ASIAN JOURNAL OF PHARMACEUTICAL AND CLINICAL RESEARCH

INNOVARE ACADEMIC SCIENCES
Knowledge to Innovation

Vol 11, Issue 11, 2018

Online - 2455-3891
Print - 0974-2441

Research Article

HEAVY METAL-INDUCED INHIBITION OF ENZYMATIC ACTIVITY IN HEN EGG WHITE LYSOZYME

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Received: 03 October 2017, Revised and Accepted: 07 July 2018

ABSTRACT

Objective: The objective of this study was to determine the content of chromium (Cr), nickel (Ni), cadmium (Cd), boron (B), mercury (Hg), and lead (Pb) in chicken hen eggs in hen's eggs and to evaluate their influence on the enzymatic activity of hen egg white lysozyme (HEWL).

Methods: In this study, we have determined the content of Cr, Ni, Cd, B, Hg, and Pb in chicken hen eggs using absorption atomic (AA) method. The residual enzymatic activity of HEWL, HEWL was evaluated using spectrophotometric method.

Results: The levels of Cr, Ni, Cd, B, Hg, and Pb in hen eggs were 1.8300, 1.1300, 0.5350, 0.5200, 0.0025, and 0.0021 mg/kg, respectively. The inhibition of enzymatic activity of HEWL, by exposure to heavy metals was studied. We found that Cr, Ni, Cd, B, Hg, and Pb produced a loss of enzymatic activity. Cr III was the metal with the highest reduction of HEWL enzymatic activity. 0.01 M of Cr III was able to reduce HEWL enzymatic activity levels up to 49% after 24 h of exposure to this metal. An exposure of 0.05 M of Cr III reduced HEWL enzymatic activity to 1%. 24 h exposure to 0.01 M of Cd presented a reduction of 37% of HEWL enzymatic activity and 0.05 M of Cd resulted in only 75% of HEWL enzymatic residual activity.

Conclusions: Content of heavy metals in hen egg from Tungurahua region was high. Cr, Ni, Cd, B, Hg, and Pb produced a loss of enzymatic activity of HEWL.

Keywords: Lysozyme, Heavy metal, Enzymatic activity, *Micrococcus lysodeikticus*.

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INTRODUCTION

Mercury, chromium, and lead are heavy metals with great impact as environmental contaminants in the ecosystems of the world. These heavy metals can contaminate the aquatic systems coming from industrial and urban residues. Many animals, such as wild birds, pigeons, and hens exposed to mercury (Hg) and lead (Pb), have negative growth, reproductive, neurological, and behavioral side effects. Cellular processes can be affected due to the exposure to different heavy metals such as Cd, Hg, and Pb with damage to enzymes, DNA, and biomolecules. The correct functioning of the central nervous system can be affected from the neurochemical changes produced by exposure to different concentrations of heavy metals [1,2]. The process of neuronal synaptic transmission in the human body can be altered after a long exposure to these heavy metals and to their compounds such as heavy metal salts. *In vivo* studies with animals have reported changes in the extracellular metabolism. The expression of components of neurotransmitter systems relates to toxic effects observed in heavy metals-exposed animals [3]. Oliveira *et al.* reported an *in vitro* and *in vivo* study regarding the effect of $HgCl_2$ on synaptosomal ATP diphosphohydrolase in the cerebral cortex of developing rats [4]. Humans are exposed to Cd in the workplace or by ingestion of Cd-contaminated food and water [5,6]. Smoking habits are a major source of Cd exposure for people around the world [7]. Depending on the dose, concentrations, route, and duration of exposure, Cd can damage different organs including kidney, lung, bones, and liver [8,9]. Cd is accumulated in the liver and produces hepatotoxicity after an acute or chronic exposure; the mechanisms of hepatotoxicity in human body are still under study [10]. Cd can interact with concentrations of biomolecules and produces important structural changes, or can replace native metal ions. In several cases, Cd can affect the biological functions. The interaction between Cd and biomolecules includes transcription factors, membrane cellular receptors, and enzymes [11,12]. It has been reported that alkaline phosphatase, a dimeric phosphomonoesterase metalloenzyme, is inactive to Cd.

