INFLUENCES OF SODIUM ARSENITE ON SOME BLOOD PARAMETERS IN *Cyprinus Carpio* EXPOSED TO ARSENIC

M. Vijaya Bhaskara Reddy*, T. Monika, P. Sasikala.,

Department of LPM, College of Veterinary University, Sri Venkateswara Veterinary University, Tirupati, India, +91-7396120530,+91-9705497313

Dept. of Animal Nutrition, College of Veterinary University, Sri Venkateswara Veterinary University, Rajendra Nagar, India, +91-9052224389

Abstract

The present study was aimed to investigate the effects of sodium arsenite on biochemical and hematologic parameters in carp (*Cyprinus carpio*, Linnaeus 1758) after exposed to arsenic. In this study fish were exposed to 0.01 mg/L arsenic. Our results indicated that significant suppression in Granulocyte, erythrocyte, hemoglobin, hematocrit values were decreased due to oxidative toxicity of arsenic in experimental group while comparing with control group. In addition levels of leucocyte, agranulocyte, MCV (mean corpuscular volume), MCH (mean corpuscular hemoglobin) and MCHC (mean corpuscular hemoglobin concentration) increased in the arsenic group ($P<0.05$) and hematological functions of common carp blood, after being exposed to arsenic.

Keywords: Arsenic, Blood parameter, *Cyprinus carpio*, Hematological parameter.

Introduction

Arsenic is a trace element that is present at low concentrations everywhere such as in air, soil and water (Gupta et al., 2005; Rana et al., 2010; Vijaya Bhaskara Reddy *et al*., 2012). Compounds of arsenic, concentrated in the environment, as a result of natural or anthropogenic sources, become a major concern for environmental and occupational health in mammals (Gupta et al., 2005; Rana et al., 2010).

Arsenic exists in different forms and oxidation status which influence its bioavailability and toxicity (Ventura-Lima et al., 2009). Inorganic compounds are more toxic than organic forms, despite differences that exist between the effects of arsenite, trivalent (As-III) and hexavalent arsenate (As-V). Arsenite can be binding to sulfphydryl (–SH) groups in proteins while arsenate interferes with phosphorylation reactions (Ventura-Lima et al., 2009). In the aquatic environment, arsenic exists either as, As-III and As-V forms which are inter converted through redox and methylation reactions with others (Hughes, 2002; Kavitha et al., 2010). These types of arsenic can accumulate in many aquatic organisms which may catalyze the oxidation of arsenite to arsenate and promote the formation of methyl arsines through biomethylation reaction (Hughes, 2002; Kavitha et al., 2010). One of the most toxic of these is arsenic trioxide and it is one of the arsenites (inorganic forms of arsenic in the trivalent state [As-III]) (Gupta et al., 2005; Ventura-Lima et al., 2009; Rana et al., 2010).

Trivalent arsenic toxicity may be through that directly connecting with –SH groups, or indirectly through production of reactive oxygen species (ROS) (Kalia et al., 2007; Banerjee et al., 2009). Eventually, oxidative stress may occur partially with arsenic toxicity (Kalia et al., 2007; Banerjee et al., 2009). Although arsenic is not biomagnified through the food chain, bioconcentration has been observed in various aquatic organisms such as a fish (Schlenk et al., 1997). Freshwater fish will uptake arsenic not only through diet by benthic-feeding but also with waterborne across the gill (Pedlar et al., 2002a,b).

Prevention of heavy metal toxicity may be accomplished by either reducing the possibility of metal interacting with critical bimolecular and inducing oxidative damage, or by bolstering the cells antioxidant defenses through endogenous and exogenous supplementation of antioxidant molecules (Kalia et al., 2007). To minimize the toxic effects and damages of arsenic compounds, cells have improved defense systems which include antioxidant molecules. When toxic agents against the natural protective systems overrun, exogenous antioxidative and protective compounds must be taken (Devillers et al., 2001).

