such as deltamethrin, which uplift the concentration of oxidized lipids in the liver, kidney, and gills of *Channa punctatus* at a dosage of 0.75 g/L for 48 hours (Sayeed *et al.,* 2003). In another study on fish where *Brycon cephalus* species were subjected to the pesticide methyl parathion (2 ppm for 96 h) revealed that the chemical caused oxidative damage in the lipids of the muscle and gills. However, the concentration of oxidised lipids in the liver did not significantly rise (Amaral Monteiro *et al.,* 2006). The concentration of oxidised lipids in the brain and kidney tissues of two fish species, *Cyprinus carpio* and *O. niloticus*, did not significantly change after exposure to the pesticide azinphosmethyl (0.23 ppm) for 96 hours; nevertheless, both species gills displayed an increase in this parameter (Ozcan and Usta, 2007). However, tests on goldfish *Carassius auratus* subjected to 2,4-D at concentrations of 1, 10, and 100 ppm for 96 hours show that the pesticide causes a large, concentration-dependent increase in oxidised lipids in the gills (Atamaniuk *et al.,* 2013). The findings of this study suggest that clarithromycin are more vulnerable to liver, kidney, gills enzymes such as; catalase, glutathione, superoxide dismutase, as compare to diazinon. An investigation by (Du and Gebicki, 2004) indicates that proteins were oxidized before lipids or DNA in myeloma cells U937 and Sp2/0-Ag14 subjected to peroxide radicals. The latter may result from protein-peroxide production. Thus, it appears that protein oxidation takes occur first, followed by the well-known oxidation of lipids and DNA in cells by reactive oxygen species. Our findings indicate that short-term Clarithromycin treatment causes considerable oxidative damage in liver, kidney, and gills of *Oreochromis niloticus*. Similarly physicochemical parameters such as increase in pH, temperature, carbon dioxide, sodium and decrease in amount of oxygen in water also caused stress to O. *niloticus*.

Conclusion

The results strongly described that different physiological doses of clarithromycin induce oxidative stressand effect of antioxidant enzymes such as; superoxide dismutase, catalase, and glutathione on liver, kidney and gills after exposure of clarithromycin in *O. niloticus*also measured due to oxidative stress. As well as during this experimental research measurements of physic-chemical parameters such as; temperature, pH, water, oxygen,calcium, magnesium, sodium and total hardness were also observed on daily basis during the exposure of fish with clarithromycin. Our results show that amount of oxygen, calcium, magnesium decreased but temperature, carbon dioxide, sodium, pH

increased as well as no change was observed in total hardness of treated groups as compare to control group. Ultimately change in water variance caused stress to *O. niloticus* during exposure of clarithromycin.

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Table 1. Effect of five days exposure of clarithromycin on superoxide dismutase activities of liver, kidney and gills in one year old fingerlings of *O. nilotocus*

