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Biochemical oxidative alterations of Nile tilapia (*Oreochromis niloticus*) exposed to sodium sulphate and *Spirulina platensis* supplementations

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A B S T R A C T

Phytofilteration using aquatic plants has promising potential for exist cleanup of polluted water.Hence,S.platensis may have potential to be used as a natural feed supplement for protecting fish against heavy metals toxicity.This study investigate the effect of sodium sulphate on Nile tilapia(*O.niloticus*) and the protective effects of S.platensis on the adverse effects of sulfur toxicity. Chronic toxicity by exposed of O.niloticus to 1/10 dose of sodium sulphate 96hours-lethal concentration 50 (LC50)(5.8mg/L) and study the changes in serum biomarkers of liver and kidney functions,total antioxidants capacity(TAC),malondialdehyde (MDA). 260 of Nile tilapia were used for determination of LC50 and chronic toxicity of sodium sulphate. the fish were allocated into four groups of 50 fish per each :control group(CTR)received basal diet, SS group (received basal diet+ 5.8mg/L sodium sulphate),SS group(received basal diet contains S.Platensis extract 1%of diet + 5.8 mg/L sodium sulphate and SE group (received basal diet contains S.Platensis extract 1% of diet with no drug). the results showed that sodium sulphate at dose 5.8mg/L the level of serum total protein,albumin,globulin, and TAC in serum of Nile tilapia were significantly decreased.The serum ALT, AST activity and LPO contents of sodium sulphate exposed group was found higher than the S.platensis supplemented groups. On the basis of present findings, it could be concluded that sodium sulphate in water cause adverse effect on fish blood biochemistry,the changes of serum biomarkers were the physiological responses of O.niloticus to the stress of sodium sulphate exposure. S.platensis can be grown to produce natural products against heavy metals toxicity.

Keywords: Oreochromis niloticus; Sodium sulphate toxicity; Spirulina platensis; Oxidative stress

1. Introduction

Fish are usually considered as organism of choice for assessing the effects of environmental pollution on aquatic ecosystem (Olushola et al. 2014). Pollution of aquatic environment with metals is common worldwide and under certain conditions aquatic fauna may concentrate large amount of some metals from water in their tissues (Kaoud and Rezk, 2011). The toxic effects of heavy metals have been reviewed, including bioaccumulations (Waqar, 2006) and are surrounded with great care and special importance due to their highly toxic effects on fish as they affect survivability, growth and reproduction (Abdel-Tawwab et al. 2004). S. platensis is a photosynthetic , filamentous, blue–green microalgae and is generally regarded as a rich source of vitamins, essential amino acids, minerals, essential fatty acids (γ-linolenic acid), and antioxidant pigments such as carotenoids and phycocyanin (Jaime- Ceballos et al. 2006).

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Hence, S. platensis may have potential to be used as a natural feed supplement for increasing fish growth. Estrada et al. (2001) demonstrated the antioxidant activity of the phycobiliproteins, phycocyanin and alophycocyanin present in Spirulina biomass. The antioxidant activity of Spirulina has been very well documented by (Athukorela et al. 2006). The results of Arun et al. (2012) revealed high percentage of NO scavenging activity in S. platensis. The antioxidant potential in the extract of Spirulina might be due to the total phycocyanin, triterpenoids and carotenoids present in the algal extracts.

This study was aimed to investigate the effects of sodium sulphate on O. niloticus, by determination of 96hour-LC50 of sodium sulphate. Chronic toxicity by exposed of O. niloticus to 1/10 dose of Sodium sulphate 96hr-LC50 values , Changes in antioxidant enzyme activities of O. niloticus gills as activities of superoxide dismutase (SOD) and catalase (CAT) were studied, In addition to changes in enzyme activities of serum as aspartate amino transferase and alanine amino transferase were determined .Also, the protective effect of Spirulina platensis to the ration of exposed O. niloticus by the rate of 1% against sodium sulphate toxicity was evaluated in the current study.

