The effect of heavy metals on the enzymatic activity of the rabbit in Libya

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ملخص البحث :

التطور الصناعي الواسع في العالم يصاحبه مشاكل في التلوث البيئي وخصوصا من العناصر الثقيلة. الدراسة الحالية تهدف إلى تسليط الضوء على تأثير كل من Cd^{+2}، Cu^{+2}, Co^{+2} على الانزيمات الآتية : ALP, CPK, LDH, GOT and GPT في بلازما الدم للأرانب.

تم إعطاء الأرانب جرعات متكررة من ماء الشرب عن طريق الفم ملوثة بتركيزين مختلفين (10ppm and 20ppm) لمدة 60 يوماً. عينات الدم تم سحبها و استخلاص البلازما منها ليتم فحصها بواسطة الاختبار الكيميائي الحيوي. نسبة التغير في نشاط الانزيمات تم حسابها و مقدار الارتباط بين تركيز الفلز الثقيل ونشاط الانزيمات تم حسابه إحصائيا بواسطة معامل ارتباط بيرسون (r) Pearson correlation factor . مقارنة بالمجموعة القياسية ، لوحظ ان نشاط كل من ALP, LDH, GOT and GPT قل بينما نشاط CPK زاد مع وجود ارتباط قوي بين تركيز العنصر الثقيل و النشاط الأنزيمي للأنزيمات محل الدراسة. النتائج تعطي دليلاً قوياً على أن هذه العناصر عالية السمية بالنسبة للأرانب حيت إنها تؤثر بشكل كبير على عمليات الأيض الحيوية بواسطة تغيير نشاط الإنزيمات المهمة حيويا في عمليات الأيض.

ABSTRACT

The vast industrial development in the world accompanied with a pollution of the environment especially from the heavy metals. The present study addresses the impact of Cd +2, Co+2 and Cu+2 on the activities of ALP, CPK, LDH, GOT and GPT in the plasma of rabbits. Rabbits received repeated oral doses of drinking water contaminated with two different concentrations (10ppm and 20ppm) over 60 days. Serum samples were collected for biochemical assay. The percentage of enzymatic activity changes was calculated and the correlation between heavy metal concentration and enzymatic activity was determined using

Pearson correlation factor (r). Compared to the control group, the ALP, LDH, GOT and GPT activities were inhibited while CPK activity was increased with a strong correlation between the heavy metals concentration to the enzymatic activity of all enzymes. The finding strongly prove that these elements are highly toxic to the rabbits as they have a great impact on the metabolic processes by altering the activities of key enzymes.

Key Words: Cadmium, Cobalt, Copper, Alkaline Phosphatase, Lactate Dehydrogenase, ALT, AST, Toxicity.

ntroduction

The pollution with heavy metals is considered as a serious problem due to their drastic effect on all creatures. (Mazrouh, 2016) Many factors have been directly related to the increases of heavy metals concentrations in the environment such as extensive population growth that accompanied with the industrial development which characterized by irrational uses of chemical fertilizers and pesticides.(Ahmed et al., 2016; Wallin, 2015)

Heavy metals are defined as inorganic elements that possess a relatively high density (Tchounwou et al., 2012) and broadly diverse in their biochemical properties and physiological functions (Espín et al., 2014; Vaishaly et al., 2015). The essential heavy metals such as copper and zinc play important biochemical roles in living organisms (Mazrouh, 2016; Tchounwou et al., 2012). And they are present naturally in the body at small concentrations, however at high concentrations they become harmful and behave as toxicants (Mazrouh, 2016; Strydom et al., 2006). Copper for instance works as a vital cofactor (Jan et al., 2015) for numerous oxidative stress-related enzymes including catalase, superoxide dismutase, peroxidase, cytochrome c oxidases, ferroxidases, monoamine oxidase, and dopamine β-monooxygenase (Tchounwou et al., 2012). On the other hand elements such as Cd, Pb, Cr and Hg are nonessential and extremely poisonous (Bat et al., 2012; Espín et al., 2014; Morsy et al., 2012; Vaishaly et al., 2015). They impede biological processes and lead to oxidative stress, inflammation, and lipid peroxidation in the organs (Kim et al., 2015)