No. of samples	**Effect on Liver**			**Effect on Kidney**			**Effect on Gills**		
	Control 0 mg/L	**T1 20mg/L**	**T2 30mg/L**	**Control 0 mg/L**	**T1 20mg/L**	**T2 30mg/L**	**Control 0 mg/L**	**T1 20mg/L**	**T2 30mg/L**
1	0.123	0.559	1.42	0.096	0.544	1.326	0.165	0.561	1.342
2	0.756	1.96	1.656	0.226	0.999	1.847	1.026	1.676	1.67
3	0.756	1.96	1.656	0.191	0.527	1.388	0.44	1.874	1.256
4	0.254	1.189	1.445	0.1	0.868	1.614	0.567	1.697	1.778
5	0.187	1.112	1.189	0.248	1.121	1.344	0.735	1.511	1.813
6	0.756	1.189	1.445	0.226	0.999	1.847	1.026	1.676	1.67
7	0.254	1.112	1.656	0.096	0.544	1.614	0.735	1.697	1.778
8	0.187	1.96	1.189	0.248	0.527	1.388	0.165	1.511	1.67
9	o.756	1.189	1.656	0.226	0.868	1.326	0.44	0.561	1.342
10	0.256	1.112	1.445	0.1	1.121	1.847	0.567	1.874	1.256
11	0.187	1.96	1.189	0.09	0.999	1.614	1.026	1.511	1.813
12	0.756	1.189	1.656	0.226	0.527	1.344	0.735	1.874	1.67
13	0.254	1.189	1.189	0.248	0.868	1.847	0.165	1.697	1.778
14	0.756	1.96	1.445	0.096	0.544	1.326	0.44	0.561	1.256
15	0.1232	0.559	1.42	0.191	1.12	1.388	1.026	0.561	1.813
Mean	**0.424+ 0.283**	**1.346+0 .493**	**1.443+ 0.186**	**0.174+ 0.067**	**0.811+0. 247**	**1.537+0. 219**	**0.617+ 0.318**	**1.389+ 0.530**	**1.593+0. 229**
SEM	**0.073**	**0.127**	**0.048**	**0.017**	**0.0639**	**0.0567**	**0.082**	**0.137**	**0.0592**

Where as : Arithmetic mean ± standard daviation , * ($p<0.01$), ** ($p<0.01$), *** ($p<0.001$)

