LIPID PEROXIDATION AND ANTIOXIDANT ENZYMES IN BLOOD AND TISSUES OF CATFISH EXPOSED TO HEAVY METAL POLLUTION

BY

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SUMMARY

The passage of different environmental pollutant to the aquatic system demonstrates the need for a comprehensive study for their effect on the living resources. The effects of exposure of catfish (Clarias lazera) to lead, nickel, and chromium for one and two months on malondialdehyde, reduced glutathione and some antioxidant enzymes in blood, liver, kidney and muscles were studied. The role of vitamins E and C as antioxidant was also investigated. It is evident that heavy metals elevated malondialdehyde, reduced glutathione concentrations, glutathione–S-transferase and superoxide dismutase activities in blood and organs. Furthermore, they inhibited glutathione peroxidase and catalase activities. Treatment by vitamins E and C reversed the effect of heavy metals on malondialdehyde, superoxide dismutase, and catalase.

INTRODUCTION

Heavy metals pollution was regarded as a sever problem because heavy metals injure the biological functions of the aquatic organisms and their accumulation in fish organs and flesh leads to serious healthy hazards to the consumers *(Daoud et al., 1999)*.

Primary source of heavy metals pollution includes industrial and agriculture discharges, chemical and chloride plants emissions, aerial fallout, phosphate fertilizers and sludge used in cultivated lands, sewage effluents, some types of plastics and pesticides, and coal and oil combustion. *(El-Nabawi et al., 1987; Sorensen, 1991; Shibamoto and Bjeldanes, 1993)*.

Heavy metals transform oxygen into reactive oxygen species (ROS), which are highly toxic and mutagenic. Heavy metals catalyze the reaction of superoxide anion and hydrogen peroxide to form hydroxyl radicals, a highly reactive and deleterious ROS that damages many biomolecules *(Winston and Di-Giulio, 1991)*.

Reactive oxygen species are continually produced in biological system as by products of normal metabolism, where they are detoxified by antioxidant defenses. Marked increase in ROS production, can tip the balance between pro-oxidant and antioxidant processes, resulting in oxidative damage to cellular processes *(Stohs and Bagchi, 1995).*

Antioxidant defense system detoxifies "ROS" and its major constituents including non enzymatic defense components such as certain vitamins like vitamin E and C, Low molecular reducing agents e.g. reduced glutathione and other thiols, and antioxidant enzymes e.g. superoxide dismutase, catalase, glutathione peroxidase and glutathione-S-transferase *(Gustafson et al., 1993).*

The present work was planned to elucidate the effect of pollution with heavy metals such as lead, nickel, and chromium on lipid peroxidation, antioxidant defense enzymes. Also to test the ability of vitamins E and C for protection of fish against the expected harmful effect induced by exposure to heavy metals.

MATERIALS AND METHODS

Three hundred apparently healthy catfish (Claris Lazera) weighting about 200-250 gram, were purchased from the local market at Giza governorate and transferred to fish disease department in Animal Health Research Institute. The fish were kept in aquaria (100x 50x50 cm) supplied by dechlorinated tape water and aerated by using air pumps. The aquarium water temperature was adjusted at (22- 26^{0}c) and pH 7.2 ± 0.2.

Fish were acclimated to the laboratory condition for 2 weeks before the beginning of the experiment, fed on balanced ration of 32% protein used in the form of artificial pellets, provided as (2-3) % of the body weight *(Eurell et al., 1978).*

One hundred and eighty fish were used for the determination of 96 hours median lethal concentration (96 h LC_{50}) for lead, chromium and nickel (60 fish for each) according to the method reported by *Behrens and Karber (1953).*

The remaining 120 fish were divided into four groups each of 30 fish. The first, second and third groups were exposed to 1/20 of 96 h LC_{50} lead (as lead nitrate), 1/10 of 96 h LC_{50} chromium (as sodium chromate) and 1/10 of 96 h LC_{50} nickel (as nickel chloride) for two months respectively, while the last group was kept as control. Each group was subdivided into three subgroups (ten fish for each). The first subgroup was exposed to heavy metal only while the second and the third subgroup were injected i.m. with 5 and 10 mg/Kg BW of vitamin E and C respectively in alternative days.