Monitoring the levels of toxic and potentially toxic elements is one of the most important aspects of the environmental quality control and food safety in the food and pharmaceutical industry. Trace elements are found naturally in the environment and their presence can be attributed to natural processes such as volcanism or geochemical activities. Ecuador is in the named fire belt of the Andes, Five Volcanoes are active in Ecuador. The central region of Ecuador has the active Tungurahua Volcano. Human activities may increase their levels in the various environmental compartments, leading inevitably to their bioaccumulation in the food chain of humans and animals. Some of these elements are essential for living organisms, including humans, while others have long been known to be highly toxic. The European Commission has stabilized maximum limits on heavy metals, such as lead, cadmium, and mercury in certain foods, but has not suggested limits of heavy metals in hen eggs [13].

Fresh hen eggs are an important food in human diet due to their nutritional components with high protein content, vitamins, and other components. Hen eggs are included as ingredients in many food products having different functions such as spumant and flavor capacity. Toxic metals such as As, Cd, and Pb can enter in the chain food and produce risks to human and animal health [14]. Trace metals such as Cr, Ni, As, Cd, and Pb have been considered as the most toxic elements in the environment by the USA environment protection agency. The risk of contamination of food and foodstuffs is high in some regions of world [15]. Monitoring their levels then is of great importance for nutritional, toxicological, and environmental purposes.

Hen egg white lysozyme (HEWL) is an enzyme of type E.C.3.2.17, N-acetylmuramic-hydrolase. HEWL is a basic protein with 129 amino acids content with a Lys placed at the N-terminal amino acid and a Leu at the C-terminal. HEWL has a molecular weight of 14.3 kDa and one structural subunit. The chain of amino acid residues is cross-linked by four disulfide bridges, which allows the enzyme to have a high thermal stability in different mediums, along with six-helix structured regions and a high isoelectric point with a value of pI (10.7) at pH 7.0 in physiological conditions [16,17].

Hen eggs are the richest source of lysozyme enzyme, accounting for 3.5% of total egg white proteins [18,19]. Lysozyme belongs to a type of enzymes that lyse the cell wall of certain Gram-positive bacteria (*Micrococcus lysodeikticus, Staphylococcus carnosus, Staphylococcus aureus, Clostridium botulinum, Clostridium tyrobutyricum*, etc.) by splitting β (1-4) linkages between N-acetylmuramic acid and N-acetylglucosamine of the peptidoglycan of the cell wall of Gram-positive bacteria. The outer membrane of Gram-negative bacteria protects the peptidoglycan from the enzymatic action of hen egg lysozyme. This enzymatic activity is named muramidase activity or lytic activity [20,21]. Different technological treatments can modify the enzymatic activity such as heat, chemical, or hydrolysis obtaining an increase of antibacterial spectrum [22]. The aim of this study was to determine the content of heavy metals in Ecuador eggs, Cr, cadmium (Cd), mercury (Hg), lead (Pb), nickel (Ni), and boron (B) and to evaluate if the exposure to these heavy metals can modify the HEWL enzymatic activity.

METHODS

A total of 48 hen egg samples were collected from small rural farms and from domestic, non-commercial producers in the area of Cotalo in the region of Tungurahua in Ecuador. After collection, the samples were sent to the laboratory for chemical analyses. The yolk and egg white were mixed, lyophilized, and stored at −80°C until samples were analyzed after atomic absorption (AA).

Analysis of content of heavy metal in hen eggs by AA

Before digestion of the lyophilized hen egg and with the aim to analyze heavy metals contents, each compost sample was dried at 65°C for 48 h. Nitric acid digestion method was used in the samples tested in this study. All samples were made in triplicate for each concentration of sample tested. 0.5 g of sample added in tubes with 10 mL of 5 M HNO3. Then, the samples were incubated at 120°C for 2 h. At the end of the digestion, the samples were allowed to cool to room temperature. Finally, the concentrations of Cr, Ni, Cd, B, Hg, and Pb in the final solutions were determined by an AA spectrometer (PG Instrument AA500, Germany) [23].

Lysozyme and materials

Lysozyme (L2879, chloride from hen egg white Grade VI, 60,000 units/mg protein, EC 3.2.1.17) and *Micrococcus lysodeikticus* were purchased from Sigma Chemical Co. (Saint Louis, MO, USA). Reagents for AAS were of analytic grade.