2. Material and methods

1-1 Ethical statement

The expirment was carried out according to the standered method of experimental design toxicity NCLS2000…..
Tricaine Methane Sulphonate MS222 used as anesthestic to decrease fish pain for tissue sampling

1-2 Chemicals and reagents

The assay kits used for biochemical measurements of (CAT), (SOD),((MDA) and (TAC), (AST); (ALT); Total protein; Albumin ; creatinine were purchased from Biodiagnostic Company, ARE. All other chemicals were of reagent grade and were commercially available from local scientific distributors in Egypt. Sodium sulphate was purchased from Lab Service Co. Egypt and dissolved in water.
S.platensis extract was purchased from Urgent® Company, Alexandria branch, Semoha, Egypt and added to the ration by 1% in ration.

1-3.Fish for experimental work

A total of 260 apparently healthy O. niloticus monosex tilapia fish at age of 2months were collected from private fish farms and previously acclimated in indoor tanks in full glass aquaria with dimensions of 70×50×60 cm. They seemed healthy and had a uniform size and weight with average body weight 40 ± 3 gram.

1-4.Fish for determination of LC50

At the first, the 60 apparently healthy O. niloticus fish were used for determination of lethal concentration 50 (LC50) by addition of Sodium sulphate with different concentrations (0, 20, 40, 60, 80, 100 mg L-1) for96hours. Sodium sulphate solutions were prepared by diluting of a stock solution with distal water. The concentration of dilution of heavy metal caused 50% mortality in fish for 96 hours was taken as the LC50 value. During the toxicity test, the fish were not fed. The numbers of dead fish were counted daily and removed immediately from the aquaria. The lethal concentration of Sodium sulphate after 96 hour (96-h LC50) of exposure was calculated according to Behreus and Karber (1953).

1-5.Fish for chronic toxicity test

Chronic toxicity test were established by addition of 1/10 LC50 of sodium sulphate (5.8 mg /L) with a trail of reducing impacts of sodium sulphate toxicity by addition of S.platensis extract by 1% in ration. A total of 200 O. niloticus with average initial weight of 40 ± 3 g. They were allocated into four equal groups (each of 50 fish). including: control group (CTR) received basal diet, SS group (received basal diet and drinking water contain 5.8mg sodium sulphate of per litter),SS group(received basal diet containsS. Platensis extract ad a concentration of 1% of diet and drinking water containing 5.8mg sodium sulphate of per litter, and SE group (S.Platensis) received basal diet contains S. Platensis extract in a concentration of 1% of diet and free access of drinking water with no drug) Each group was reared in a glass aquarium (70×50×60 cm) that was supplied with an aerator and acclimatized for two weeks before the beginning of the experiment. Fish were given a diet of 25% crude protein two times per day at feeding levels of 3% from the live body weight, 7 days per week.

1-6.Fish diets:

Fish were fed on a commercial fish diet containing 25% crude protein. The diet was daily provided at a fixed feeding ratio of 3 % of body weight of fish as described by Eurell et al. (1978). The constituents of the basal diet were illustrated in theTable(1).

1-7 Chemical analysis of S. platensis

The S.Platensis was analysed according to method described by Associated official analytical chemists (AOCA), 1995

1-8- Experimental design of chronic experiment: Four aquaria were used for experimented O. niloticus and divided to four equal groups (50 fish per each) and kept for 8 weeks. The design of the chronic experiment was summarized in Table(2).

1-9-Sample collection and preparation

At 2nd, 4th, 6th and 8th weeks during the experimental period, 4 ml blood samples were collected from different groups via the caudal vessels from 3 fish using disposable syringe (Hawak et al., 1965). Also, sampling was carried out for the analysis of blood parameters. Half the blood sample was then transferred immediately in sterile Eppendorf tubes at -20°C until used for assay (Lied et al., 1975). At same time of blood sampling the specimen of gills, liver, kidney and musculature were collected at 2nd, 4th, 6th and 8th weeks during the experimental period for measurement of different antioxidant parameters from different groups.

1-10 Biochemical analyses

Determination of serum aspartate amino transferase (S.AST), and serum alanineamino transferase (S.ALT) according to the method of Reitman and Frankle(1957).

