Plant Archives Vol. 20, Supplement 2, 2020 pp. 936-940 **e-ISSN:2581-6063 (online), ISSN:0972-5210**

CURATIVE ROLE OF VITAMIN (C) IN REDUCTION OF CADMIUM TOXICITY ON THE LEVELS OF SOME LIVER FUNCTIONS, LIPID PEROXIDATION AND ANTIOXIDANTS ENZYMES IN *IN VIVO* CONDITION

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Abstracts

The presents study included the reduction of cadmium toxicity by vitamin C on the levels of liver functions such as alkaline phosphatase ALP, alanine transaminase ALT, aspartate transaminase AST, lactate dehydrogenase LDH and lipid peroxidation such as malondialdehyde MDA, and some antioxidants enzymes such as superoxide dismutase SOD and catalase CAT after exposure to sublethal concentration of cadmium (1ppm) for 14 days. The results of presents study showed significant differences at level $P \leq 0.05$ between control and treatments groups with cadmium. All biochemical markers such as alkaline phosphatase ALP, alanine transaminase ALT, aspartate transaminase AST and malondialdehyde MDA, and some antioxidants enzymes such as superoxide dismutase SOD and catalase CAT in treated groups with cadmium significantly increased when compared with control groups and vitamin C alone has no effects on biochemical markers While in vitamin C + cadmium groups, all activities of alkaline phosphatase ALP, alanine transaminase ALT, aspartate transaminase AST, lactate dehydrogenase LDH at 7 and 14 days in treatments groups with vitamin C + cadmium were returned the values of liver function ALP, ALT, AST, LDH and lipid peroxidation marker such as malondialdehyde MDA and some markers of antioxidants markers such as superoxide dismutase SOD and catalase CAT to the normal values as that of controls.

Keywords **:** Cadmium toxicity, Vitamin C, Liver Function, Lipid peroxidation and Antioxidants enzymes.

Introduction

The aquatic ecosystem contamination by industrial and agricultural pollutants effects on the health of fish, either directly by uptake from the water, or indirectly through their diet of vegetation, invertebrates or smaller fish so that fish are part of the natural diet of both aquatic mammals and birds, as well as providing an increasingly important protein source for humans, their population and health is of major concern (Aschner *et al.,* 2010 and Aljuboori, 2017). The important environmental pollutants are those that tend to accumulate in organisms, those that are persistent because of their chemical stability or poor biodegradability and those that are readily soluble and therefore environmentally mobile (Bagdonas and Vosyliene, 2006). Heavy metals cannot be destroyed through biological degradation, accumulation in organs of aquatic animals heavy metals (Bayen *et al.,* 2005 and Benedetti *et al.,* 2007). Heavy metals accumulated in the tissues of fish catalyze redox reactions that generate reactive oxygen species (ROS) which may lead to environmental oxidative stress and, therefore, cause biochemical, molecular and morphological alterations in fish (Cao *et al.,* 2010). Environmental oxidative stress is commonly observed when pro-oxidants, such as free radicals, reactive oxygen and reactive nitrogen species (i.e. superoxide anion, hydroxyl radical, hydrogen peroxide and nitric oxides) overwhelm cellular antioxidant systems and induce cellular damage, cellular injury is primarily due to the inability of the anti-oxidants to neutralize the effects of the oxygen radicals (Çimen, 2008). The body is however equipped with antioxidant systems to combat the menace posed by the reactive oxygen species these include the antioxidant enzymes and molecules, the antioxidant enzymes include superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase, etc (Khadairi *et al.,* 2017). The antioxidant molecules include glutathione, ceruloplasmin, albumin, vitamin C and E, and β-carotene, they act to protect the body against oxidative damage by either scavenging for the free radical and/ or repairing the damage caused by the ROS (Fatima *et al.,* 2015). The antioxidant vitamin C is a highly potent reducing agent which is found naturally and abundantly in vegetables and fruits (Khadairi *et al.,* 2018). It is a low-molecular weight antioxidant that protects the cellular compartment against hydrophilic oxygen-nitrogen radicals; a property that makes it an efficacious antioxidant of the hydrophilic phase (Fatima *et al.,* 2015). Vitamin C is an important vitamin for fish and plays an important role in non-enzymatic antioxidant systems ,this vitamin is also known as a valuable free radical scavenger in biological systems, It can reduce the toxic effects of environmental pollutants on animals (Acharya *et al.,* 2008 and Harabawy and Mosleh, 2014) Therefore, increasing the bioavailability of vitamin C may reduce the effects of environmental toxins on fish, the problem, however, lies in the fact that this vitamin is very sensitive to light, temperature, humidity, and pH, Furthermore, vitamin C may quickly be destroyed during preparation or storage of feed, Nevertheless, it may be possible to increase the bioavailability and half life of vitamin C in feed through using a drug carrier, Vitamin C (L-ascorbic acid) has a simple biochemical structure and small molecular weight despite its high density of negative charges due to the presence of acid and carbonyl groups. Hence, it is