Enzymatic activity assay of hen lysozyme

Lysozyme 1 mg/ml was solved in buffer phosphate 10 mM at pH 6.24 without heavy metals. This sample was used as control. Lysozyme at 1 mg/ml was solved in buffer phosphate 10 mM at pH 6.24 incubated with heavy metals (Cr, Cd, Hg, Pb, Ni, and B) [$CrCl_3.6H_2O$; CrO_3; CdO; $HgCl_2$; $(CH_3COO)_2Pb.3H_2O$; $NiCl_2.6H_2O$; H_3BO_3] 0.01, 0.02, 0.03, 0.04, and 0.05 M for 4, 12, and 24 h at room temperature. A 2.96 ml volume of *Micrococcus luteus* was pipetted into a cuvette and the reaction was started with 0.01 ml of lysozyme solution. After incubation, the enzymatic activity of lysozyme was determined by monitoring the decrease in turbidity of a suspension of *M. lysodeikticus* cell (dissolved in buffer phosphate 10 mM at pH 6.24) spectrophotometrically at 450 nm using a spectrophotometer (Thermo Fisher Scientific Evolution 200 UV/Vis, Waltham, MA, USA) at 25°C, according to Carrillo *et al*. [22]. The enzymatic activity of each sample was assayed 3 times. The parameter ΔA/min was calculated from the linear part of the curve. The activity was calculated using the equation: % Enzymatic activity=$\Delta A_{450}B\ min^{-1} - \Delta A_{450}S\ min^{-1}/\Delta A_{450}B\ min^{-1}$.

Where, $\Delta A_{450}B\ min^{-1}$=Variation of the absorbance at 450 nm of blank.

$\Delta A_{450}S\ min^{-1}$=Variation of the absorbance at 450 nm of sample.

RESULTS

Content of heavy metal in eggs

The content of heavy metals in egg samples was analyzed using the AA method. The values were presented as the average with their standard deviation (Table 1). The order of content of heavy metals was Cr>Ni>Cd>B>Hg>Pb mg/kg in hen eggs. Content of Cr and Ni was the highest ones with a value of 1.8300 mg/kg and 1.1300 mg/kg, respectively. The lowest content of heavy metal was Pb with a value of 0.0021 mg/kg.

Enzymatic activity of HEWL in presence of heavy metals

The enzymatic activity of HEWL was evaluated in the presence and absence of heavy metal using the turbidimetric spectrophotometric method. The decline of absorbance for 8 min was measured at 450 nm.

The percentage of enzymatic activity of standard HEWL incubated with heavy metal was determined using the spectrophotometric method. We can see that at a high concentration of metal, the loss of enzymatic activity was high. The loss of the enzymatic activity of HEWL was also proportional to the concentration of metals. The loss of enzymatic activity increased with the time of incubation. At 24 h of incubation with heavy metals, the loss of enzymatic activity was the highest. This occurred with all heavy metals assayed and occurred with the 3 times tested in this study (Figs. 1-7).

Fig. 1 shows the HEWL enzymatic residual activity incubated with Cr III at 0.01 M - 0.005M for 4, 12, and 24 h. At 24 h of incubation with Cr III, HEWL only keeps 1% of the enzymatic activity having then a loss of 99% of enzymatic activity.

Two-way ANOVA of HEWL enzymatic activity showed statistical differences against the control. Statistical differences were also observed when the groups were compared to the solution of Cr III 0.01 M. Fig. 2 shows HEWL residual enzymatic activity incubated with Cr VI. The loss of all concentrations tested was less of 30%. At 4 h of incubation with 0.005 M of Cr VI, HEWL only conserved 27% of enzymatic activity. At 12 h of exposure to the metal, HEWL conserved 22% of enzymatic activity. At 24 h of HEWL incubation with Cr III, HEWL only conserved 20% of the enzymatic activity, having then 80% of loss of the enzymatic activity. The longest the time of exposure of HEWL to the heavy metal, the highest the decline in the enzymatic activity. The test two-way ANOVA indicates the statistical differences between the different groups.

Fig. 3 shows the HEWL enzymatic activity when exposed to cadmium. We can see that at 4, 12, and 24 h exposure, all concentrations present a loss in the HEWL enzymatic activity below 50%. At 24 h exposure with 0.005 of Cd, the loss of enzymatic activity was extremely high, having only 7% of HEWL enzymatic activity with then a 93% of loss of activity.

Table 1: Heavy metals content in eggs collected mg/kg

Metals	Mean±SD
Cr	1.8300±0.0245
Ni	1.1300±0.0115
Cd	0.5350±0.0324
B	0.500±0.0021
Hg	0.0025±0.0312
Pb	0.0021±0.0000

SD: Standard deviation

Fig. 4 shows HEWL enzymatic activity when exposed to mercury metal. After HEWL being exposed for 4 h to 0.01M, HEWL presents 71% of enzymatic activity, whereas the HEWL incubated with mercury for 12 h presents 63% of enzymatic activity. Finally, 24 h of exposure of HEWL to 0.01 M of mercury presented 36% of enzymatic activity. After 24 h of incubation with the metal, HEWL only retained 17% of its activity. The two-way ANOVA analysis indicated statistical differences between positive control and all groups tested.