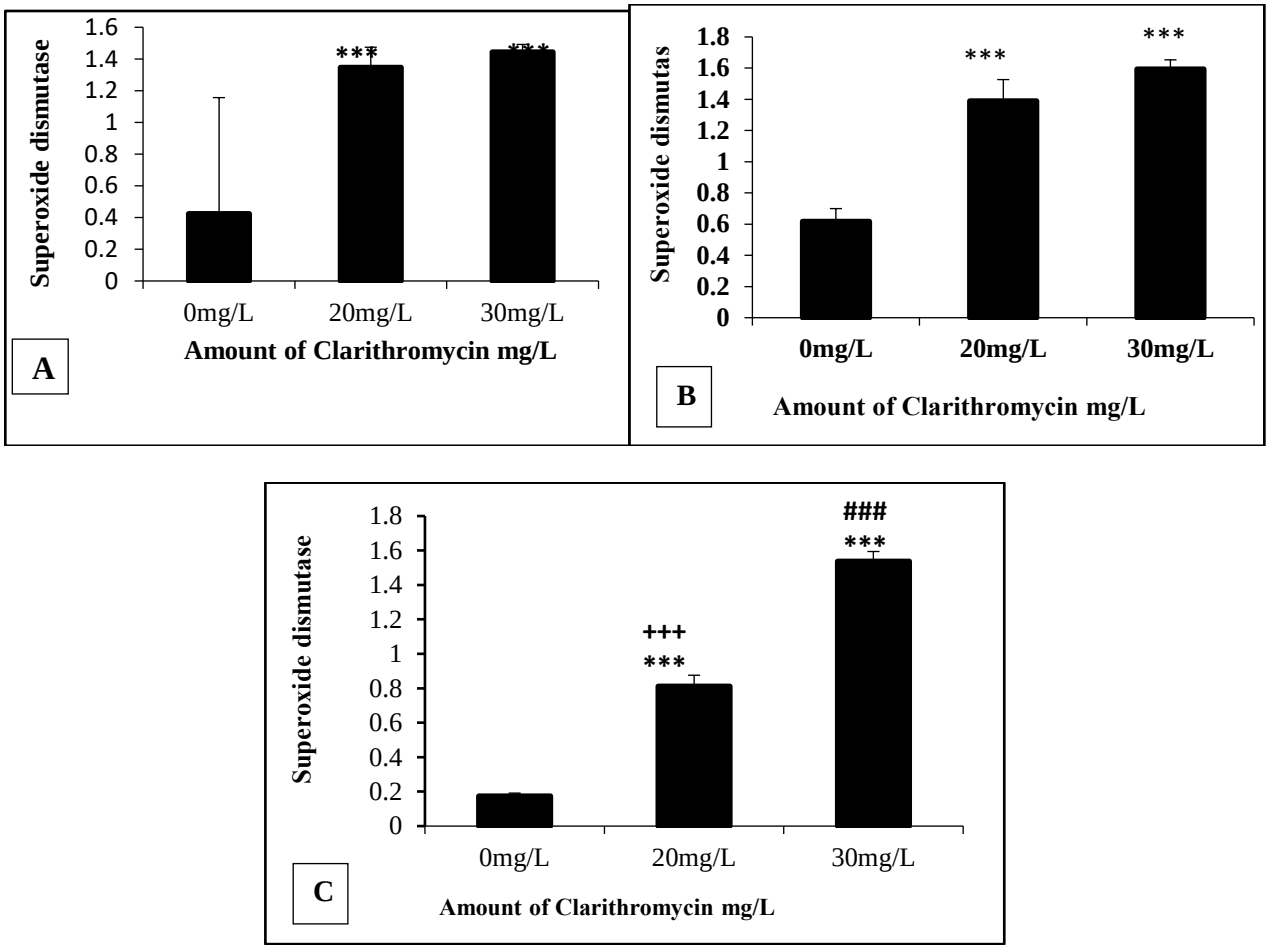

Fig 1.Effect of five days exposure of clarithromycin on superoxide dismutase activities of liver, kidney and gills in one year old fingerlings of *O. nilotocus*

Table 2.Effect of five days exposure of clarithromycin on catalase activity of liver, kidney and gills in one year old fingerlings of *O. nilotocus.*

Samples	**Effect on Liver**			**Effect on Kidney**			**Effect on Gills**		
	0mg/L	**20mg/L**	**30mg/L**	**0mg/L**	**20mg/L**	**30mg/L**	**0mg/L**	**20mg/L**	**30mg/L**
1	0.25	3.47	3.624	0.13	3.295	3.645	0.173	3.554	3.683
2	0.095	3.497	3.544	0.237	3.398	3.666	0.102	3.505	3.676
3	0.187	3.471	3.889	0.15	3.291	3.556	0.17	3.478	3.724
4	0.171	3.386	3.538	0.09	3.363	3.626	0.128	3.681	3.669
5	0.269	3.54	3.449	0.217	3.287	3.578	0.359	3.352	3.853
6	0.095	3.497	3.544	0.237	3.398	3.666	0.102	3.505	3.676
7	0.171	3.386	3.538	0.09	3.363	3.626	0.128	3.681	3.669
8	0.25	3.47	3.634	0.217	3.295	3.645	0.173	3.554	3.683
9	0.269	3.54	3.449	0.13	3.287	3.578	0.359	3.681	3.853
10	0.095	3.386	3.538	0.15	3.291	3.556	0.102	3.505	3.676
11	0.25	3.497	3.544	0.237	3.363	3.666	0.359	3.352	3.724
12	0.171	3.54	3.624	0.09	3.398	3.626	0.17	3.681	3.669
13	0.187	3.471	3.449	0.217	3.295	3.645	0.128	3.505	3.853
14	0.269	3.47	3.538	0.15	3.87	3.578	0.173	3.352	3.683
15	0.25	3.386	3.544	0.13	3.291	3.556	0.359	3.554	3.724
mean	**0.198+0.065**	**3.467+0.056**	**3.563+0.107**	**0.164+0.056**	**3.326+0.046**	**3.614+0.042**	**0.199+0.103**	**3.529+0.117**	**3.721+0.071**
SEM	**0.016**	**0.014**	**0.027**	**0.014**	**0.012**	**0.011**	**0.026**	**0.030**	**0.018**

Whereas : Arithmetic mean ± standard daviation , * ($p<0.01$), ** ($p<0.01$), *** ($p<0.001$)