combined well with cadmium (Harabawy and Mosleh, 2014 and Khadairi *et al.,* 2017).

Material and Methods

Chemicals and reagents

Cadmium chloride ($CdCl_2$) and vitamin C were purchased from advance of environmental laboratory in science of Babylon university and dissolved in water by 1 mg/l and vitamin c (L-ascorbic acid) added to water in ratio 50 mg/l. All other chemicals were of reagent grade and were commercially available from local scientific distributors in Iraq.

Animals and Experimental design

A total of 75 healthy adult individuals of common carp *Cyprinus carpio* (60 ± 5 g bwt) were obtained from agriculture in Babylon city. The fish were acclimatized for 3 days prior to the experiment in glass aquaria (dimensions 90 × 30 × 50 cm) filled with 120 L de-chlorinated tap water; the aquaria were aerated and the *C. carpio* were maintained in a laboratory environment with a photoperiod (12-h light and 12-h dark cycle) and a temperature of 29 ± 2°C. Dissolved oxygen 6.5 mg/L, pH 6.9 ± 0.4 and electrical conductivity 219 ± 2 µmho/cm of aquaria water were determined by using multi meter; conditions were closely monitored and kept stable during the experiments. They were divided into four groups and putting in each group 15 individual. Control group were given tap water only. Second group were given L-ascorbic acid at a dose 50 mg/L. Third groups were given cadmium at a dose 1 mg/l and four groups were (Vitamin C and cadmium) were given Vitamin C 50 mg/L prior three hours of cadmium in concentration 1 mg/L administration .The experiments were performed in accordance with the Guidelines for the Care and Use of Laboratory Animals of the National Institutes of Health (NIH)

Serum biochemical assay

Biochemical testing of common carp *C. carpio* were achieved on the surviving fish, Blood samples were collected from the caudal vessel by using disposable 3-cc syringes and 21-gauge needles, were transferred into Eppendorf tubes without anticoagulants for serum separation to determine serum of alkaline phosphatase ALP, Alanine transaminase ALT, Aspartate transaminase AST, malondialdehyde MDA, lactate dehydrogenase and some antioxidants enzymes.

Liver function and Lactate dehydrogenase assay

The activity of alkaline phosphatase ALP, Alanine transaminase ALT and Aspartate transaminase AST were measured for serum of blood fish by reflotron plus roche and lactate dehydrogenase by a specific Kit BioScience for each biomarker.

Lipid peroxidation assay

Lipid peroxidation was estimated by the Thiobarbituric acid assay for Malondialdehyde (MDA) concentration according to Aust, (1985)

Antioxidants Enzyme Measurements

Superoxide dismutase activity SOD was determined by autooxidation of Pyrogallol according to Marklund and Marklund, (1974). While Catalase activity was measured according to procedure of Clariborn, (1985) and Aebi, (1974)

Statistical analysis

All data analysis was analyzed according to the system of statistical package for social science (SPSS) to found the significant difference between the different groups within different periods of exposure. All data are expressed as means ± standard deviation (SD) of the means, and the levels of significance were represented for each other. A *p*-value less than 0.05 are considered statistically significant by ANOVA