Fig. 5 shows the HEWL enzymatic activity when exposed to 0.01 M, 0.02 M, 0.03 M, 0.04 M, and 0.05 M of Pb for 4, 12, and 24 h. The loss of enzymatic activity increases with the concentration and the time of exposure to the metal. All concentrations were able to produce inhibition of the HEWL enzymatic activity. At 24 h of exposure to Pb, the loss of the HEWL enzymatic activity varied according to the concentration. The highest the Pb concentration, the highest the loss of HEWL enzymatic activity. Fig. 5 shows that at 0.01 M of Pb, HEWL presents an enzymatic activity of 48%, at 0.02 M of Pb presents a 33% of HEWL residual enzymatic, at 0.03 M of Pb, HEWL presented a value of 31% of enzymatic activity, at 0.04 M of Pb, HEWL presented a value of 25% of enzymatic activity, and finally, at the concentration of 0.05 M of Pb, HEWL registered a value of 19% of enzymatic activity. Two-way ANOVA test indicates statistical difference between positive control versus groups and 0.01 M versus other groups. All heavy metal tested were able of producing a loss of HEWL enzymatic activity. Cr VI was the metal with the highest reduction of the HEWL enzymatic activity.

Fig. 6 shows the HEWL enzymatic activity when exposed to 0.01 M, 0.02 M, 0.03 M, 0.04 M, and 0.05 M of Ni for 4, 12, and 24 h. The loss of enzymatic activity increases with the concentration and the time of exposure to the metal. All concentrations were able to produce inhibition of the HEWL enzymatic activity. At 24 h of exposure to Ni, the loss of the HEWL enzymatic activity varied according to the concentration. Fig. 3b shows that at 0.01 M of Ni, HEWL presents an enzymatic activity of 21%, at 0.02 M of Ni presents a 19% of HEWL residual enzymatic, at 0.03 M of Ni, HEWL presented a value of 19% of enzymatic activity, at 0.04 M of Ni, HEWL presented a value of 19% of enzymatic activity, and finally, at the concentration of 0.05 M of Pb, HEWL registered a value of 17% of enzymatic activity.

Fig. 7 shows the HEWL enzymatic activity when exposed to 0.01 M, 0.02 M, 0.03 M, 0.04 M, and 0.05 M of B for 4, 12, and 24 h. The loss of enzymatic activity increases with the concentration and the time of exposure to the metal. All concentrations were able to produce inhibition of the HEWL enzymatic activity. At 24 h of exposure to B, the loss of the HEWL enzymatic activity varied according to the concentration. Fig. 4 shows that at 0.01 M of B, HEWL presents an enzymatic activity of 48%, at 0.02 M of B presents a 41% of HEWL residual enzymatic, at 0.03 M of B, HEWL presented a value of 30% of enzymatic activity, at 0.04 M of Ni, HEWL presented a value of 23% of enzymatic activity, and finally, at the concentration of 0.05 M of B, HEWL registered a value of 14% of enzymatic activity.

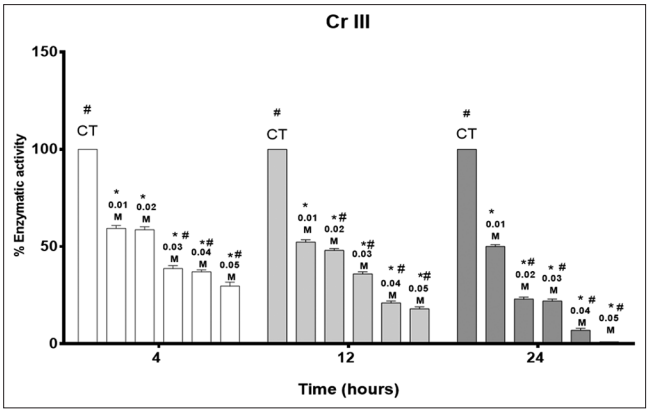

Fig. 1: Evaluation of the enzymatic activity of hen egg white lysozyme (HEWL) incubated with chromium (Cr AIII) solution at concentrations of 0.01 M, 0.02 M, 0.03 M, 0.04 M, and 0.05 M for 4, 12, and 24 h at room temperature. CT (positive control) HEWL without chrome. Results represent the average of three determinations±standard deviation (n=3). *Denotes p<0.05 as compared to control (HEWL without Cr VI) (two-way ANOVA/Tukey). #Denotes p<0.05 as compared to 0.01 M Cr VI (two-way ANOVA/Tukey)