1-11 Assessment of antioxidant parameters

The antioxidants analysis was carried out as; Total antioxidant capacity(TAC) according to the method of (Galaktionova et al. 1998). Malondialdehyde (MDA) according to the method of Buege and Aust (1978).

1-12-Statistical analysis

All data are expressed as the mean ± standard deviations (SDs), and the levels of significance are cited. SPSS statistical package version 17.0 for Windows (IBM, Armonk, NY, USA) was used for all data analysis. Differences in values were analyzed by one-way analysis of variance (ANOVA), followed by Duncan's multiple range tests. Differences were deemed significant when $P<0.05$.

3. Results

Chemical composition of S. platensis

The data illustrated in Tables (3) revealed the chemical composition of S. platensis which contains crude protein (64%),Fat (8%),total carbohydrate(10%), fiber(8%), and minerals (10%). LC50 sodium sulphate The obtained results showed that the lethal concentration 50 (LC50)of sodium sulphate in tilapia was 58 mg/L; so the 1/10 dose of LC50 of sodium sulphate in tilapia to induce chronic toxicity was 5.8 mg/L.

Effect of sodium sulphateand/or S.platensison alanine aminotransferase (IU/L) in serum of O.niloticus

Our results indicated statistically significant increases in the activities of ALT in the serum of O.niloticus expose to sodium sulphate Fig (1). But levels of the activities of ALT in the serum S.platensis supplemented group decreased significantly in comparison with the control group. In relation to control group, sodium sulphate significantly ($P<0.05$) increased the serum levels of ALT at 6thand 8th weeks of exposure period of 5.8 mg/L of sodium sulphate. S.platensis alone has no effect on the levels of ALT, fortunately it returned the increased levels of ALT to their normal values in sodium sulphate-treated and S.platensis supplemented fish

Effect of sodium sulphateand/or S.platensison aspartate aminotransferase in serum of O.niloticus

Sodium sulphate induced increase in the activities of aspartate aminotransferases were reported, in the serum samples of O.niloticus Fig (2). Our results indicated statistically significant increases in the activities of AST in the serum of O.niloticus exposed to sodium sulphate. But the activities of AST in the serum of S.platensis supplemented group decreased significantly in comparison withthe control group. In relation to control group, sodium sulphate significantly ($P<0.05$) increased the serum activities of AST at 6thand 8th weeks of exposure period of 5.8mg/L of sodium sulphate. S.platensis alone has no effect on the activities of AST, fortunately it returned the reversed the AST activities to their normal values in –treated and S.platensis supplemented fish

Effect of sodium sulphateand/or S.platensison total protein (mg/dL) in serum of O.niloticus

Fig (3) has also been shown to lead to a decrease in total protein levels in serum of O. niloticus exposed to 5.8 mg/L sodium sulphate for 8th weeks. We also observed a strong linear relationship between sodium sulphate exposure periods and the biochemical parameters in the serum. However, these biochemical endpoints are potential biomarkers for sodium sulphate exposure in O. niloticus. Furthermore, there were statistically significant changes in levels of total protein in serum of groups exposed to sodium sulphate and / or S.platensis groups ($P<0.05$).

Effect of sodium sulphateand/or S.platensison albumin (mg/dL) in serum of O.niloticus

Our results indicated that there were statistically significant increases in the levels of albumin in the serum of sodium sulphate and/or S.platensis group ($P<0.05$) (Fig, 4). But levels of albumin in the serum of sodium sulphate group decreased significantly in comparison with the control group.

Effect of sodium sulphateand/or S.platensison globulin in serum of O.niloticus

Fig (5) shown significant decreases in globulin levels in serum of O. niloticus exposed to 5.8 mg/L sodium sulphate for 8th weeks. We, also observed a strong linear relationship between sodium sulphate periods of exposure and the biochemical parameters in the serum. However, these biochemical endpoints are potential biomarkers for sodium sulphate exposure in O. niloticus. Furthermore, there were statistically significant changes in levels of globulin in serum of groups exposed to sodium sulphateand/or S.platensis groups
($P<0.05$).