Results

The results of presents study were determined the impact of cadmium toxicity on *c. carpio* and reduced its toxicity by ascorbic acid on the levels of biochemical markers , the markers of liver function in presents study were showed significantly differences at $p \leq 0.05$ between control groups and treatments, ascorbic acid alone has no effect on all biochemical markers as compared with treatments of cadmium however, the activity of alkaline phosphatase ALP in cadmium exposure was significantly increased at 7 and 14 days and reached to (1.9-2.5) U/l While alkaline phosphatase activity in treatment with ascorbic acid plus cadmium exposure was significantly decreased at 7 and 14 days (0.8 – 1.1) U/l as compared with control groups at 7 and 14 days (0.7-0.73) U/l respectively figure (1), Whereas Alanine transaminase activity ALT in control was reached to (46.5-45.9) U/l at 7 and 14 days, While in cadmium exposure, its activity was significantly elevated to (82.6–95.7) U/l at 7 and 14 days respectively as compared with treatment groups by ascorbic acid which its activity significantly reduced at 7 and 14 days (47.2-49.6) U/l respectively figure (2) and the activity of aspartate transaminase AST in cadmium exposure was significantly increased at 7 and 14 days to reach (90.5 – 121.7) U/l respectively While aspartate transaminase AST activity in treatment by ascorbic acid plus cadmium exposure was significantly decreased at 7 and 14 days (48.9 – 49.2) U/l as compared with control groups at 7 and 14 days (45.8-46.2) U/l respectively figure (3). The activity of lactate dehydrogenase LDH in the control was 6 – 6.5 U/l at 7 and 14 days and its activity in cadmium treatment were significantly decrease to reach 2.2- 1.8 U/l and vitamin C+ cadmium treatments were (4.8- 4.2)U/l at 7 and 14 days respectively Figure (4). the malondialdehyde concentration in cadmium exposure was significantly increased at 7 and 14 days and reached to (2.3 – 5.2) µmol/ml While its concentration in treatment with ascorbic acid plus cadmium exposure was significantly decreased at 7 and 14 days (0.8 – 0.9) µmol/ml as compared with control groups at 7 and 14 days (0.65- 0.68) µmol/ml respectively figure (5)

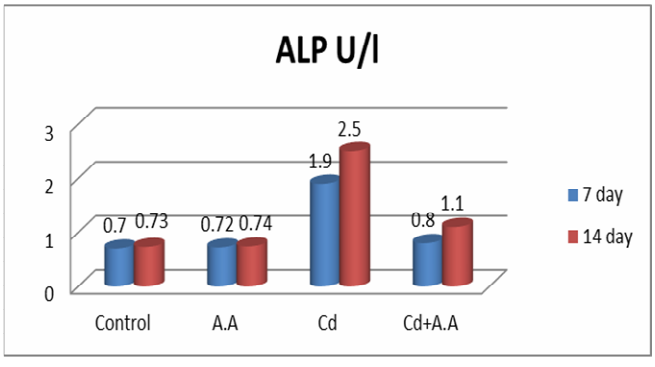

Fig. 1 : Showed alkaline phosphatase ALP activity in serum of *C. carpio* during period of exposure

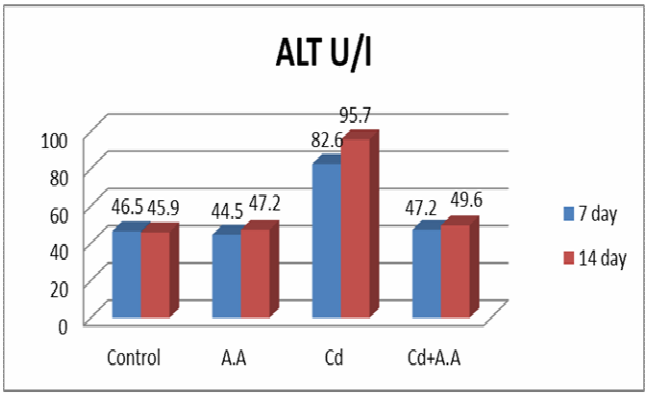

Fig. 2 : Showed alanine transaminase ALT activity in serum of *C. carpio* during period of exposure

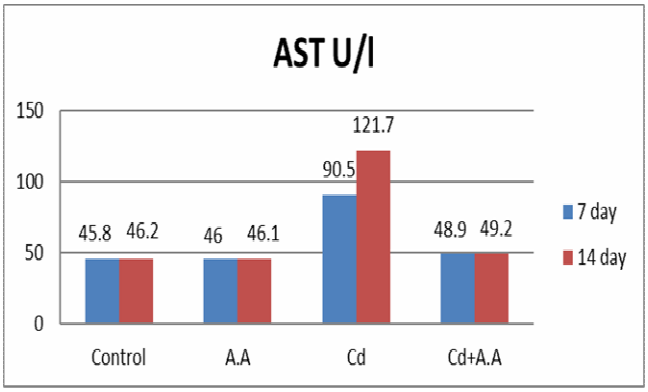

Fig. 3 : Showed aspartate transaminase AST activity in serum of *C. carpio* during period of exposure