Eventually, to search new antioxidants as potential therapeutic agents is an active field of biochemistry. In recent years, several organic forms of antioxidant molecules have been studied as preventive agents and natural therapeutics. In particular, researchers have been interested in

propolis, which plays an important role among these natural agents (Kanbur et al., 2009). Propolis (bee glue) is a natural dark-coloured, resinous sticky substance produced by honey bees by mixing their own waxes with resins collected from plants, and is used as a sealant and sterilant in their nests (Sforcin et al., 2000; Moreira et al., 2008). Propolis has been used since ancient times as a medicine owing to those biological properties as an antifungal, antiprotozoal, antimicrobial, and antiviral agent (Sforcin et al., 2000; Moreira et al., 2008).

The fish products have attracted a source of high amounts of significant nutritional components and considerable source of protein in the human diet (Ozogul et al., 2005; Duran and Talas, 2009; Yousefian et al., 2011). The main aim of the present study was investigating the effects of propolis on hematological and blood biochemical in carp (*Cyprinus carpio*, Linnaeus 1758) exposed to arsenic (As2O3).

Materials and methods

The carp were obtained from fisheries department of Chittoor District. Fish were fed for 15 days in an 8 x 5 x 1.5 m stock pond to be acclimated. They were transferred to a 200 L tank filled with water. Airflow in the tank was continuously provided and fish were given artificial dry food once daily. Physical and chemical properties of the water are depicted as shown below.

Parameter mg/L	Before treatment	After treatment
Dissolved oxygen	7.9 ± 0.02	7.4 ± 0.01
Chemical oxygen demand	15.91 ± 0.11	16.92 ± 0.12
Suspended solids	37.9 ± 1.02	41.1 ± 1.27
Calcium	128.0 ± 1.16	112.11 ± 1.01
Sodium	24.04 ± 0.18	18.07 ± 0.07
Chloride	16.10±1.05	18.20±1.04
Total nitrogen	5.98 ± 0.2	6.88 ± 0.3
Hardness (CaCO3)	174.93 ± 3.11	168.92 ± 2.18
Temperature (oC)	18.95 ± 1.0	20.01 ± 0.05
pH	7.5 ± 0.1	7.6 ± 0.1

Experimental design

In the present research work, a total of 16 healthy fish were used and they were divided into two groups, each consisting of eight animals. The average mass of fish was determined as 450-500 g. Fish in the first group were used as a control and there was no application and in the second group for one week and they were not fed for 12 h before; 0.01 mg/L (Schlenk et al., 1997) of arsenic (As2O3) (from sigma Aldrich, 98 % purity), was treated to the fish

Hematological analyses

Blood samples were collected prior to anesthetizing fish to prevent hemolysis (McKnight, 1966) and they were transferred into tubes. Hematometric parameters were immediately determined to red blood cell counting after 1:200 dilution into Hayem solution was done (Blaxhall and Daisley, 1973). One drop of hemolyzed blood was transferred onto Thoma lamella and examined under a light microscope (Soif, XZS-107B model) with a magnification of X400 (Blaxhall and Daisley, 1973). Leucocytes counting were done in blood samples after proper dilution into Turck solution (Blaxhall, 1981). Hemoglobin (HGB) concentration was determined according to the cyano-methemoglobin procedure (Blaxhall and Daisley, 1973). In this case nonclotted blood (20 µl aliquots) was diluted with 1 mL of Drabkin solution and left to stand for 10 min at room temperature. The absorbance was read at 540 nm and the amount of hemoglobin was calculated against a hemoglobin standard (Azizoglu and Cengizler, 1996). Hematocrit was determined as in Wilhelm et al. (1992). Nonclotted blood was transferred to a microhematocrit capillary, afterward centrifuged at 14000 g for 5 min and read against a standard cart.

Statistical analysis

SPSS (Statistical Package for Social Sciences) 9.0 for Windows statistic program was used for analyses of data. Biochemical and hematological data were analyzed using SPSS 9.0 for Windows using one-way analyses of variance (ANOVA). Differences between means were determined using Duncan's multiple range tests in which the significance level was defined as $P< 0.05$.