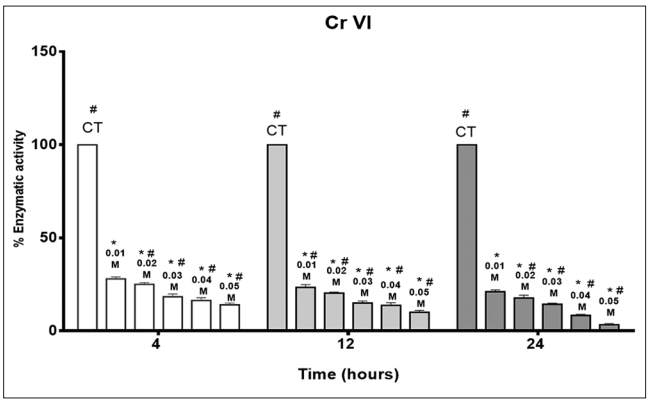

Fig. 2: Evaluation of the enzymatic activity of hen egg white lysozyme (HEWL) incubated with chromium (Cr VI) solution at concentrations of 0.01 M, 0.02 M, 0.03 M, 0.04 M, and 0.05 M for 4, 12, and 24 h at room temperature. CT (positive control) HEWL without chrome. Results represent the average of three determinations±standard deviation (n=3). *Denotes p<0.05 as compared to control (HEWL without Cr VI) (two-way ANOVA/Tukey). #Denotes p<0.05 as compared to 0.01 M Cr VI (two-way ANOVA/Tukey)

DISCUSSION

Content of heavy metals

In this study, the concentration of heavy metals from hen eggs was measured by atomic absorption (AA) technique. The concentration of chromium determined in eggs from Tungurahua region was higher than the one reported by different authors in the literature. Esposito *et al*. [24] have reported a value of (0.008 mg/kg) chromium present in hen egg collected in the Campania region in Italy. Uluozlu *et al*. have reported a value of 0.06 mg/kg of chromium present in eggs in Turkey [25]. Khan and Naeem reported the content of chromium in eggs in Pakistan with a value of 0.732 mg/kg of chromium [26]. Ul Islam *et al*. reported 0.002 mg/kg of chromium in eggs in Pakistan [14]. Parvin and Rahman, 2014, reported chickens of different regions of Bangladesh were analyzed for their Cr (VI) content in the different body parts such as liver, gizzard, flesh, and brain [27]. Hui reported 0.23, 0.26, and 0.23 ppm in pigeon eggs in three cities of the USA [28]. In 2014, EFSA in their scientific report: Scientific opinion on dietary reference values for chromium EFSA panel on dietetic products, nutrition and allergies indicated that there were no evidences to consider chromium (III) as an essential element with biological activity [29]. Thus, it was not recommended to ingest daily doses of chromium. The existing regulations are of Cr (III), the UK allows the use of Cr (III) chloride and phosphate in food manufacturing [30]. Chromium is passed from hen to egg [31-33]. Ingestion of Cr-VI by the hen reduces egg hatchability. Chromium in the hens of roseate terns (*Sterna dougallii*) and herring gulls (*Larus argentatus*) exposes embryos to the metal as chromium is passed to eggs, being eggs a method of excretion of chromium [34].

The nickel content in the present study (1.130 mg/kg) was higher than values reported in different studies. Siddiqui *et al.* [35] reported 0.277 mg/kg of nickel in egg-free collected in a supermarket in London. Esposito *et al*. [24] reported a content of nickel of 0.096 mg/kg in hen egg in the Campania region in Italy. Van Overmeire *et al*. [36] reported 0.036 mg/kg of nickel in hen eggs. Only Ul Islam *et al*. [37] reported a higher value with 1.9 mg/kg in hen eggs in Bangladesh.