Effect of sodium sulphate and/or S.platensison creatinine (mg/dL) in serum of O.niloticus

A significant increase of creatinine (mg/dL) in serum of O.niloticus occurred at sodium sulphate exposure of O.niloticus to 5.8 mg./L. for 8th weeks ($P<0.05$) (Fig6). A significant reduction of creatinine (mg/dL) in serum of O.niloticus was found at S.platensis supplement group alone .Moreover, an increase in the level of creatinine (mg/dL) in serum of O. niloticus exposed to sodium sulphate and/or S.platensis, but it was significantly higher ($P<0.05$) only at sodium sulphate exposure of O.niloticus to 5.8 mg/Lat the8th week.

Effect of sodium sulphateand/or S.platensison malondialdehyde (MDA)activity in the serum of O.niloticus

Malondialdehyde (MDA) in the sodium sulphate-exposed group was found higher than in the S.platensis supplemented groups ($P<0.05$). A slight non-significant increase was observed in MDA levels in the serum, liver, and kidney of O.niloticusat the2th week of exposure to sodium sulphate. However,at4th,6th, and 8th week of exposure the levels of MDA were increased significantly ($P<0.05$) as presented in Fig 7-8-9.

4. Discussion

The antioxidant activity of Spirulina has been very well documented by (Athukorela et al. 2006). The activities and expression levels of antioxidant enzymes and metabolic were used as biomarkers to evaluate the influence of pollution on the biochemical pathway and enzymatic

function in fish (Correia et al. 2007) and also for monitoring unacceptable levels of environmental contamination (Svoboda, 2001). The obtained results showed that the LC50 of sodium sulphate in O. niloticus was 58 mg/L; so the 1/10 dose of LC50 of sodium sulphate in O. niloticus to induced chronic toxicity was 5.8 mg/L. The acute toxicity (LD50) of sodium sulfate has not been reliably established but is probably far in excess of 5000 mg / kg Wang et al. (2015). In this study, we examined the effects of spirulina on serum AST and ALT levels, and found that, the levels of AST and ALT were decreased by spirulina supplementation, no significant differences were observed among treatments. Fish mostly rely on innate immunity in comparison to mammals (Swain et al., 2007). Hussein et al. (2012) recorded that the fish exposed to Hg resulted in significant changes in plasma aspartate aminotransferase (AST), alanine aminotransferase (ALT) were observed in fish exposed to Hg. Results also, indicated that Spirulina platensis was effective in removing Hg from water. The addition of dried Spirulina platensis improves the haematological parameters (RBCs, Hb and Hct) and ameliorates the toxic effect of Hg which indicating the capability of S. platensis to chelate Hg from the media The quantitative measurements of enzymes AST and ALT, in serum showed increased which may be due to the reduced oxidation of glucose while decreased in protein concentration may be related to produce the energy demand to overcome the stress for survival of fish (Ganeshwade et al., 2012). This is supported by the facts that when an organ is directly exposed to toxicants, enzymes activity may be increased or decreased due to denaturation of active sites (Aziz, 2012). The activity of AST and ALT enzymes in blood may also be used as a stress indicator. The significant changes in the activities of these enzymes in blood plasma indicate tissue impairment caused by stress (Svoboda, 2001). In the present study, there were significant changes in AST and ALT activities in serum of fish exposed to sodium sulphate compared to the supplemented S.platensis and control group. The increase in concentration of AST and ALT in serum indicates impairment of parenchymatous organs mainly liver. In addition, the increase of serum AST and ALT may be attributed to the hepatocellular damage or cellular degradation in liver, spleen or muscles (Kaoud et al., 2011). Hepatic AST and ALT activities were decreased at high dose of Hg O. niloticus exposed to inorganic Hg (Abdel-Tawwab et al., 2004)