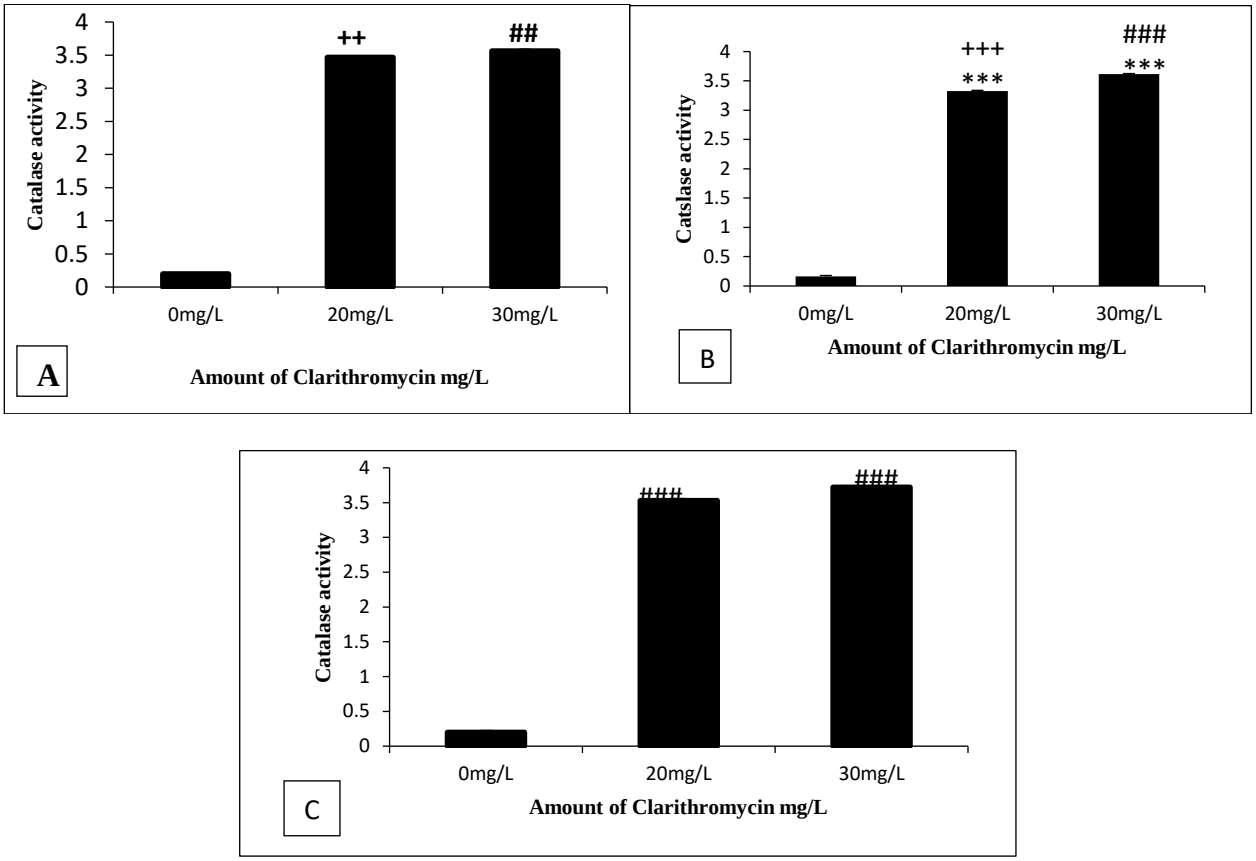

Fig 2.Effect of five days exposure of clarithromycin on catalase activity of liver, kidney and gills in one year old fingerlings of *O. nilotocus.*

Table 3.Effect of five days exposure of clarithromycin on glutathione peroxide activity of liver, kidney and gills in one year old fingerlings of *O. nilotocus.*