Biochemical analysis:

Five samples of whole blood, kidney, liver and muscles were collected from each subgroup at the end of the first and second months from the beginning of experiment. Blood and organs were prepared and used for determination of malondialdehyde *(Albro et al., 1986),* reduced glutathione *(Chanarin, 1989),* glutathione peroxidase (Gpx, EC 1.11.1.9) *(Paglia and Valentine, 1967),* glutathione-S-transferase (GST, EC 2.5.1.18) *(Habig et al.*

1974), superoxide dismutase (SOD, EC 1.15.1.1) (*Misra and Fridovich, 1972;* and *Packer and Glazer, 1990),* catalase (CAT, EC 1.11.1.6) (*Aebi, 1974),* hemoglobin in blood hemolysate was determined according to *Wintrobe, (1965),* protein in tissue homogenate (*Lowry et al. 1951),* heavy metal residues was determined by atomic absorption according to *Jackson, (1973).*

RESULTS AND DISCUSSION

The data shown in Table (1) indicated that malondialdehyde concentration in plasma and organs of catfish exposed to lead, chromium and nickle was significantly increased with more pronounced effect after two months than one month. Peroxidation of lipid has been suggested to be one of the primary mechanisms of cell injury by xenobiotics *(Recknagel and Glende, 1977).*Transition metals are though to initiate the lipid peroxidation by catalizing the production of reactive oxygen species *(Green and Hill, 1984)* The stimulatory effect of xenobiotics on lipid peroxidation have been also attributed to impairment of cystolic protective systems including low molecular weight antioxidant such as ascorbic acid *(Wright et al., 1981)*and cystolic enzyme such as glutathione peroxidase *(Flohé,et.al 1976)*and catalase *(Palace,et.al 1993)* .

Administration of vitamin E during lead exposure originally decreased malondialdehyde (MDA) concentration in plasma and all organs after one and two months. In chromium and nickel intoxicated fish, vitamin E significantly decreased malondialdehyde concentration in plasma, kidney and muscles after one and two months while in liver this effect appears at the end of two months.

Vitamin E offers protection against peroxidative damage to the membrane, as it is believed to quench and react with singlet oxygen and therefore protect the membrane against ROS. It also reacts with lipid peroxyl radicals to form vitamin E radicals that are insufficiently reactive to abstract hydrogen radical from the membrane lipids *(Gutteridge, 1978).* The effect of vitamin C against the peroxidative effect of heavy metals was obvious in plasma and organs of lead and nickel treated fish while in chromium treated fish, this effect appears in plasma and muscles only. Vitamin C acts as a free radical scavenger, neutralizing reactive oxygen species as superoxide, hydrogen peroxide and hypochlorous acid and being converted into dehydroascorbic acid *(Nygaard and Simic, 1983)*

Reduced glutathione plays an important role in metabolism of peroxides and free radicals, protecting cells from lipid peroxidation *(Heath, 1995).* Reduced glutathione (GSH) concentration was significantly increased in blood and all organs of lead intoxicated fish after one and two months similar results were recorded in blood, liver and kidney of chromium and nickel treated fish. While in muscles, the concentration was significantly increased after two months. Our findings agree with that obtained by *Thomas and Juedes (1992).* They recorded a significant increase in glutathione concentration in the liver and kidney of Atlantic croaker exposed to lead. *Allen*

(1995) found that cadmium, mercury and lead significantly elevated reduced glutathione concentration in the kidney of Oreochromis niloticus fish.

The increase in the concentration and content of GSH that occur in the present study (table 2) may be due to induction of the synthesis of the rate-limiting enzyme, γ-glutamyl-cysteine synthetase by heavy metals *(Zalups and Lash, 1996)*. Treatment of catfish with vitamin E and vitamin C had no effect on GSH concentration in blood and all organs that agree with *(Lygren et al., 2000)*.

Glutathione peroxidase (Gpx) is the major effector in relieving oxidative stress by the conversion of GSH to GSSG with concomitant reduction of hydrogen peroxide *(Stephensen et al., 2002)*. Lead and chromium significantly decreased glutathione peroxidase (Gpx) activity in blood after first and second month of exposure while in organs after two months. On the other hand, nickel exposure significantly decreased the enzyme activity in blood and liver after one month and in kidney and muscles after two months. The reduced activity of Gpx by lead, chromium and nickel exposure observed in the present investigation may contribute greatly to peroxidative damage as described by *Sugiyama (1994)*. Lastly, heavy metals may alter the tertiary and quaternary structure of the enzyme *(Bem et al., 1985)*. In addition, heavy metals probably interact with tissue selenium or with the seleno-prosthetic group precursor available for enzyme synthesis *(Omaye and Tappel, 1975)*. Vitamin E injection significantly increased Gpx activity in liver and muscles of chromium and nickel exposed fish after two months respectively. On the other hand vitamin C had no reversal effect to that of heavy metals.