Data showed that creatinine was significantly increased in sodium sulphate treated groups compared with the S.platensis and control groups. Mukhopadhyay et al. (1982) reported that there is an intimate relationship between serum transaminases levels and liver integrity A noticeable increase in lipid peroxidation biomarker MDA was observed in Paramecium cells treated with different concentrations of cypermethrin. These findings are in good agreement with those of (Xiangguo et al., 2011) who recorded a significant increase in MDA level in embryo-larval stages of zebrafish intoxicated with different doses of cypermethrin. Also, (Oliveira et al., 2012) reported the significant increase of MDA level in the common prawn Palaemon serratus intoxicated with deltamethrin, a type II pyrethroid. Our results showed that sodium sulphate treatment may result in peroxidation of polyunsaturated fatty acids, leading to the degradation of phospholipids and ultimately result in cellular deterioration (Tappel, 1973) .Addition of dried S. platensis at 10 g/kg of diet for 2 months in feeding of O. niloticus during sodium sulphate toxicity is increase (MDA) activity in the serum, gills, liver, kidney and muscular tissue of O. niloticus. Upasani and Balaramana (2003) investigated the protective effect of Spirulina on lead induced changes in the levels of (MDA) and endogenous antioxidants in liver, lung, heart, kidney and brain of rats. Levels of elemental lead were decreased in the organs of rats in all experimental groups. This evaluation was done by measuring the malondialdehyde (MDA), reduced glutathione (GSH), 4-hydroxy-2-nonenal (4- HNE) and nitric oxide (NO) content of the tissue as markers of lipid peroxidation. The results suggest that ascorbic acid and water extract of Spirulina platensis could suppress the 5-FU-induced lipid peroxidation to a significant extent. Similarly, Shelke (2016) and Priya et al. (2018) mentioned that the Spirulina provides

protection against mercuric chloride-induced oxidative stress and alteration of antioxidant defence mechanism in the liver.

5. Conclusions

On the basis of present findings it could be concluded that increased sodium sulphate content in water of aquaculture causes adverse effect on fish. The changes of plasma biomarkers as antioxidant enzymes; were the physiological responses of O. niloticus to the stress of sodium sulphate exposure. Spirulina platensis can be grown to produce more natural products and environmentally friendly materials.

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Table (1): Design of the chronic experiment

Groups	Treatments	Number of fish	Dose of Sodium sulphate and other additives	Reference
G (1)	Control (without treatment)	50	0	
G (2)	Sodium sulphate only	50	5.8 ppm (1/10dose of LC_{50})	Behreus, A. S. and Karbeur, L. (1953)
G (3)	Sodium sulphate plus *S .platensis* extract	50	5.8 ppm+ *S .platensis* extract by 1% in ration	
G (4)	*S .platensis* extract	50	*S .platensis* extract by 1% in ration	

Table (2) : The ingredient composition (%) of the basal diet (without supplementation of spirulina platensis extract

Ingredients	**%**
Fish meal (65%)	20
Barley	30
Soybean meal	30.7
Bone meal	1.18
Wheat bran	14.5
Premix	0.3
Limestone	1.8
DL- methionine	0.55

Table (3): The Biochemical analysis of S. platensis

Component	concentration
Crude protein	64%
Fat (lipids)	8%
Total carbohydrate	10%
Fiber	8%
Minerals	10%