Samples	Effect on Liver			Effect on Kidney			Effect on Gills		
	0mg/L	20mg/L	30mg/L	0mg/L	20mg/L	30mg/L	0mg/L	20mg/L	30mg/L
1	0.196	2.624	3.542	0.126	2.645	3.558	0.13	2.683	3.652
2	1.144	2.544	3.4	0.078	2.666	3.627	0.081	2.676	3.478
3	0.13	2.378	3.502	0.13	2.669	3.568	0.072	2.556	3.504
4	0.085	2.724	3.642	0.085	2.853	3.635	0.09	2.626	3.517
5	0.072	2.669	3.496	0.126	2.645	3.558	0.08	1.112	1.189
6	1.144	2.544	3.4	0.078	2.889	3.449	0.756	2.449	3.641
7	0.085	2.724	3.642	0.085	2.853	3.627	0.081	2.676	3.478
8	0.196	2.544	3.502	0.126	2.669	3.635	0.09	2.626	3.478
9	0.13	2.624	3.496	0.13	2.666	3.568	0.13	2.683	3.478
10	0.072	2.669	3.542	0.078	2.889	3.449	0.072	2.676	3.641
11	1.144	2.378	3.642	0.085	2.853	3.627	0.081	2.626	3.517
12	0.085	2.669	3.542	0.126	2.669	3.635	0.09	2.683	3.478
13	0.072	2.544	3.4	0.13	2.853	3.449	0.08	2.556	3.478
14	0.13	2.624	3.496	0.085	2.666	3.558	0.13	2.626	3.652
15	0.196	2.724	3.502	0.078	2.645	3.627	0.072	2.683	3.504
mean	**0.325+ 0.426**	**2.598+ 0.111**	**3.516+ 0.080**	**0.103+ 0.02**	**2.742+ 0.104**	**3.571+ 0.070**	**0.090+ 0.021**	**2.618+ 0.081**	**3.546+ 0.075**
SEM	**0.110**	**0.028**	**0.020**	**0.006**	**0.027**	**0.018**	**0.005**	**0.020**	**0.0193**

Where as : Arithmetic mean ± standard daviation , * ($p<0.01$), ** ($p<0.01$), *** ($p<0.001$)