Glutathione-S-transferases (GST) catalyses the conjugation of a wide variety of electrophilic compound including xenobiotics with glutathione *(Arias and Jakoby, 1976)*. This action is very important because it prevents xenobiotics from reacting with nucleophilic centers of proteins, DNA and RNA *(Ronisz et al., 1999)*. The data recorded in table (4) revealed a significant elevation in GST activity in blood and organs of catfish exposed to lead, chromium and nickel after two months of exposure with non significant change after one month, treatment by vitamin E and vitamin C did not alter GST activity. This data coincided with *Tuvikene et al (1999),* who recorded a significant elevation in hepatic GST in rainbow trout, perch and roach exposed to heavy metals pollution.

SOD activity in blood was significantly increase after one and two months of lead, chromium, and nickel exposure while in organ after two months only (Table 5),The significant increase in SOD may be attributed to the fact that xenobiotics as heavy metals enhance the formation of superoxide radicals *(Lemaire and Livingstone 1993)* while elicited a significant increase in SOD as adaptive response*(Pedragas et al., 1993)* Vitamin E administration reduced SOD activity in the blood and liver of catfish exposed to lead for two months and in blood and muscles of catfish exposed to chromium for one and two months respectively and in muscles of catfish exposed to nickel for two

months. The protective effect of vitamin C appears in blood and muscles of chromium treated fish only. These results are in accordance with that of *Palace et al. (1993)*, they found that deficiency in ascorbic acid and tocopherol increases hepatic SOD activity in rainbow trout (Oncorhynchus mykiss) fish. Vitamin E and vitamin C serve as antioxidants against lipid peroxidation *(Murata et al., 1994)* so their administration along with heavy metals probably decreases the demand for enzymatic antioxidants such as SOD.

Exposure of catfish to lead, chromium and nickel significantly decrease catalase activity in blood and organs. Possible mechanisms by which the heavy metals inhibit CAT activity include direct metal mediated structural alteration of the enzyme (*Arillo et al., 1984*) and depression of CAT synthesis (*Pruell and Engelhardt, 1980*). Administration of vitamin E significantly increased CAT activity in muscles of all treated groups, in blood of lead and chromium groups and in kidney of chromium and nickel groups. Vitamin C significantly increased CAT activity in muscles after one month of lead, nickel exposure and after one and two month in chromium treated fish. These results agree with that of *Andersen et al. (1998),* they found that ascorbic acid polyphosphate increased kidney catalase in salmon smolts.

Lead concentration was significantly increased in blood and kidney after one and two months of lead exposure and in liver and muscle after two months. Chromium and nickel concentration was significantly increased in blood, liver and kidney after one and two months and in muscle after two months. Accumulation of lead and chromium was high in kidney while nickel was more pronounced in liver (table 7).

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Table (1) Malondialdehyde (MDA) concentration in plasma (nM / ml) and organs (nM/mg protein) of catfish intoxicated with lead, chromium and nickel and treated by vitamin E and vitamin C.

Tissue / Treatment	Plasma		Liver		Kidney		Muscles	
	1 month	2 months	1 month	2 months	1 month	2 months	1 month	2 months
Control	# 4.52 ± 0.08 A	# 4.63 ± 0.08 A	# 1.18 ± 0.03 A	# 1.19 ± 0.05 A	# 2.15 ± 0.07 A	# 2.17 ± 0.08 A	# 4.52 ± 0.09 A	# 4.64 ± 0.09 A
Vitamin E treated	3.91 ± 0.09 aB	3.65 ± 0.07 aB	0.91 ± 0.04 aB	0.89 ± 0.04 aB	1.74 ± 0.08 aB	1.64 ± 0.08 aB	3.69 ± 0.09 aB	3.52 ± 0.09 aB
Vitamin C treated	4.07 ± 0.11 aC	3.96 ± 0.11 aC	1.03 ± 0.05 aC	1.01 ± 0.05 aC	1.86 ± 0.09 aC	1.75 ± 0.09 aC	3.94 ± 0.10 aC	3.84 ± 0.07 aC
Pb treated	6.46 ± 0.16ab cD	6.83± 0.19ab cD	1.46± 0.06ab cD	1.97± 0.07ab cD	2.73± 0.08ab cD	3.18± 0.08ab cD	6.54± 0.15ab cD	7.63± 0.19ab cD
Pb and vitamin E treated	5.34± 0.09ab d	5.74± 0.18ab d	1.30± 0.05bd	1.64± 0.08ab d	2.46± 0.08ab d	2.64± 0.07ab d	5.59± 0.15ab d	5.91± 0.18ab d
Pb and vitamin C treated	5.76± 0.11ac d	6.65± 0.11ac	1.38± 0.05ac	1.68± 0.05ac d	2.53± 0.08ac	2.82± 0.12ac d	5.71± 0.17ac d	6.28± 0.08ac d
Cr treated	6.55± 0.14ab cD	7.08± 0.17ab cD	1.45± 0.05ab c	1.65± 0.06ab cD	2.65± 0.0abc D	2.95± 0.09ab cD	6.21± 0.19ab cD	6.71± 0.21ab cD
Cr and vitamin E treated	5.51± 0.12ab d	5.72± 0.15ab d	1.37± 0.08ab	1.48± 0.07ab d	2.34± 0.08bd	2.66± 0.09ab d	5.26± 0.11ab d	5.38± 0.11ab d
Cr and vitamin C treated	5.86± 0.12ac d	6.24± 0.14ac d	1.34± 0.04ac	1.55± 0.05ac	2.45± 0.09ac	2.74± 0.08ac	5.36± 0.09ac d	6.33± 0.18ac
Ni treated	7.33± 0.15ab cD	7.62± 0.17ab cD	1.52± 0.05ab c	1.84± 0.04ab cD	2.94± 0.07ab cD	3.11± 0.21ab cD	6.56± 0.13ab cD	7.12± 0.15ab cD
Ni and vitamin E treated	6.26± 0.16ab d	6.49± 0.15ab d	1.39± 0.05ab	1.59± 0.05ab d	2.44± 0.08ab d	2.65± 0.07ab d	5.42± 0.11ab d	6.10± 0.14ab d
Ni and vitamin C treated	6.33± 0.12ac d	7.01± 0.14ac d	1.55± 0.05ac	1.65± 0.04ac d	2.58± 0.08ac d	2.76± 0.08ac d	5.54± 0.11ac d	6.35± 0.14ac d