The toxicity of heavy metals hinges mainly on two factors, specifically, metal ion type and its concentration (Sa'idi, 2010). Their effect on enzyme depend on the ability of metal ion to react with ligands, which is crucial for the enzyme to perform its function normally (Sandhya M Sonawane, 2017; Suresh et al., 2016). The enzymes are particularly vulnerable to the heavy metals, as their level and activities varying considerably (Ahmed et al., 2016). This variation

could be linked directly to the tissue damage. Because of this sensitivity they can be utilized as a biomarker for metal contamination (Ahmed et al., 2016; Chen et al., 2013; Mazrouh, 2016). As a result, it could be useful in environment monitoring studies (Ahmed et al., 2016; Sarosiek et al., 2009)

Enzymes are biological catalytic agents which follow definite general rules. They catalyzed reactions occur at physiologically low temperatures (37oC) and demand extremely small quantities of enzymes. From a chemical prospective, they are complex protein molecules synthesized inside the cells. The place where they work as biocatalysts to execute several physico-chemical reactions.(Sandhya M Sonawane, 2017a, 2017b; Vaishaly et al., 2015) Alkaline phosphatase (ALP) is an enzyme which participated in numerous metabolic processes, such as bone mineralization, molecule permeability, cell growth and differentiation, osmoregulation, and regeneration The measurement of this enzyme activity is commonly performed in clinical and ecotoxicological researches..(Chen et al., 2013) Glutamic oxaloacetic (GOT) and glutamic pyruvic (GPT) transaminases are among many enzymes that have an important function in both protein and carbohydrate metabolism (Chen et al., 2013; Mazrouh, 2016). Glutamic oxaloacetic transaminase (GOT), glutamic pyruvic transaminase (GPT), and lactate dehydrogenase (LDH) are generally found in a low concentration in the blood. Scientists used the alterations in plasma enzyme activity as a general parameter for detection of biochemical changes brought by toxicants (Chen et al., 2013). GOT and GPT are not specific for liver and their activities may become higher as a result of other organs damage (Chen et al., 2013; Mazrouh, 2016). LDH level was found to be much greater in liver than in six other tissues of mullet, a marine species. Its activity was found to be boosted by copper treatment, and inhibited by lead (Chen et al., 2013).

Tarhuna city is located 65 Km to the southeast of Tripoli (the capital city of Libya). Economically it depends mainly on its agriculture and animal wealth. (Wikipedia contributors, 2019, November 18) This study is aimed to measure the effect of the following elements, cadmium (Cd), cobalt (Co), and copper (Cu) on enzymatic activity mainly ALP, CPK ,GOT, GPT and LDH in rabbit serum.

1. Method

2. Experimental animals

The study was started after getting an ethical approval from ethical committee at Mesallata faculty of art and science. Seven rabbits were collected form Amamra area of Trahona city. They were placed in special cages and monitored for two

months period under same condition. The health status of the rabbits was clinically assessed by a veterinarian prior to blood sampling. This clinical assessment includes the evaluation of general body conformation, posture, attitude, stimulus response and character of respiration. They were divided in to three groups A, B and C each group consists of two rabbits. As seen from figure 1. The group A was treated with a drinking water containing Cadmium at two different concentrations 10 ppm and 20 ppm, the group B was treated with a drinking water contaminated with Cobalt at two different concentrations 10 ppm and 20 ppm, and the group C was treated with a drinking water polluted with Copper at two different concentrations 10 ppm and 20 ppm. the seventh rabbit was considered as a standard (negative control), where it is treated with uncontaminated water. The food used during the trial period was natural herbs and animal feed.

