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SOME STUDIES ON HEMATOloLOGICAL AND SOME BIOCHEMICAL CHANGES INDUCED BY HEAVY METAL IN CATTLE REARED IN BAHR EL-BAKAR REGION WITH SPECIAL REFERENCE TO ITS RESIDUES IN MILK

(With 6 Tables)

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بعض الدراسات على التغيرات الدموية والبيوكيميائية المحدثة بواسطة المعادن الثقيلة في الأبقار المرباة في منطقة بحر البقر ومدى تواجد بقاياها في اللبن

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أجري هذا البحث على الأبقار التي يتم تربيتها في منطقتى فاقوس وبحر البقر بمحافظة الشرقية وذلك لتقدير بقايا المعادن الثقيلة في اللبن ألبقري وكذلك معرفة تركيز تلك المعادن فى مياة المنطقتين وتأثير تلك المعادن الثقيلة على بعض الوظائف البيوكيميائية حيث يعتبر مصرف بحر البقر هو المكان الرئيسي الذي يتم فية صرف مياة الأرض الزراعية والصرف الصحي ومياة الصرف لبعض المصانع بمحافظة الشرقية.لذلك تم تجميع ٥٠ عينة عشوائية (١٠ عينة لبن خام ، ١٠ عينة دم من ابقار ترعى فى منطقة مدينة فاقوس و٥ عينة مياة من منطقة فاقوس ومثل تلك العينات من منطقة مدينة بحر البقر) وتم تحليل عينات اللبن والمياة باستخدام مقياس الامتصاص الذرى الطيفي للوقوف على مستويات الرصاص والكادميوم والزئبق والنحاس والزنك. واستخدمت عينات الدم لمعرفة تاثير تلك المعادن على صورة الدم وبعض الوظائف البيوكيميائية وخطورة بقايا المعادن الثقيلة في الألبان على الصحة العامة. وقد أسفرت النتائج عن تواجد الرصاص والكادميوم والزئبق والنحاس والزنك فى مياة مدينة فاقوس قدره بمقدار ٠,٩٠، ٠,٧٤، ١,٠٧،٠,٠٩٢ و ٣,٣٤ جزء في المليون على التوالي وأيضا النسب المرادفة في مياة منطقة بحر البقر أعلى ٢,٨٩، ٠,٠٢٣، ٠,٨١، ٢,٩٥ و ٤,٧٢جزء في المليون على التوالي بينما أسفرت النتائج عن تواجد بقايا المعادن الثقيلة الرصاص والكادميوم والزئبق والنحاس والزنك في اللبن الخام المأخوذ من أبقار تربى على الحشائش والمحاصيل التي تنمو في مدينة فأقوس بنسب قدرها ٠,٨٢، ٠,٣٣، ٠,٠٧٤، ٠,٠١٩،و ١,١٨ جزء في المليون على التوالي بينما النسب في اللبن الخام المأخوذ من أبقار تربى على الحشائش والمحاصيل التي تنمو فى مدينة بحر البقر كانت أعلى١,٩٠، ٠,٦٩، ٠,١٢ ، ٠,٠٣٦،و ٣,١٩ جزء في المليون على التوالي. وقد أتضح أيضا أن المأخوذ اليومي من المعادن الثقيلة (الرصاص والكادميوم والزئبق والنحاس والزنك) للإنسان البالغ من اللبن قدره٧٦، ١٩,٧١، ١,٧٢. ٤٨ ، ٠,٢٠٥ و ٠,٩١١%) من المأخوذ اليومي المسموح به عالميا. وتشير ا لنتائج أن المعادن الثقيلة أدت إلى حدوث نقص معنوي فى العدد