Each value represents mean ± S.E.; n = 5.
#: Significant variation between groups by ANOVA at P ≤ 0.05.
Small letters a, b, c and d represent a significant change to capital letters A, B, C and D respectively by LSD at P ≤ 0.05.

Table (2) Reduced glutathione (GSH) concentration in blood (µM / ml) and organs (µM /mg protein) of catfish intoxicated with lead chromium and nickel and treated by vitamin E and vitamin C.

Tissue / Treatment	Plasma		Liver		Kidney		Muscles	
	1 month	2 months	1 month	2 months	1 month	2 months	1 month	2 months
Control	# 0.38 ± 0.03 A	# 0.38 ± 0.01 A	#85.55 ± 0.80 A	# 85.14 ± 0.95 A	# 65.95 ± 1.36 A	# 65.75 ± 0.85 A	# 33.14 ± 1.09 A	# 33.06 ± 0.87 A
Vitamin E treated	0.39 ± 0.02 B	0.41 ± 0.03 B	86.68 ± 1.13 B	87.44 ± 0.92 B	66.70 ± 1.44 B	67.14 ± 1.23 B	35.11 ± 0.95 B	35.30 ± 1.34B
Vitamin C treated	0.39 ± 0.02 C	0.40 ± 0.03 C	85.93 ± 1.57 C	86.62 ± 1.12 C	66.29 ± 1.02 C	66.92 ± 1.30 C	34.07 ± 1.36 C	34.62 ± 0.84 C
Pb treated	0.48± 0.02 abc	0.51± 0.02ab c	96.14± 2.33ab c	103.22 ± 1.30ab c	75.97± 1.18ab c	78.85 ± 0.89 abc	39.81 ± 0.86 abc	43.52± 2.50 abc
Pb and vitamin E treated	0.52 ± 0.03 ab	0.56 ± 0.03 ab	98.31 ± 3.13 ab	106.79 ± 1.66 ab	77.49 ± 1.28 ab	80.54 ± 0.91 ab	41.11 ± 0.80 ab	45.28 ± 0.74ab
Pb and vitamin C treated	0.50 ± 0.03 ac	0.54 ± 0.02 ac	97.08 ± 1.31 ac	105.02 ± 1.43 ac	76.15 ± 1.13 ac	79.33 ± 0.84 ac	40.71 ± 1.57 ac	44.43 ± 0.90ac
Cr treated	0.48 ± 0.01 abc	0.53 ± 0.02 abc	93.07 ± 2.36 abc	103.48 ± 2.46 abc	74.56 ± 1.77 abc	81.38 ± 1.55 abc	36.08 ± 1.05	38.80 ± 0.90 abc
Cr and vitamin E treated	0.50 ± 0.01 ab	0.55 ± 0.01 ab	95.40 ± 3.23 ab	104.89 ± 2.66 ab	75.40 ± 1.73 ab	82.19 ± 1.78 ab	37.99 ± 1.08	40.95 ± 0.88ab
Cr and vitamin C treated	0.50 ± 0.01 ac	0.54 ± 0.02 ac	94.74 ± 2.12 ac	104.20 ± 2.31 ac	75.28 ± 1.62 ac	81.79 ± 1.11 ac	37.10 ± 0.94	40.40 ± 1.44ac
Ni treated	0.49 ± 0.02 abc	0.54 ± 0.02 abc	93.18 ± 1.27 abc	104.06 ± 2.05 abc	76.07 ± 1.12 abc	80.17 ± 1.13 abc	37.43 ± 1.52	40.28 ± 1.31 abc
Ni and vitamin E treated	0.52 ± 0.02 ab	0.56 ± 0.02 ab	94.50 ± 1.15 ab	105.50 ± 2.28 ab	77.11 ± 0.92 ab	81.50 ± 1.07 ab	38.29 ± 1.46	41.50 ± 111ab
Ni and vitamin C treated	0.50 ± 0.01 ac	0.56 ± 0.02 ac	93.90 ± 1.16 ac	104.62 ± 1.83 ac	76.89 ± 1.14 ac	80.80 ± 1.19 ac	37.99 ± 1.24	41.15 ± 1.84ac