Results:

In the present investigation Hematological parameters were statistically significant suppressed levels of hematocrit, hemoglobin, erythrocyte counts and granulocyte counts in the exposed arsenic group compared to the control group ($P<0.05$). There were significant increases in levels of leucosyte, agranulocyte, MCV, MCH and MCHC in the arsenic group. The levels of leucocyte, agranulocyte, MCV, MCH and MCHC were significantly increased by arsenic due to extensive oxidative stress ($P<0.05$) (Table 1)

Table 1: Effect of Arsenic on the hematologic parameters in blood of carp experimental and control groups

Hematological parameters	Control	Arsenic (0.01mg/L)
Leukocyte	8.21 ± 0.48	13.29 ± 0.32
Granulocytes (%)	92.10 ± 0.11	58.99 ± 1.37
Agranulocytes (%)	18.80 ± 0.12	41.95 ± 1.35
ErythrocyteCount (mm3/106)	1.69 ± 0.04	0.88 ± 0.05c
Hemoglobin (g/dL)	9.19 ± 0.11	7.87 ± 0.87
Hematocrit (%)	38.02 ± 0.90	26.88 ± 0.61
Erythrocytes indexes		
MCV (μ3)	213.40 ± 2.67	217.761 ± 2.89
MCH (μg)	54.99 ± 1.67	55.01 ± 1.74
MCHC (%)	25.29± 0.92b	25.33 ± 0.81

Discussion

In the present study, levels of leucocytes, agranulocytes, MCV, MCH and MCHC in the arsenic group increased, in contrast to decreased levels of erythrocyte count, granulocytes, hemoglobin and hematocrit. These values can be a marker of anemia with subsequent result of inhibition of erythropoesis in the hemopoietic system (Lavanya et al., 2011). Furthermore, the reason of increase in the MCV, MCH and MCHC values may be macrocytic type anemia. In addition, high leukocyte values depend on stress factors resulted in regulatory effects of toxic

substances on the immune system (Das and Mukharjee, 2003; Dobsikova et al., 2006). There have been many studies on showing alike changes in blood parameters depending on various stress factors. For example, *Salmo gairdneri* exposed to lead indicated a decrease in blood parameters, such as erythrocyte number, hemoglobin level and hematocrit value (Johansson-Sjobeck and Larsson, 1978). It was also reported that there was an important decrease in the hematocrit value of *Salmo gairdneri* blood exposed to cadmium (Haux and Larsson, 1984). Also, exposure of *Oncorhynchus kisutsh* to zinc caused important decreases in hemoglobin and hematocrit values (McLeay, 1975). Our results are also in accordance with these previously reported results (Modi et al., 2006; Kalia et al., 2007; Lavanya et al., 2011). Arsenic is known to generate free radicals (Rana et al., 2010). It is well known that propolis is an antioxidant (Kanbur et al., 2009; Moreira et al., 2008).

Arsenic may cause oxidative stress in the liver of fish and bring about alterations in hematological parameters. Arsenic exposure may cause decreases in the counting of white and red blood cells (Kavitha et al., 2010). Hematological profiles of fish are widely used to demonstrate the environmental pollution in aquatic ecosystems (Carvalho and Fernandes, 2006). These parameters are also used to notice the physiological status of lives and indicators of stress (Adhikari et al., 2004; Lal Shah, 2010). Tripathi et al. showed that hemoglobin levels of *Clarias batrachus* exposed to arsenic decreased (Tripathi et al., 2003). Work on the elemination of toxic substance like arsenic from fish is very important for human health (Adhikari et al., 2004). Certain antioxidant agents can be used to eliminate and suppress the damages of toxic matters such as arsenic.

Our study indicated that can damages and the deterioration caused by arsenic in fish which was demonstrated by analyses of hematological and biochemical parameters. In future, this work may shed light on investigations on who to reduce the oxidative arsenic effects on aquatic organisms.

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