الكلى كرات الدم الحمراء، العدد الكلى لكرات الدم البيضاء، تركيز الهيموجلوبين، حجم خلايا الدم المرصوصة، الجليكوز، البروتين الكلى، الزلال، الجلوبيولينات بينما كان هنـاك زيـادة معنوية في نشاط إنزيمات الترانس امينيزسس (الأسبرتيت امينوترانسفريزAST- الألانـين أمينوترانسفريز ALT) والفوسفاتيز القاعدي وكذلك اليوريا والكرياتينين في مصل دم الأبقار بالمقارنة بالمجموعة-الضابطة. يتبين لنا من تلك الدراسة أن المعادن الثقيلة موجودة فى مياة منطقة بحر البقر أعلى من الحد المسموح به مما أدى إلى وجود تأثير سمي ويـؤدى إلـى تغيرات كبيرة فى صورة الدم وبعض الوظائف البيوكيميائية. وكانت نسبة متبقيات تلك المعادن الثقيلة فى الألبان المأخوذة من ابقار منطقة بحر البقر أعلى من الحد المسموح به.

SUMMARY

Pollution of water has increased due to the industrial effluents, municipal waste and agricultural drainage. The aim of this work was to evaluate the haematobiochemical effect of heavy metal as well as its residues in milk in Bahar El Baker region in which Bahar El Baker drainer sea is considered the main place for agriculture drain, sewage discharge, waste water and industrial effluents in Sharkia Governorate but the water in Fakous region from Nile River. Cattle reared in Bahar El Baker exhibited some clinical symptos represented by anemia, abdominal colic, liver dysfunction, renal damage, skeletal disorder with symptoms resemble osteomalachia. A total of 50 random samples (20 each of each of raw cows milk, blood and 5 water samples were collected from cattle in Fakous and Bahar El Baker City Sharkia Governorate. Milk samples where placed in an ice box and immediately send to laboratory for determination of some heavy metals (lead, cadmium, mercury, copper and zinc) concentration by Atomic Absorpation Spectrophotometry. Blood samples were taken in two poration, first blood samples were collected in heparinized tube for total erythrogram and total leukocytic and the second sample was taken in centrifuge tube for obtain clear serum for biochemical analysis. The analyzed data revealed that the mean values in the examined water samples collected from Fakous City amounted 0.90 ppm lead, 0.74 ppm cadmium, 0.092 ppm mercury, 1.07 ppm copper and 3.34 ppm zinc. Examined water samples collected from Bahar El Baker city conteaned 2.89 ppm lead, 0.023 ppm cadmium 0.81 ppm mercury, 2.95 ppm copper and 4.72 ppm zinc. Also,the mean values of estimated element in the examined raw milk samples collected from Fakous City were 0.82 ppm lead, 0.33 ppm cadmium,(0.074) ppm mercury, 0.19 ppm copper and 1.18 ppm zinc while respective values in examined raw milk samples collected from Bahar El Baker City were 1.90 ppm lead, 0.69 ppm cadmium, 0.12 ppm mercury, 0.036 ppm copper and 3.19 ppm zinc. The present work revealed that, erythrogram (total erythrocytic count, haemoglobin % and packed cell volume), total leukocytic count, blood serum glucose, total proteins, albumin and globulins values all were significantly decreased in cattle reared in Bahar El Bakar region with significant increase in serum levels of aminotra-nsferase (ALT-AST) alkaline phosphatase activity level, urea and creatinine levels. It could be concluded that, water in Bahar El Baker and milk obtained from cattle reared in Bahar El Baker contain high concentrations of heavy metals and induce adverse effect in hemato-biochemical Parameters s in lactating cows.

Key words: *Haematology, heavy metals, milk, cattle.*

INTRODUCTION

Heavy metals are persistent contaminant in the environment and come to the forefront of dangerous substances causing serious health hazard in human. Copper, chromium, cadmium, zinc and lead are among the most dangerous of these elements (Samaha and Haggag, 2004). Environmental pollution is deleterious to the biological life. Pollution due to chemical substances such as pesticides, inorganic heavy metals,organic compounds, toxic gases and fumes widely spread and threatens the biological balance (Forstner and Wittmann, 1983), However heavy metals in soil and water may enter the food chain through the biological cycle which includes bioconcentration by plant and animals (Goyer, 1992) Moreover heavy metals have a tendency to accumulate in tissues and organs of animals (Friberg, *et al.,* 1986) and cadmium are among the dangerous heavy metals. Many results studied lead concentration in tissues of different animal species and results pointed out to the fact that lead was accumulated in kidney, liver and bone in significant concentration than in the muscle tissue (Leita, *et al.,* 1991). Chronic lead poisoning may induce clinical signs manifested by anemia and renal damage. Although copper and zinc are essential elements to life, they are toxic when exceed the permissible limits. Acute copper exposure causes hypertension and hemolytic anemia, while the oral zinc intoxication leads to enteritis, bloody watery diarrhea and abdominal pain (Gossel and Bricker, 1990) with liver dysfunction (Goldfrank, *et al.,* 1990).