Each value represents mean ± S.E.; n = 5.
#: Significant variation between groups by ANOVA at P ≤ 0.05.
Small letters a, b, c and d represent a significant change to capital letters A, B, C and D respectively by LSD at P ≤ 0.05.

Table (3) Glutathione peroxidase (Gpx) activity in blood (eu/g haemoglobin) and organs (eu /g protein) of catfish intoxicated with lead, chromium and nickel and treated by vitamin E and vitamin C.

Tissue / Treatment	Plasma		Liver		Kidney		Muscles	
	1 month	2 months	1 month	2 months	1 month	2 months	1 month	2 months
Control	#326.08 ± 6.84A	#327.70 ± 9.33A	106.02 ± 3.93	#105.48 ± 4.11A	129.72 ± 4.30	#130.63 ± 3.35A	25.46 ±1.40	#25.16 ±1.02A
Vitamin E treated	339.37 ± 10.99B	340.66 ± 10.12B	110.19 ± 2.90	111.04 ± 2.99B	135.46 ± 3.97	138.67 ± 3.77B	28.47 ± 2.14	29.86 ± 1.23B
Vitamin C treated	330.93 ± 4.99C	332.29 ± 5.63C	107.66 ± 2.96	107.73 ± 3.24C	131.62 ± 3.11	132.52 ± 3.06C	26.50 ± 2.28	26.59 ± 1.95C
Pb treated	299.75 ± 5.83abc	292.01 ± 5.20abc	97.22 ± 3.81	90.20 ± 1.43abc	117.99 ± 3.79	108.60 ± 3.97abc	22.28 ± 2.65	19.78 ± 1.16abc
Pb and vitamin E treated	313.56 ± 8.06b	307.70 ± 9.55b	102.21 ± 4.75	98.00 ± 4.79b	122.71 ± 4.87	115.35 ± 4.79ab	23.01 ± 1.70	21.29 ± 1.46b
Pb and vitamin C treated	309.68 ± 8.05	302.75 ± 7.88ac	100.57 ± 5.11	95.84 ± 6.94	120.17 ± 7.17	107.13 ± 5.62ac	22.64 ± 1.23	20.26 ± 1.24ac
Cr treated	301.89 ± 8.06abc	296.32 ± 7.12abc	94.16 ± 5.22	89.19± 3.06abcD	119.36 ± 3.51	116.41 ± 3.65abc	22.19 ± 0.73	19.79 ± 0.74abc
Cr and vitamin E treated	315.55 ± 7.26b	312.91 ± 7.97b	103.63 ± 3.91	101.07 ± 2.96d	123.23 ± 5.68	126.06 ± 3.62b	23.00 ± 0.99	21.26 ± 1.03ab
Cr and vitamin C treated	309.89 ± 5.58	305.75 ± 9.61c	96.64 ± 6.52	95.21 ± 5.02c	119.90 ± 6.23	120.37 ± 5.55c	22.33 ± 1.63	20.68 ± 1.39ac
Ni treated	296.66 ± 6.26abc	288.95 ± 6.76abc	93.29± 3.93abc	90.19± 3.58abc	118.47 ± 4.83	112.04 ± 5.40abc	22.29 ± 1.39	19.76± 0.85abcD
Ni and vitamin E treated	314.94 ± 6.29b	303.57 ± 6.14ab	101.55 ± 3.42	94.89 ± 3.04ab	124.33 ± 6.80	121.47 ± 5.79b	24.68 ± 0.81	23.71 ± 0.80bd
Ni and vitamin C treated	310.472 ± 9.136	300.77 ± 8.96ac	96.74 ± 4.32c	92.93 ± 4.29ac	122.70 ± 3.28	118.73 ± 3.44c	23.23 ± 1.37	21.15 ± 1.42ac

Each value represents mean ± S.E.; n = 5.
#: Significant variation between groups by ANOVA at $P \leq 0.05$.
Small letters a, b, c and d represent a significant change to capital letters A, B, C and D respectively by LSD at $P \leq 0.05$.