Fig. (1): S.ALT (IU/L) in the serum of O.Niloticus

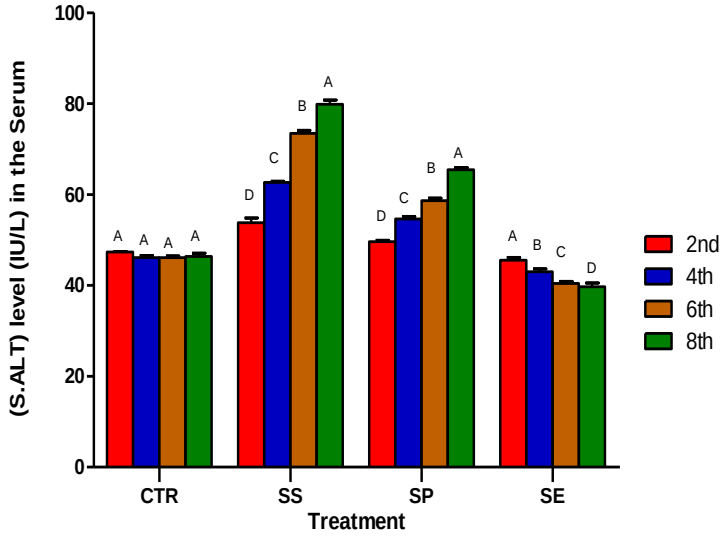

Fig. (2): S.AST (IU/L) in the serum of O.niloticus

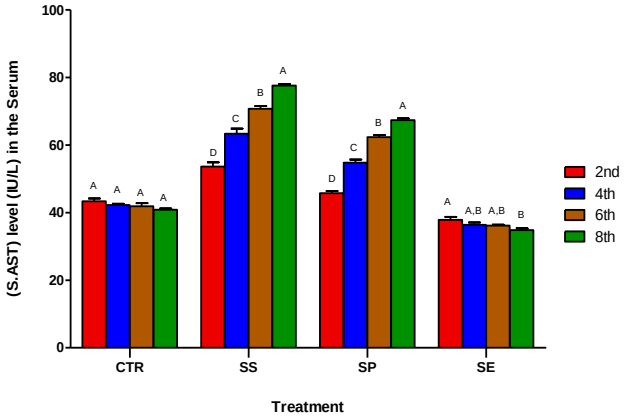

Fig. (3):Total protein (mg/dL) in the serum of O.niloticus

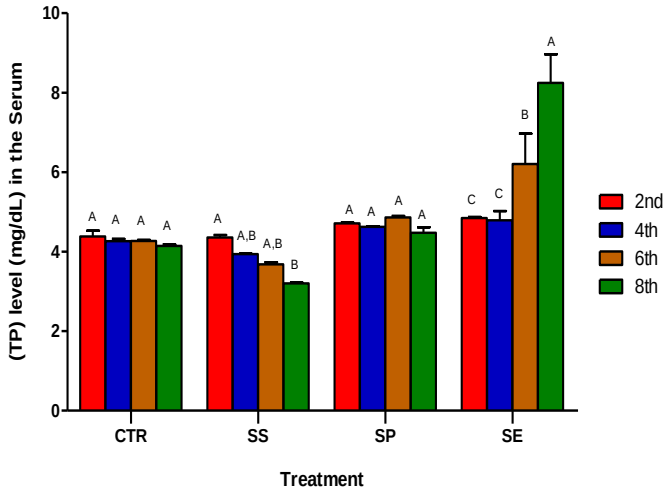

Fig. (4):Albumin (mg/dL) in the serum of O.niloticus

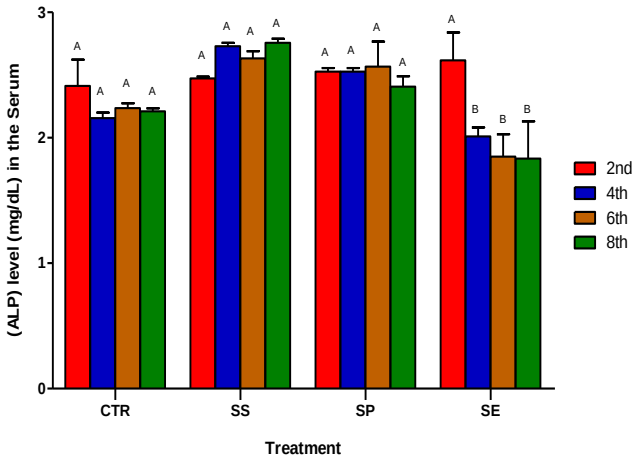

Fig. (5): Globulin (mg/dL) in the serum of O.niloticus

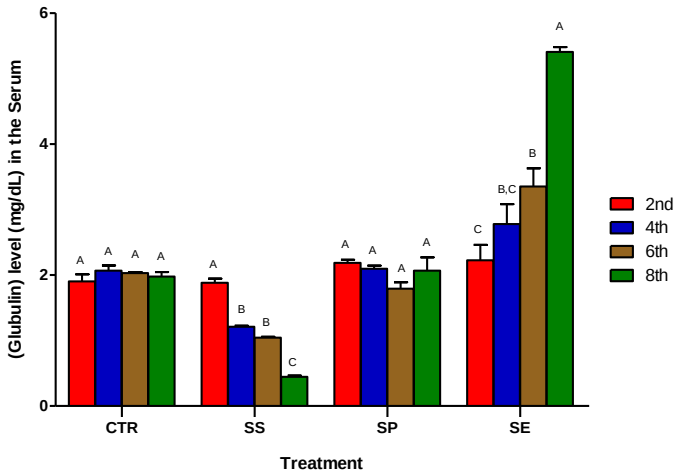