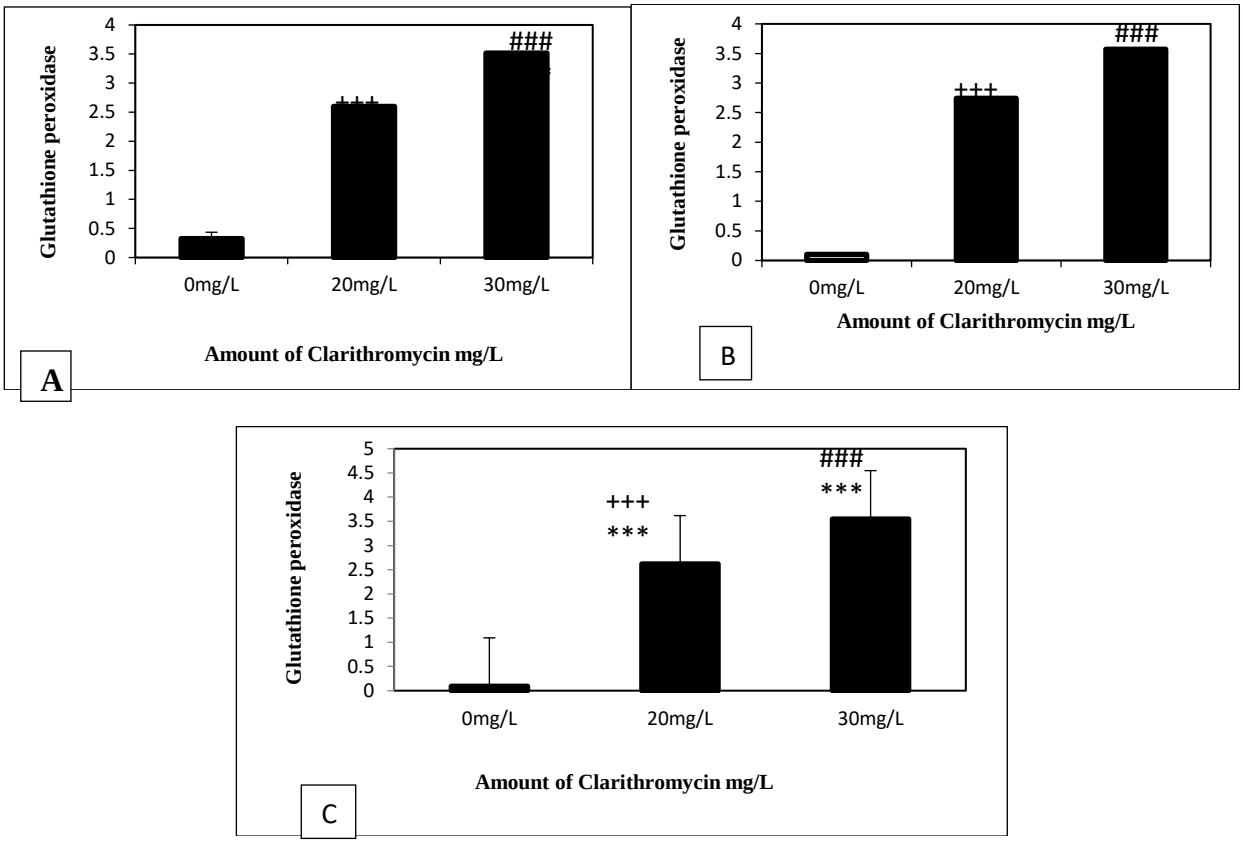

Fig 3.Effect of five days exposure of clarithromycin on glutathione peroxidase activity of liver, kidney and gills on one year old fingerlings of *O. nilotocus.*

Table 4 Analysis of variance (mg/L) of the test media pH, Temperature, Carbon dioxide, Dissolved Oxygen

Samples mg/L	pH of Test Media			Temperature of Test Media			Carbon dioxide of Test Media			Dissolved oxygen of Test Media	
	0mg/L	20mg/L	30mg/L	0mg/L	20mg/L	30mg/L	0mg/L	20mg/L	30mg/L	Omg/L	20mg/L 30mg/L
1	8.00	8.01	8.02	28	28	29	0.23	0.26	0.25	0.45 0.44	0.44
2	8.01	8.02	8.01	28	29	28	0.25	0.25	0.24	0.47 0.43	0.45
3	8.01	8.02	8.03	28	28	29	0.21	0.23	0.23	0.45 0.46	0.45
Mean±SD	8.0± 0.01	8.01±0 .01	8.02± 0.01	28±0 .00	28.05 ±0.00	28.05± 0.00	0.23± 0.02	0.24± 0.01	0.25± 0.01	0.45±0.01 0.44±0.01	0.44±0.01

Table 5 Analysis of variance on contents of sodium, magnesium, calcium, total hardness (mg/L) of the test media

Samples mg/L	Sodium in Test Media			Magnesium in Test Media			Calcium in Test Media			Total hardness in Test Media	
	0mg/L	20mg/L	30mg/L	0mg/L	20mg/L	30mg/L	0mg/L	20mg/L	30mg/L	Omg/L	20mg/L 30mg/L
1	44	45	45	25	26	26	7.00	7.30	7.30	250 250	250
2	45	46	47	24	26	26	7.30	7.35	7.35	250 250	250
3	44.5	47	46	24.7	25.7	25.7	7.5	07.59	7.59	250 250	250
Mean±SD	44.66 ±0.57	461±1	46±1	25.56 ±0.51	25.09± 0.17	25.9±1 7	7.26± 0.25	7.41±0. 15	7.41±0 .15	250±0.00 250±0.00	250±0.00