Table (4) Glutathione -S- transferase (GST) activity in blood (eu/g haemoglobin) and organs (eu /g protein) of catfish intoxicated with lead, chromium and nickel and treated by vitamin E and vitamin C.

Tissue / Treatment	Plasma		Liver		Kidney		Muscles	
	1 month	2 months	1 month	2 months	1 month	2 months	1 month	2 months
Control	14.62 ± 0.54	#14.78 ± 0.44A	211.96 ± 6.74	#214.79 ± 4.04A	190.32 ± 8.84	#191.26 ± 4.22A	28.78 ± 1.14	#28.99 ± 0.97A
Vitamin E treated	14.21 ± 0.60	14.17 ± 0.54B	206.39 ± 8.78	204.92 ± 6.15B	187.04 ± 4.39	184.70 ± 4.89B	28.31 ± 1.06	28.17 ± 1.06B
Vitamin C treated	14.53 ± 0.46	14.39 ± 0.46C	208.75 ± 6.92	207.60 ± 4.60C	189.56 ± 8.05	188.59 ± 8.18C	28.63 ± 0.97	28.45 ± 0.96C
Pb treated	15.60 ± 0.60	16.65± 0.48abc	223.51 ± 6.25	229.72 ± 2.24abc	205.57 ± 6.42	214.52 ± 4.20abc	31.52 ± 0.59	32.50± 0.74abc
Pb and vitamin E treated	15.20 ± 0.42	15.93 ± 0.45b	216..49 ± 8.51	222.62 ± 4.09b	199.08 ± 10.18	208.51 ± 6.48b	30.85 ± 1.14	32.00 ± 1.03ab
Pb and vitamin C treated	15.33 ± 0.68	16.14 ± 0.49c	218.75 ± 4.94	224.39 ± 4.58c	201.28 ± 8.58	211.90 ± 10.10ac	31.22 ± 0.96	32.17 ± 0.95ac
Cr treated	15.76 ± 0.52	16.39± 0.28abc	225.80 ± 4.03	232.83 ± 3.40abc	205.65 ± 4.05	216.16 ± 3.92abc	31.29 ± 0.34	32.26± 0.35abc
Cr and vitamin E treated	14.85 ± 0.46	15.59 ± 0.42b	219.31 ± 3.50	229.57 ± 3.70ab	196.95 ± 4.59	211.52 ± 6.63ab	30.54 ± 0.43	31.67± 0.53ab
Cr and vitamin C treated	14.94 ± 0.52	15.87 ± 0.43c	222.02 ± 3.84	230.19 ± 3.72ac	199.83 ± 5.06	213.84 ± 5.27ac	30.83 ± 0.59	32.14 ± 0.48ac
Ni treated	15.53 ± 0.36	16.49± 0.42abc	225.03 ± 3.50	232.41 ± 3.75abc	206.08 ± 3.32	216.24 ± 3.20abc	31.22 ± 0.71	32.75± 0.37abc
Ni and vitamin E treated	15.24 ± 0.31	16.27 ± 0.32ab	218.06 ± 3.86	226.51 ± 4.23b	199.32 ± 3.30	210.02 ± 3.48ab	30.99 ± 0.77	32.20 ± 0.39ab
Ni and vitamin C treated	15.32 ± 0.43	16.39 ± 0.33ac	220.35 ± 4.34	227.05 ± 4.37c	203.68 ± 3.64	212.86 ± 3.74ac	31.16 ± 0.68	32.44 ± 0.27ac

Each value represents mean ± S.E.; n = 5.
#: Significant variation between groups by ANOVA at P ≤ 0.05.
Small letters a, b, c and d represent a significant change to capital letters A, B, C and D respectively by LSD at P ≤ 0.05.

Table (5) Superoxide dismutase (SOD) activity in blood (eu/mg haemoglobin) and organs (eu /mg protein) of catfish intoxicated with lead, chromium and nickel and treated by vitamin E and vitamin C.