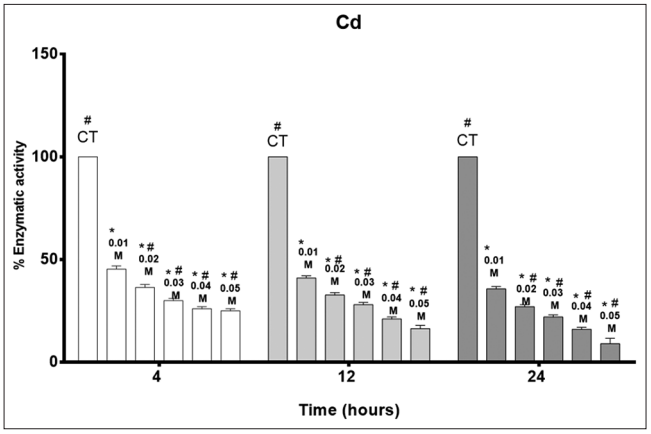

Fig. 3: Evaluation of the enzymatic activity of hen egg white lysozyme (HEWL) incubated with cadmium solution at concentrations of 0.01 M, 0.02 M, 0.03 M, 0.04 M, and 0.05 M for 4, 12, and 24 h at room temperature. CT (positive control) HEWL without chrome. Results represent the average of three determinations±standard deviation (n=3). *Denotes p<0.05 as compared to control (HEWL without Hg) (two-way ANOVA/ Tukey). #Denotes p<0.05 as compared to 0.01 M Cr+3 (two-way ANOVA/Tukey)

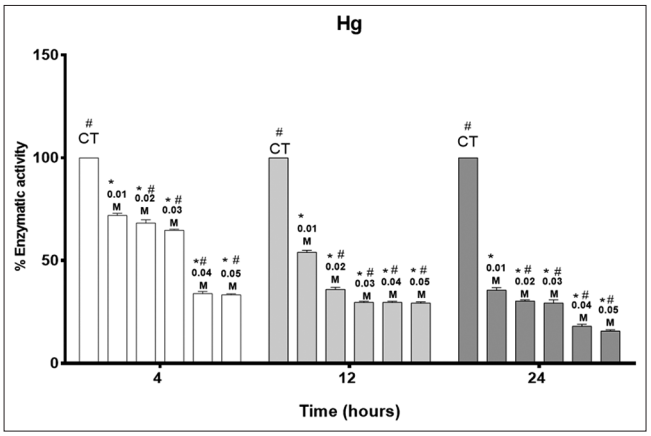

Fig. 4: Evaluation of the enzymatic activity of hen egg white lysozyme (HEWL) incubated with mercury solution at concentrations of 0.01 M, 0.02 M, 0.03 M, 0.04 M, and 0.05 M for 4, 12, and 24 h at room temperature. CT (positive control) HEWL without chrome. Results represent the average of three determinations±standard deviation (n=3). *Denotes p<0.05 as compared to control (HEWL without Hg) (two-way ANOVA/ Tukey). #Denotes p<0.05 as compared to 0.01 M Cr+3 (two-way ANOVA/Tukey)

The next heavy metal with a high concentration was cadmium with a value of 0.5350 mg/kg of cadmium. This value is above the reference value of 0.00035 mg/kg per day [38]. Lead and cadmium are heavy metals that occur naturally in the environment and as pollutants released from industrial and agricultural industries. Food is the main source of human exposure to these elements, being responsible for the accumulation in the body, having serious effects in the central nervous system and kidneys. Lead content of 0.0021 mg/kg was above the reference value (0.0015 mg/kg per day: Cardiovascular effects and 0.00063 mg/kg per day: Nephrotoxicity) [39]. Our value was, however, lower when compared to other reports such as Esposito *et al.* [24] who reported 0.019 mg/kg of lead in eggs in Italy. Van Overmeire *et al*. [36] reported 0.068 mg/kg of lead in eggs in Belgium. In Pakistan, Khan and Naeem have reported a high value of lead in hen eggs, 0.59 mg/kg [26].