2.1.The procedure:

At the beginning, the samples were taken from all rabbits. These samples used to determine the level of the enzymatic activity before the contaminated water administration. And it will be considered as a baseline data to assess the effect of heavy metals on these enzymes. A week later, groups A, B and C were supplied with different concentrations of cadmium, cobalt and copper through water contaminated with the concentrations mentioned earlier on daily basis. The activity of the enzymes was estimated before starting the heavy metals administration process and also after given them by two months. The blood samples were withdrawn from rabbits and placed in a clean test tube. The sample was then placed in the centrifuge at 3500 rpm for half an hour. The serum was separated in all blood samples. After the separation process, the samples were placed in the freezer under 0 ° C until the time of analysis. The blood was extracted from rabbits by a specialist in this field. Also, another sample was taken from the standard rabbit, the rabbit whose drinking uncontaminated water and has not been treated with contaminants, to ensure that the level of the enzymatic activity are same as in the beginning of the experiment. This is an essential step to prove that there is no other factors affect their enzymatic activities. The data were entered and analyzed using SPSS version 20. The percentage of the enzymatic activity were calculated using this formula.

$$percetage\ (\%) = \frac{E\ test - E\ standard}{E\ standard}\ X\ 100$$

Where;
E test is the enzymatic activity after exposure to contaminants.
E standard is the enzymatic activity of the standard animal.
The relationship between the concentration of heavy metal and the enzymatic activity were assessed using Pearson correlation method.

3. Results

Table (1) shows the activities of enzymes in plasma of standard rabbit which did not treated with the contaminants. The results of the study show that the values of these enzymes were close proving the presence of rabbit under the same natural conditions.
As shown in table 2, the activity of ALP and CPK were increased with the increase of the Cd concentration to (313, 330)U/L and (855, 1035) U/L respectively. However, a noticeable decrease in the activity of LDH, GOT and GPT as the percentage values were in negative sign compared to the standard rabbit level of enzymatic activity (-32%, -40%, -33%) at 20 ppm of cadmium respectively.
The activity of CPK was increased dramatically at a 10 PPM of Co to 1163 U/L and its activity decreased to reach 920 U/L at higher concentration of cobalt, similarly, GPT increased at 10 PPM to 85 U/L compared to the drastic decrease which occurred due to exposure to a higher concentration 35 U/L. However, the activity of ALP, LDH and GOT had a similar pattern as the concentration of the cobalt increased the activity decreased. More details are presented in table 3
After exposure to cupper at concentration of 10 and 20 ppm, the activity of ALP was decreased to 93 U/L and 150 U/L respectively compared to the value of control group. And the effect of the exposure to 10 on the ALP was greater than the effect which happened due to the higher concentration. On the similar way a noticeable decrease on the activity of both LDH and GPT was also occur. The only difference was the effect of 20 ppm was higher than the effect of 10 ppm. On the other hand, the activity of both CPK and GOT were increased on the rabbit which received 10 ppm of cupper and their activities were dramatically decreased due to the exposure to 20 ppm of cupper

4. Discussion

The pollution of the environment largely affect the physiological state of the animals as it causes a disturbance in the enzymatic activities. it affects directly or

indirectly the living cells and causes distortion either by elevation or inhibition of various enzymes which are vital for life. In this study, the effect of cadmium, cobalt and copper on the activities of some enzymes ,which considered as biomarkers for the diagnosis of certain diseases, in rabbit serum were observed.

There was a significant change in the activity of ALP in the serum due to the exposure to the toxicants. Only cadmium cause elevation in the ALP activity while other elements cause inhibition to its activity. This finding was not in good agreement with the previous studies (Sandhya M Sonawane, 2017; Suresh et al., 2016). Sandhya M Sonawane, (2017) and Suresh, Jaganath Bose, & Deecaraman (2016) studies reported that the activities of ALP was decreased after they exposed to the cadmium. The raise in the ALP level was also reported by Mary, Bhuvaneswari, & Anandan, (2015) and it was probably occurred due to the direct toxic effect on the cells which lead to the necrosis and hence the leakage of the enzyme to the plasma will lead to increase its level (Mary et al., 2015). The inhibition of ALP activity which caused by cobalt and copper may be occurred due to the direct reaction of the heavy metals with the active site of the enzyme leading to form nonfunctional metal enzyme complex similar result was reported by El-Demerdash (2001)(El-Demerdash, 2001). As a result the enzyme fail to perform its catalytic function. The ALP play a vital role in the phosphorylation of organic compound and bone formation in the higher animals (Suresh et al., 2016). It is a nonspecific enzyme present in many tissue especially liver and bone. therefore any changes in its level will be helpful in detecting damage to these organs.