Tissue / Treatment	Plasma		Liver		Kidney		Muscles	
	1 month	2 months	1 month	2 months	1 month	2 months	1 month	2 months
Control	#6.78 ± 0.09 A	#6.74 ± 0.07A	24.34 ± 1.05	#24.56 ± 1.31A	11.86 ± 0.23	#11.94 ± 0.25A	5.39 ± 0.07	#5.40 ± 0.06A
Vitamin E treated	6.32 ± 0.09aB	6.14 ± 0.20aB	23.78 ± 0.87	22.76 ± 1.22B	11.39 ± 0.48	11.12 ± 0.51B	5.31 ± 0.10	5.24 ± 0.09B
Vitamin C treated	6.58 ± 0.13C	6.57 ± 0.13C	24.02 ± 1.10	23.72 ± 1.24C	11.48 ± 0.31	11.30 ± 0.30C	5.39 ± 0.17	5.36 ± 0.07C
Pb treated	7.70± 0.10abc	8.04± 0.17abc D	28.65 ± 1.05	33.52± 1.08abc D	12.86 ± 0.86	13.26± 0.39abc	5.54 ± 0.18	5.94± 0.11abc
Pb and vitamin E treated	7.32± 0.20ab	7.42± 0.17abd	27.46 ± 2.88	29.78± 1.50abd	12.03 ± 1.56	12.13 ± 0.53	5.42 ± 0.15	5.67 ± 0.12b
Pb and vitamin C treated	7.44± 0.14ac	7.73± 0.20ac	26.27 ± 0.96	30.37± 1.03ac	12.33 ± 1.25	12.68 ± 0.45c	5.48 ± 0.15	5.83± 0.11ac
Cr treated	7.68± 0.10abc D	7.89± 0.13abc	26.75 ± 1.18	28.93± 1.00abc	12.65 ± 0.37	13.37± 0.37abc	5.56 ± 0.16	6.03± 0.10abc D
Cr and vitamin E treated	7.14± 0.13abd	7.60± 0.16ab	25.58 ± 0.96	27.65± 0.99b	12.12 ± 0.45	12.69± 0.33b	5.44 ± 0.13	5.66± 0.07abd
Cr and vitamin C treated	7.31 ± 0.12 acd	7.62 ± 0.11 ac	26.17 ± 0.95	28.15 ± 0.89ac	12.38 ± 0.55	12.56 ± 0.36c	5.54 ± 0.16	5.67 ± 0.07acd
Ni treated	7.64 ± 0.11 abc	7.84 ± 0.14 abc	27.31 ± 1.07	29.63 ± 1.05 abc	12.37 ± 0.36	13.32 ± 0.31 abc	5.52 ± 0.17	6.02 ± 0.09abc D
Ni and vitamin E treated	7.31± 0.12ab	7.64± 0.13ab	25.47 ± 1.10	27.71 ± 1.18b	12.05 ± 0.47	12.67 ± 0.48 b	5.44 ± 0.15	5.74 ± 0.08 abd
Ni and vitamin C treated	7.52± 0.18ac	7.71± 0.15ac	26.64 ± 1.18	28.71± 1.16ac	12.35 ± 0.39	12.85± 0.40ac	5.48 ± 0.14	5.88± 0.10c

Each value represents mean ± S.E.; n = 5.
#: Significant variation between groups by ANOVA at $P \leq 0.05$.
Small letters a, b, c and d represent a significant change to capital letters A, B, C and D respectively by LSD at $P \leq 0.05$.

Table (6) Catalase (CAT) activity in blood (eu/mg haemoglobin) and organs (eu / mg protein) of catfish intoxicated with lead, chromium and nickel and treated by vitamin E and vitamin C.

Tissue / Treatment	Plasma		Liver		Kidney		Muscles	
	1 month	2 months	1 month	2 months	1 month	2 months	1 month	2 months
Control	#63.61 ± 1.74A	#63.43 ± 2.02A	#48.06 ± 0.97A	#48.18 ± 0.97A	#40.50 ± 1.16A	#40.24 ± 1.13A	#3.99 ± 0.07A	#3.94 ± 0.08A
Vitamin E treated	64.13± 1.40B	65.09± 1.37B	49.29± 1.27B	49.55± 1.35B	42.56± 1.02B	43.54± 1.09B	4.04± 0.06B	4.11± 0.14B
Vitamin C treated	64.11± 0.95C	64.36± 1.33C	48.21± 0.92C	49.20 ± 1.20C	41.15 ± 1.13C	41.95± 1.13C	4.02± 0.06C	4.02± 0.06C
Pb treated	55.28± 1.11abc D	51.89± 1.40abc	41.26± 1.51abc	36.05± 1.65abc	35.78± 1.27abc	32.52± 1.25abc	3.46± 0.12abc D	3.12± 0.09abc D
Pb and vitamin E treated	59.42± 1.16abd	55.94± 1.08ab	44.30± 1.72b	39.83± 1.73ab	39.15 ± 1.78	35.07± 1.79ab	3.89± 0.17d	3.73± 0.16bd
Pb and vitamin C treated	56.60± 1.51ac	52.71± 1.64ac	43.10± 1.42ac	38.03± 1.41ac	37.48 ± 1.11	33.93± 1.05ac	3.73 ± 0.12	3.64± 0.11cd
Cr treated	53.76± 3.24abc D	51.74± 1.33abc	42.21± 1.60abc	37.92± 1.74abc	36.66± 0.91abc D	33.42± 1.01abc	3.33± 0.09abc D	3.23± 0.07abc D
Cr and vitamin E treated	58.71± 1.07abd	55.62± 1.17ab	44.62± 1.35b	40.66± 1.12ab	40.94 ± 0.69d	36.33 ± 1.24ab	3.74± 0.13bd	3.63± 0.08abd
Cr and vitamin C treated	56.96± 0.96ac	53.75± 1.36ac	43.92± 1.64ac	40.26± 1.78ac	39.34 ± 1.01	35.15± 1.02ac	3.68± 0.08acd	3.55± 0.09acd
Ni treated	52.82± 1.20 abc	49.92± 1.15abc	41.09± 1.37abc	38.22± 1.06abc	34.73± 1.08abc D	31.71± 1.12abc D	3.43 ± 0.10 abcD	3.12± 0.11 abcD
Ni and vitamin E treated	56.52± 1.52 ab	54.41± 1.48ab	44.46± 1.53ab	41.38± 1.54ab	38.60± 1.52bd	36.65± 1.62bd	3.72± 0.11abd	3.52± 0.10abd
Ni and vitamin C treated	54.97± 1.77ac	53.32± 2.04ac	43.10± 1.03ac	40.43± 1.11ac	37.56± 1.42	35.07± 1.43ac	3.62± 0.08ac	3.46± 0.13acd