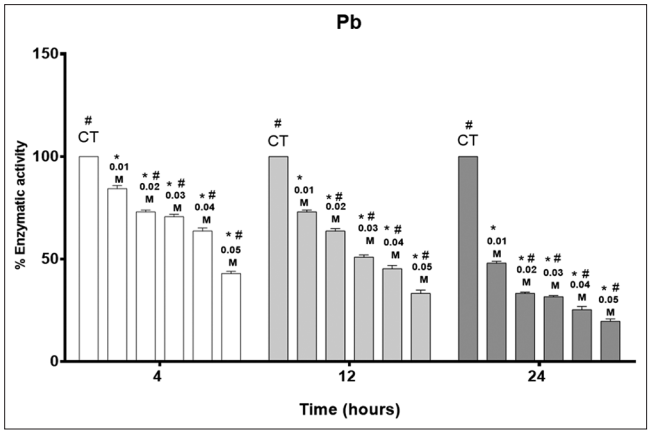

Fig. 5: Evaluation of the enzymatic activity of hen egg white lysozyme (HEWL) incubated with lead solution at concentrations of 0.01 M, 0.02 M, 0.03 M, 0.04 M, and 0.05 M for 4, 12, and 24 h at room temperature. CT (positive control) HEWL without chrome. Results represent the average of three determinations±standard deviation (n=3). *Denotes p<0.05 as compared to control (HEWL without Ni) (two-way ANOVA/Tukey). #Denotes p<0.05 as compared to 0.01 M Ni (two-way ANOVA/Tukey)

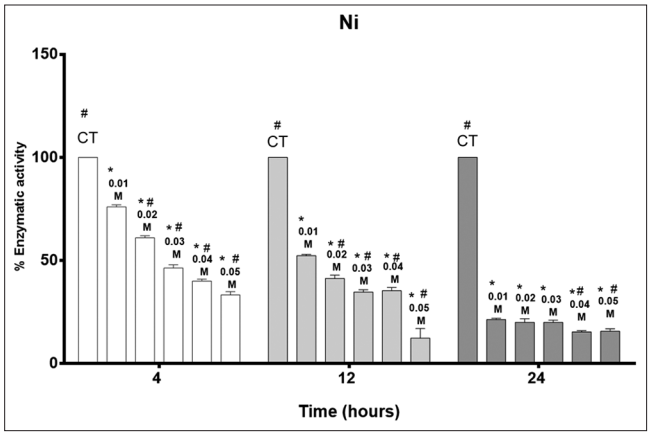

Fig. 6: Evaluation of the enzymatic activity of hen egg white lysozyme (HEWL) incubated with nickel solution at concentrations of 0.01 M, 0.02 M, 0.03 M, 0.04 M, and 0.05 M for 4, 12, and 24 h at room temperature. CT (positive control) HEWL without chrome. Results represent the average of three determinations±standard deviation (n=3). *Denotes p<0.05 as compared to control (HEWL without Ni) (two-way ANOVA/Tukey). #Denotes p<0.05 as compared to 0.01 M Ni (two-way ANOVA/ Tukey)

On the other hand, our results of cadmium in eggs in Ecuador were very high with a value of 0.535 mg/kg of cadmium. Esposito *et al.* [24] reported 0.003 mg/kg of cadmium in eggs in Italy. Uluozlu *et al.* reported value of 0.003 mg/kg of cadmium in hen eggs in Turkey [25].

Our values for boron also were high with a value of 0.5200 mg/kg of Boron. In the case of mercury, our value was of 0.0025 mg/kg, being this value above the reference value of 0.00057 mg/kg per day [40].

Residual enzymatic activity of HEWL

In this study was evaluated the residual enzymatic of HEWL after exposure with different heavy metals. The measure was assayed with *in vitro* spectrophotometer method. HEWL in presence of Cr III loss 99% of their enzymatic activity. Chromium can exist showing an oxidation between Cr^{+0} and Cr^{+6}. The three most stable forms in which chromium occurs in the environment are the 0, +3, and +6 valence states; metal

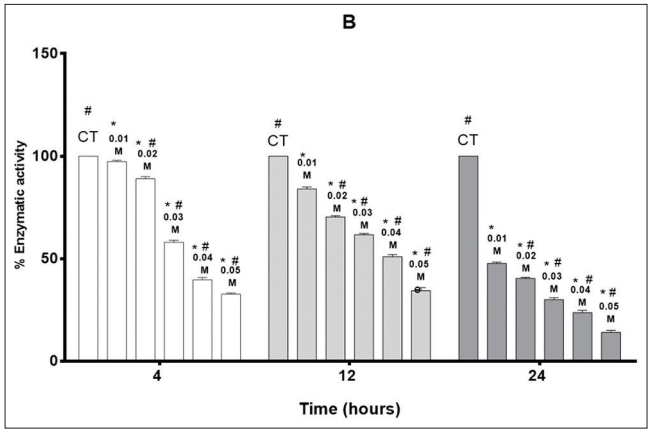

Fig. 7: Evaluation of the enzymatic activity of hen egg white lysozyme (HEWL) incubated with boron solution at concentrations of 0.01 M, 0.02 M, 0.03 M, 0.04 M, and 0.05 M for 4, 12, and 24 h at room temperature. CT (positive control) HEWL without chrome. Results represent the average of three determinations±standard deviation (n=3). *Denotes p<0.05 as compared to control (HEWL without B) (two-way ANOVA/Tukey). #Denotes p<0.05 as compared to 0.01 M B (two-way ANOVA/Tukey)

and alloys, trivalent chromium, and hexavalent chromium, respectively. Elemental chromium (Cr^0) does not occur naturally. Chromium compounds with an oxidation state below +3 are limited, and above +3, they are in the oxidizing process. The occurrence of hexavalent chromium compounds is rare and nearly always humanmade in industrial activities such as the tannery industry. Cr III is the form of chromium found in food and supplements [30].