The LDH enzyme plays a vital role in the carbohydrate metabolism. In the current research LDH activity revealed a significant decrease due to the exposure to the heavy metal stress(Sandhya M Sonawane, 2017b) and this effect was dependent on the concentration of the pollutants. Similar findings were also identified in the previous studies (Sandhya M Sonawane, 2017b) (Suresh et al., 2016) where Sonawane (2017) proposed that this inhibitory effect of heavy metals maybe similar in such way to the inhibitory effects which result from the organophosphate compounds as the formation of enzyme inhibitor complex will take place this complex is not easily dissociate back. However the exact mechanism of heavy metals on the enzyme activities still unclear (Sandhya M Sonawane, 2017a). The decrease in the activity of this enzyme proposes that there is a reduction in the conversion of the lactate to pyruvate which result in

the accumulation of the lactic acid showing the predominance of anaerobic glycolysis process (Aanand et al., 2010).

The activities of GOT and GPT in general were decreased after exposed to the Cadmium and Cobalt at both tested concentration (10ppm and 20 ppm). similar finding was reported due to lead exposure(Mary et al., 2015). The significant inhibition could be resulted by the formation of irreversible complex between the enzymes and the pollutants . The activities of these enzymes were increased only after exposure to cupper at 10 ppm. This was not consistent with the previous studies performed on European eel (Anguilla anguilla) at Lake Edku (Mazrouh, 2016), Nile tilapia fish (Oreochromis niloticus) in Lake Manzala, Egypt (Abumourad et al., 2014) and another study on three fish species in Nile river (Ibrahim & Mahmoud, 2005)which conducted to assess the level of pollution by the nickel, copper and zinc and their effect on the GOT and GPT.(Abumourad et al., 2014; Ibrahim & Mahmoud, 2005; Mazrouh, 2016) the activities of both enzymes were significantly increased which indicate sever hepatocellular damage. the inconsistence may be attributed to the synergistic effect of the multiple heavy metal on the cells of the liver compared to the mono metal effect in the present study.

5. Conclusion

These days, the investigation of ecological pollution cannot be grounded entirely on a chemical examination. Because these chemical tests do not offer a clear sign about the poisonous consequences of pollutant on the living creatures. In the current research, the exposure of rabbits to different concentrations of heavy metals toxicity namely cadmium (Cd), cobalt (Co), and copper (Cu) on some key enzymes ALP, CPK ,GOT, GPT and LDH over 2 months period. The studied heavy metals triggered substantial changes in the enzymatic activities of the animals which essential for the life as they responsible for the main metabolic activities.

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Author contributions

All authors were involved in conceiving the study. I. A. designed the experiments. F.A, M.D. and N.W. conducted the experiments F.A, M.D. and N.W. analyzed the samples. F.A, M.D., N.W. and S.D. analyzed the data. S.D.

wrote the manuscript with significant assistance and comments from all the other authors. All authors approve the final version of the manuscript.

Declaration of Competing Interest

None.

