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In-vitro effect of lead and temperature in blood of broiler chicken (Hybro hisex)

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Abstract- Environmental pollution as a result of anthropogenic activity has raised the possibility of pollution with heavy metals. A serious threat in general for health of living organisms is pollution with lead, because lead has a wide range of use and it is listed after iron, copper and zinc. The level of inhibition of ALA-D activity in erythrocyte is used as a biological index for exposure to lead not only in human population but also in wild species, which are environmentally exposed to lead. The aim of current research is to investigate in-vitro effect of lead in different concentration (2-10μ% and 20-100μ %) in activity of ALA-D in blood hemolysate of broiler chicken (Hybro hisex). It is also analyzed the effect of higher temperature in activity of ALA-D (37°C, 42°C, 50°C and 60°C). The research was performed in broiler chicken blood which were grown in a professional farm and feed with commercial food. It was demonstrated that in all concentration of lead, ALA-D is slightly inhibited. The high temperature (50°C and 60°C) has increased activity of enzyme compared with activity of enzyme in 37°C and 42°C.

Index Terms- blood, lead, ALA-D, chicken

I. INTRODUCTION

Pollution with lead in general is a serious threat for health of all living organisms. Hence, lead is one of the limited class of elements that can be described as purely toxic. Lead is distributed in environment because it has wide range of use and it is listed after iron copper and zinc. Lead has multiple negative effects in all living organisms affecting nervous system, hematological system, reproduction system and their behavior (Demay et all, 1982, Eisler, 1988).

In living organisms, lead enters through inhalation (by breathing), or by ingestion (during feeding) and blood is the main carrier of lead from gastrointestinal system in all other systems of the body (Anders et al, 1982).

Inhibition of ALA-D activity has become as standard method to verify intoxication with lead not only in human population but also in animals. In avian lead is found to be more potent inhibitor of ALA-D activity than copper, cadmium or mercury (Scheuhammer, 1987). Chickens are also very sensitive in lead intoxication and amount of 1.0 mg/kg of lead in their food regime can cause depression of growth and reduction of ALA-D activity. Clinical signs of lead intoxication in chicken include muscle weakness anorexia followed with body weight lose and eventually with interruption of egg production. Young chickens are found to be more sensitive in lead intoxication than older.

Ingested lead in chicken is deposited in bones, in soft tissues and egg, and results with high lead level in blood (Vengris and Mare, 1974). Eggs accumulate lead in shell, albumins and in egg yolk (Trampel et al, 2003).

Investigation of Stone et al (1976) done in Japanese Quial showed that consume of 25 ppm of lead does not show any effect in body weight, size of kidneys or lung, but ALA-D activity was inhibited for 45 % compared with the control group.

Many reports (Ohi et al; 1974, Huton and Goodman, 1980; Gonzales and Tejodor, 1992), confirm hypothesis that avian are sensitive indicator of environmental pollution with lead and they respond with inhibition of ALA-D quickly than other species.

The aim of current investigation is to verify in-vitro effect of lead in different concentrations (2-10μ% and 20-100μ %) in ALA-D activity in blood hemolysate of chicken and effect of high temperature (50°C and 60°C) in the reactivation of the enzyme.

II. MATERIALS AND METHODS

Blood samples were taken from chickens which were grown in professional farm and feed with the commercial food. Experiments were performed with more than one individual. The age of individuals was not taken in consideration. The blood lead level was determined in advance. Blood was treated with lead acetate, and for that purpose lead acetate stock solution was prepared and was diluted twice with destilated water in portion 1:9.

With heparinisied syringe by punctuation, blood was taken directly from hart and then is carried into test tubes which were wrapped with aluminium folio and heprinizied with 0.002 ml heparin/ml blood. For different lead concentration, two series of test-tubes were prepared with nine tubes for each series. In a first experiment, blood was treated with lead in cocnetrations from 2-10μ% and in second experiment blood was treated with lead from 20-100μ%, after lead treatment, blood was stored in refrigerator till day of analyses.

In each of two experiments, blood hemolysate was incubated in different temperatures (37°C, 42°C, 50°C and 60°C) for sixty minutes.

Blood lead level was determined with flame atomic absorption spectrometry, method by Milic (Milic, 1985).

The activity of D-ALA (EC.4.2. 1.24) was estimated according to the European standardized method of Berlin and Schaller (1974):

$$ALA-D\ (U/LE)=\frac{A_{555}\ corrected}{\%\ Hct}\ x\,1881{,}7$$

Statistical software Sigmastat was used for processing results of investigation and presented as average values with corresponding standard deviations.

III. RESULTS

Effect of low concentration of lead (2-10µ%) and influence of different temperatures (37°C, 42°C, 50°C and 60°C) in activity of ALA-D activity in blood hemolysate of chicken is presented in Fig. 1.