Each value represents mean ± S.E.; n = 5.
#: Significant variation between groups by ANOVA at $P \leq 0.05$.
Small letters a, b, c and d represent a significant change to capital letters A, B, C and D respectively by LSD at $P \leq 0.05$.

Table (7) Heavy metals concentration in blood (ug/ml) and organs (ug/g wet weight) of catfish

Tissue \ Treatment		Lead		Chromium		Nickel	
		Control	Exposed	Control	Exposed	Control	Exposed
Blood	1 month	0.430 ± 0.009	0.462 ± 0.010*	0.842 ± 0.020	0.916 ± 0.025 *	0.183 ± 0.008	0.212 ± 0.008 *
	2 month	0.426 ± 0.012	0.472 ± 0.013*	0.826 ± 0.021	0.948 ± 0.032 *	0.177 ±0.008	0.221 ± 0.010 **
Liver	1month	0.351 ± 0.011	0.373 ± 0.013	0.608 ±0.015	0.662 ±0.015 *	0.174 ±0.007	0.206 ±0.008 *
	2 month	0.341 ± 0.012	0.384 ± 0.014*	0.596 ±0.016	0.693 ±0.020 **	0.168 ±0.007	0.223 ±0.008 **
Kidney	1 month	0.366 ± 0.014	0.410 ± 0.012*	0.722 ±0.022	0.802 ±0.024 *	0.191 ±0.006	0.226 ±0.007 **
	2 month	0.349 ± 0.011	0.425 ± 0.013**	0.704 ± 0.022	0.831 ±0.025 **	0.186 ±0.007	0.234 ±0.007 **
Muscles	1 month	0.327 ± 0.012	0.353 ± 0.013	0.605 ±0.018	0.647 ±0.020	0.170 ±0.006	0.184 ±0.006
	2 month	0.325 ± 0.011	0.365 ± 0.012*	0.591 ±0.017	0.664 ±0.020 *	0.165 ±0.006	0.192 ±0.006 *

Each value reprecnts mean ± S.E.; n = 5.
*: Significant difference by t - student test at P ≤ 0.05.
**: Significant difference by t - student test at P ≤ 0.01.

الملخص العربي

تم دراسة تأثير تعريض أسماك القراميط للرصاص والكروم والنيكل لمدة شهر وشهرين علي المالونالدهيد والجلوتاثيون المختزل وبعض الإنزيمات المضادة للأكسدة في دم وأكباد وكلي وعضلات الأسماك. وتم أيضاً دراسة تأثير فيتاميني هـ ، ج. وقد أظهرت الدراسة أن تعريض الأسماك للمعادن الثقيلة أدي إلي زيادة تركيز المالونالدهيد والجلوتاثيون المختزل ونشاط إنزيمي الجلوتاثيون-إس-ترانسفيراز والسوبرأوكسيد ديسميوتاز وتثبيط نشاط إنزيمي الجلوتاثيون بيروكسيداز والكتالاز في دم وأعضاء الأسماك. وقد أدي العلاج بفيتاميني هـ ، ج إلي تأثير معاكس لتأثير المعادن الثقيلة علي المالونالدهيد وإنزيمي السوبرأوكسيد ديسميوتاز والكتالاز.

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