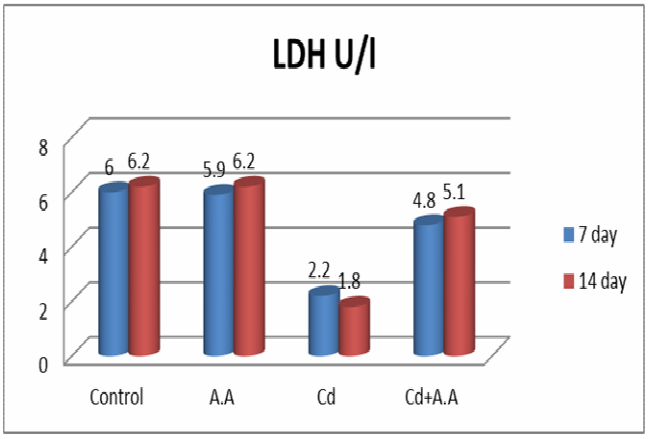

Fig. 4 : Showed lactate dehydrogenase LDH activity in serum of *C. carpio* during period of exposure

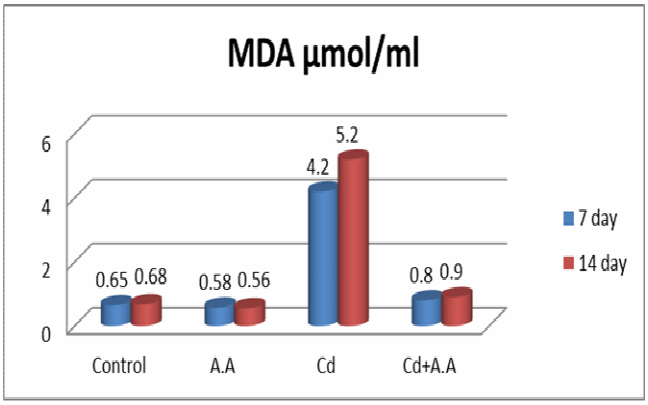

Fig. 5 : Showed malondialdehyde MDA concentration in serum of *C. carpio* during period of exposure

The superoxide dismutase SOD activity in control was ranged between (5.6- 9.1)U/mg at 7 and 14 days respectively, While its activity in both treated serum with cadmium was significantly increased to (10.2- 13.4) U/mg and in Vit C+ cadmium reach to (6.9-7) U/mg at 7 and 14 days respectively figure (6), the catalase activity was ranged between (19.2-19.7)U/mg in control, Whereas in treated with cadmium, the activity of CAT were was significantly decreased to reach at 7 days 14.8 U/mg while at 14 days was reached to 9.4 U/mg and in Vit C+ cadmium treatment was ranged between (17.6-18.1) U/mg at 7 and 14 days respectively figure (7).

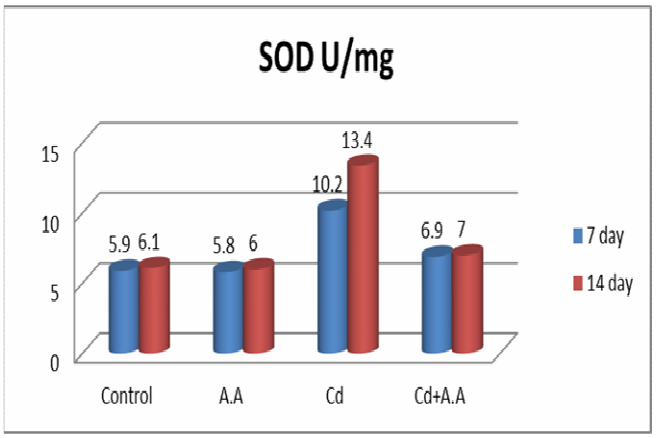

Fig. 6 : Showed superoxide dismutase SOD activity U/mg in serum of *C. carpio* during period of exposure