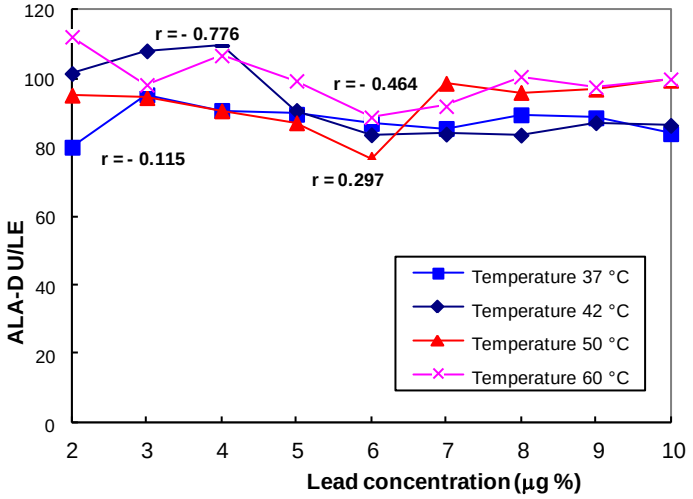

Fig. 1: Correlation between ALA-D activity and lead concentration (2-10 µg%) in different temperatures (37°C,42°C, 50°C and 60°C)

Figure 1 shows that lowest activity of ALA-D is marked in 37 °C and it is 80 - 95 ALA-D U/LE. In 42°C which is normal body temperature of avian in beginning enzyme activity is higher than in previous temperature but after concentration of 5 µg% until 10µg% of lead it fall down and it remains almost unchanged.
Enzyme activity is shown to be lower in 50°C than in 42°C and it is almost same in all concentrations of lead.
An increase of ALA-D activity is marked in 60°C.

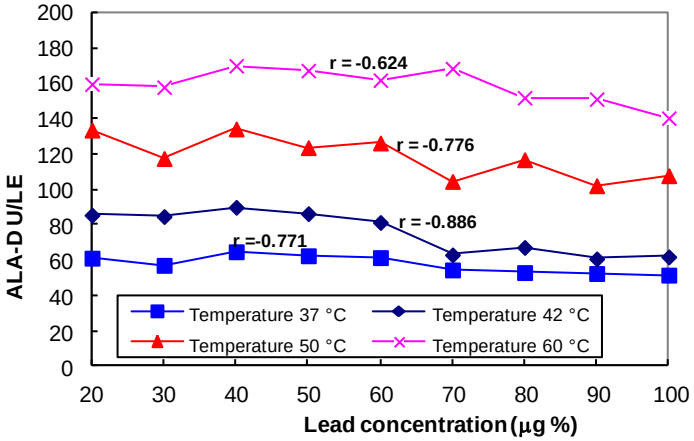

Fig. 2: Correlation between ALA-D activity and lead concentration (20-100 µg%) in different temperatures (37°C,42°C, 50°C and 60°C)

Effect of high lead concentration in activity of ALA-D is presented in Fig.2. It is shown that in temperature of 37°, activity of ALA-D is very low and almost same in all concentration. In temperature of 42°C activity of ALA-D is in negative correlation with lead concentration. Further fig. 2 indicates that in 50°C activity of enzyme is higher than in 42°C but with the same trend of ALA-D inhibition in both temperatures.
The highest activity of ALA-D activity is marked in temperature of 60°C.

IV. DISCUSSION

The level of inhibition of ALA-D activity in erythrocyte is used as a biological index for exposure to lead not only in human population but also in wild species, which are environmentally exposed to lead. Test of ALA-D inhibition as index of lead exposure has many advantages than other biochemical tests because:

1. ALA-D is inhibited before any other effect appears.
2. In human population ALA-D is inhibited even in low concentration.
3. Inhibition is indirect proportional with lead level.
4. Spontaneous regeneration of inhibited ALA-D is very slow.

In current investigation in which in-vitro effect of lead was investigated is demonstrated that the test of inhibition of ALA-D is not reliable.
Results of the present study has shown slight inhibition of ALA-D activity in all concentration of lead (2-100µ%) after incubation of hemolysate in different temperatures (°C, 42°C, 50°C and 60°C). These results are compatible with results of Pauza, et al (2005) obtained from their investigation in chicken embryo treated with lead.
High activity of ALA-D after incubation in high temperatures obtained in current research is in harmony with the results of Scheuhammer, (1987), who has observed increase of activity for 20% in 42°C compared to 37°C, and also observed that lead is 10-100 times more potent inhibitor of ALA-D activity than Cu, Cd or Hg.
However many reports of different authors showed that lead is more inhibitor of ALA-D activity in-vivo than in in-vitro in different living organisms.
Elezaj et al (2004) has observed negative correlation of ALA-D activity and lead in blood of pigeon (Columbia livia) collected in three different areas (Mitrovica, Prishtina and Zatriq).
The research done by Bakalli (1990) with chicken (Hybro hisex) exposed to lead in region of Mirtovica shows negative correlation of ALA-D activity and lead compared to the control group.
In current investigation use of ALA-D activity as index of lead exposure in context of environmental studies could not be reliable because the ALA-D shown to be not sensitive in in-vitro exposure with lead. Results shows lower activity of ALA-D in blood hemolysate of chicken treated with lead than ALA-D activity of human blood hemolysate in same condition.

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