Fig. (6): Creatinine (mg/dL) in the serum of O.niloticus

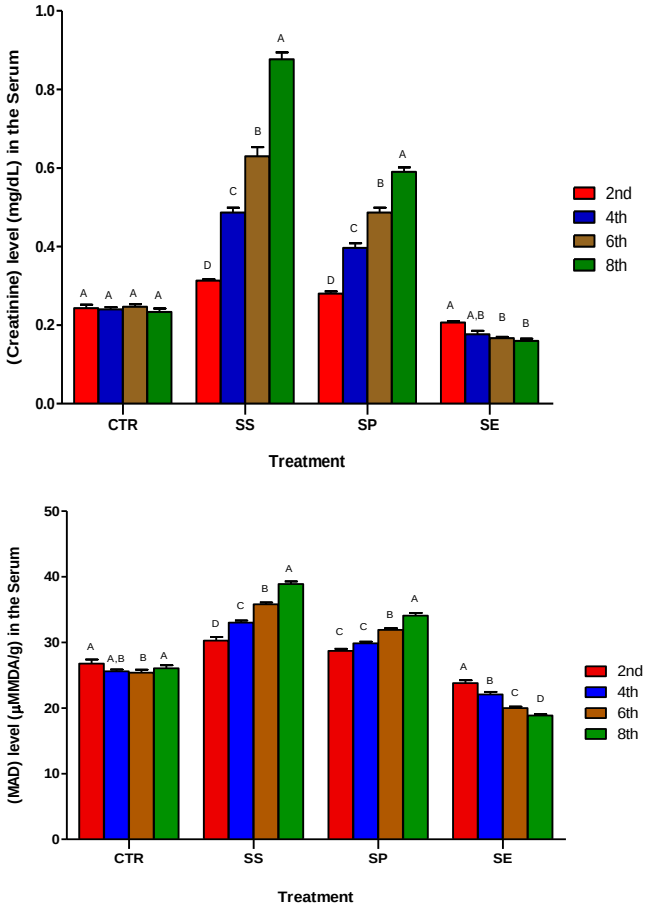

Fig. (7): Lipid peroxidase (MDA) activity (nmol (µM MDA/mg protein) /ml.) in serum of O.niloticus.

Fig. (8): Lipid peroxidase (MDA) activity (nmol (µM MDA/mg protein) /g wet tissue) in liver of O.niloticus.

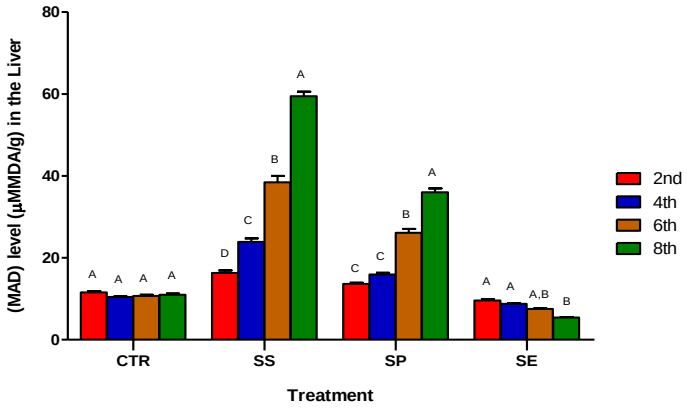

Fig. (9): Lipid peroxidase (MDA) activity (nmol (µM MDA/mg protein) /g wet tissue) in kidney of O.niloticus.