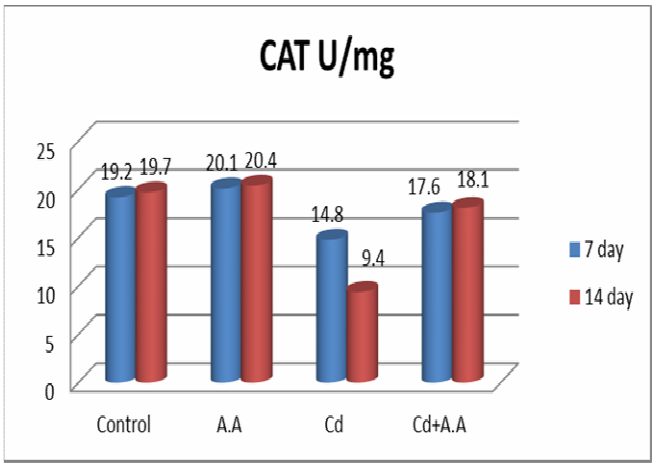

Fig. 7 : Showed Catalase CAT U/mg in serum of *C. carpio* during period of exposure

Discussion

The heavy metals contamination of water is a worldwide environmental problem, Heavy metals, such as cadmium, are released into the environment by industries and reach streams and rivers via run-off from unregulated waste disposal (WHO, 2003). Cadmium is a highly toxic heavy metal because it is unessential elements, accumulation in adipose tissue, un-degraded so that cadmium cause deleterious effects in organisms at low levels of exposure (Gupta *et al*., 2009). The present study showed the effect of sub-lethal Cd exposure for 14 days on several indicative biomarker enzymes and the possible protective effects of Vitamin C in *C. carpio*. Serum biomarkers such as alkaline phosphatase ALP, alanine transaminase ALT, aspartate transaminase AST, malondialdehyde MDA and lactate dehydrogenase LDH have been used to detect cellular damage in blood and liver, and measure the responses to

metals (Yang *et al.,* 2003). It emphasized that their measurement can be useful as a diagnostic tool in fish toxicology to identify their general health status and target organs affected by toxicants (Zikic *et al*., 2001). In the present study, exposure of Common carp to sublethal concentration of Cd for 14 days significantly increased the serum activity of ALP, ALT, AST and MDA While lactate dehydrogenase LDH was significantly decreased after 7 and 14 days as compared with control groups and ascorbic acid alone groups due to liver toxicity that cause by oxidative stress that occur through generation of reactive oxygen species ROS lead to attributed to lipid peroxidation, protein oxidation and DNA damage or cellular degradation induced by the cadmium (Oner *et al*., 2008). The results of present study were agreed with results that conducted by Shalaby, (1997) who showed the activities of AST and ALT that is significant increases in common carp after 7 and 15 days and *O. niloticus* after 15 and 30 days after exposure to sublethal concentration of Cd (Mekkawy *et al*. 2010). The activity of superoxide dismutase SOD in presents study was significantly increased in treatment groups with cadmium after 7 and 14 days when compared with control groups and vitamin C + cadmium because of the SOD plays essential role in scavenging of superoxide free radical, which helps to maintain a balance between oxidants and antioxidants to protect the cell from damage (Prieto *et al*., 2006). While catalase activities significantly decreased in treatment groups with cadmium after 7 and 14 days when compared with control groups and vitamin C + cadmium due to high production of reactive oxygen species such as hydrogen peroxide lead to imbalance between generation of H_2O_2 and catalase production (Liu *et al*., 2016 and Aljuboori, 2017). The results of presents study were showed that all activities of alkaline phosphatase ALP, alanine transaminase ALT, aspartate transaminase AST, lactate dehydrogenase LDH at 7 and 14 days in treatments groups with vitamin C + cadmium were returned the values of liver function ALP, ALT, AST, LDH and lipid peroxidation marker such as malondialdehyde MDA and some markers of antioxidants markers such as superoxide dismutase SOD and catalase CAT to the normal values as that similar in controls due to the essential role of vitamin C as non-enzymatic antioxidant systems in scavenger free radical in biological systems to reduce the toxic effects of environmental pollutants on animals, Therefore, increasing the bioavailability of vitamin C may reduce the effects of environmental toxins on fish (Acharya *et al*., 2008 and Harabawy and Mosleh, 2014).

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