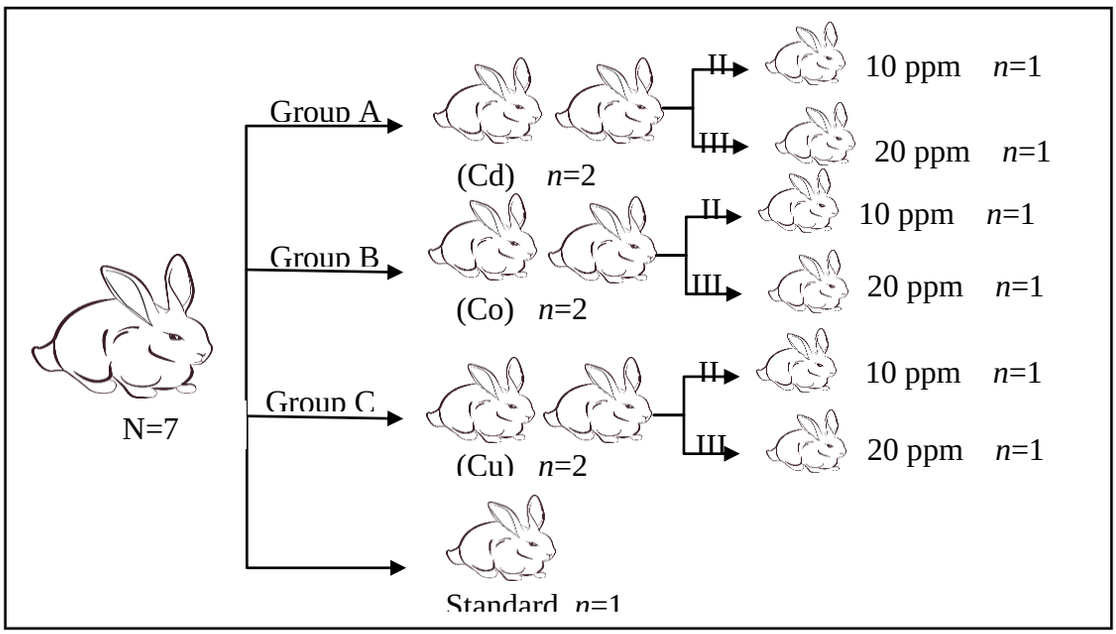

Table 1 the enzymatic activity of different enzymes in rabbit serum taken from the standard sample before exposure to pollutants in units U/L .

Sample	L)/Type of enzyme and its enzymatic activity (U				
	ALP	CPK	LDH	GOT	GPT
Standard Rabbit	308	685	866	96	78

Table 2 the enzymatic activity of different enzymes in rabbit serum after exposure to different concentration of Cd for two months

Sample	Conc. Ppm	L)/Type of enzyme and its enzymatic activity (U									
		ALP (%)		CPK (%)		LDH (%)		GOT (%)		GPT (%)	
Rabbit I	0	308	(0.0)	685	(0.00)	866	(0.0)	96	(0.0)	78	(0.00)
Rabbit II	10	313	(1.6)	855	(24.8)	685	(-21)	70	(-27)	66	(-15.4)
Rabbit III	20	330	(7.1)	1035	(51.1)	588	(-32)	57	(-40)	52	(-33.3)
r		0.954		1		-0.985		-0.985		-0.999	

Table 3 the enzymatic activity of different enzymes in rabbit serum after exposure to different concentration of Co for two months

Sample	Conc ppm	L)/Type of enzyme and its enzymatic activity (U									
		ALP	(%)	CPK	(%)	LDH	(%)	GOT	(%)	GPT	(%)
Rabbit I	0	308	(0.0)	685	(00.0)	866	(0.0)	96	(0.0)	78	(0.0)
Rabbit II	10	74	(-76.0)	1163	(69.8)	461	(-46.8)	92	(-4.17)	85	(8.97)
Rabbit III	20	85	(-72.4)	920	(34.3)	275	(-68.3)	21	(-78.1)	35	(-55.1)
R		-0.844		0.492		-0.978		-0.889		-0.794	

Table 4 the enzymatic activity of different enzymes in rabbit serum after exposure to different concentration of Cu for two months

Sample	Conc. ppm	L)/Type of enzyme and its enzymatic activity (U									
		ALP	(%)	CPK	(%)	LDH	(%)	GOT	(%)	GPT	(%)
Rabbit I	0	308	(0.0)	685	(0.0)	866	(0.0)	96	(0.0)	78	(0.0)
Rabbit II	10	93	(-69.8)	1081	(57.8)	429	(-50.5)	129	(34.4)	99	(26.9)
Rabbit III	20	150	(-51.3)	961	(40.3)	396	(-54.3)	40	(-58.3)	56	(-28.2)
r		-0.709		0.680		-0.896		-0.622		-0.512	

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