HEWL in the presence of cadmium has high loss of enzymatic activity. Olmo *et al*. (2001) reported that cadmium produces a loss of enzymatic activity in lysozyme [41]. This enzymatic activity loss was almost 50% with respect to a positive control. The largest activity decreases took place at the initial salt concentration used and activity was relatively constant with increasing salt concentrations.

Olmo *et al.* (2001) [41] reported loss of enzymatic activity for exposure with Zn and Hg. Lysozyme presents 35% of enzymatic activity with the presence of Zn and with the presence of Hg the loss was total.

Toxic heavy metals including copper (Cu), zinc (Zn), iron (Fe), aluminum (Al), cadmium (Cd), lead (Pb), and mercury (Hg) have been associated to degenerative diseases such as Alzheimer's disease [42,43], and some of these metals have the ability to aggregate amyloid beta peptide *in vitro* [44]. Chin-Chan *et al.* (2015) [45] reported a reduction of enzymatic activity of neprilysin, a metalloprotease involved in Alzheimer's disease. A decrease of antioxidants and catalase (CAT) enzyme in thalassemic patients leads to an increased hydrogen peroxide, resulting in lipid peroxidation in β-thalassemic patients [46]. Toxic levels of heavy metal affect a variety of processes in plants [47]. One of the major consequences is the enhanced production of reactive oxygen species (ROS). This situation produces an unbalance between production of ROS and the antioxidative system. For example, a mix of enzymic antioxidants such as CAT, peroxidases and superoxide dismutases, and non-enzymic scavengers, for example, glutathione can produce an increase of these enzymes due to the exposure of the plant to heavy metals such as Pb, Cd, and Hg [48].

Senger *et al*. (2006) stated that the exposure to Hg and Pb inhibits NTPDase and ecto-5`-nucleotidase activities in zebrafish central nervous system (*Danio rerio*) [49]. Cu and Zn can alter the conformation of lysozyme producing the formation of amyloid fibrils, which are implicated in Alzheimer disease [50,51]. Brandao and Nogueira (2011) reported inhibition of enzymatic activity of δ-aminolevulinate dehydratase by action of $HgCl_2$ [52].

Hens fed with supplement of heavy metals (Ni, Cd, Hg, and Pb) in their diets were affected in their eggs production and in the quality of eggs. Yolk and white were affected with oral administration of heavy metals [52-54]. The internal components, proteins, enzymes, and lipids, can be affected, and the biological function can be modified. In our study, HEWL was affected to the exposure of heavy metals, Cr, Ni, Cd, B, Hg, and Pb.

CONCLUSIONS

Eggs can be affected with the contamination of heavy metals and this contamination can inhibit their biological enzymes and proteins. At present, there are no regulations for the maximum content of heavy metal in hen eggs. Eggs are an important food in the human diet, a regulation of the content of heavy metal is needed, especially in places where leather industries and with volcanic activity as in Ecuador. The levels of heavy metals Cr, Ni, Cd, B, Hg, and Pb in hen eggs from Ecuador were 1.8300, 1.1300, 0.5350, 0.5200, 0.0025, and 0.0021 mg/kg, respectively. Enzymatic activity of HEWL was affected by the presence of heavy metals as Cr, Ni, Cd, B, Hg, and Pb. An exposure of 0.05 M to Cr III reduced 1% HEWL enzymatic activity. 24 h exposure to 0.01 M Cd presented a reduction of 37% of HEWL enzymatic activity and 0.05 M Cd resulted only in 75% of HEWL enzymatic residual activity.

ACKNOWLEDGMENTS

This study was supported by Universidad Técnica de Ambato, Ecuador (Project CPU-1373-2014-UTA), (Project Canje de Deuda España-Ecuador), and (Project Universidad Estatal de Bolívar). This work has been reviewed in the English edition by Emilio Labrador.

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