Heavy metals are recognized as acumulative toxic substances due to its low elimination rates from the body. Moreover the heavy metals could not be metabolized, thus they persist in the body and exert their toxic effects which result in serious health hazards to human, depending on their levels of contamination (Lucky and Venugopal, 1977). Among these metals, lead, cadmium and mercury have a great concern due to a variety of their uses that increases their level in environment. They cause several clinical problems due to their competiton with the essential elements for binding sites and their interference with the sulphahydryl groups and structural protein (Dasilva and Williams (1991).

Milk and milk products represent an important part of the human food especially children's diet. So, the contamination of milk and its products by heavy metals is one of the major threats confronting the public health (Jensen, 1995). The animals receive the heavy metals through air, water and the ingestion of contaminated feed stuffs has been considered as the main source of metal residues in the secreted milk (Vidovic, *et al.* (2005).

Therefore, this work was undertaken to determine the haemato-biochemical effect of heavy metal as well as its residues in milk in Sharkia Governorate.

MATERIALS and METHODS

Cattle reared in this region exhibited some clinical symptos represented by anemia, abdominal colic, liver dysfunction, renal damage, skeletal disorder with symptoms resemble osteomalachia. A total number of 40 random samples (20 of raw cows milk and 20 blood samples) were collected from different region in Sharkia Governorate (Fakous and Bahar El Bakar region) 10 raw milk samples and 10 blood sample from each region.

Samples

1. Water samples:

Ten water samples were collected from Fakous city (used as control cattle) and 10 water samples from Bahar El Bakar region (used as tested cattle). Water samples were collected in glass bottles (each of one liter capacity) for each sample. The samples were taken at a depth of half meter from the water surface (APHA, 1985) and transported to the laboratory for chemical analyses.

Chemical analysis of water:

The analysis of water samples was carried out according to APHA (1985). The water samples were filtered through a 0.45µ membrane filter.The required volu-me (100 ml) of filtrate was collected and preserved by 0.3 ml of 1:1 HNO3. Quantitative determination of heavy metals in filtered water was carried out by using Perkia-Elmer 2380 Atomic Absorption Spectrophotometer.

2. Milk samples

Milk samples were collected from 10 cattle reared in Fakous City (used as control cattle) and 10 cattle reared in Bahar El Bakar region (used as tested cattle). Milk sample from each animal was collected in a sterile Macarteny bottle for chemical examination. All samples were transported to the laboratory. Each sample was labeled to identify the source, site and date of sampling. Delayed samples were stored in ice bag.

Chemical analysis of raw milk:-

Raw milk samples were digested according to method described by Tsoumbaris and Papadopoulou, (1994) where 10 ml solution of concentrated nitric acid and perchloric acid (1:1) were added to 10 ml of throughly homgenized milk sample. The samples were cold digested overnight followed by mild increase in temperature till heating at 100°C in water bath for 3-4 h. 4-5 drops of H2O2 (30%) were added and heating continued till the brown nitrous gasses were expelled and the specimens became clear. After cooling, each digest was diluted to 25 ml with deionized water and filtered through Whatman filter paper No.42. The clear filtrate of each sample was kept in refrigerator to avoid evaporation. The filtrated samples were analysed by using Perkia-Elmer Atomic Absorption Spectrophotometer.

3. Blood samples

Two blood samples were collected from 10 cattle reared in Fakous City (used as control cattle) and 10 cattle reared in Bahar El Bakar region (used as tested cattle)

First blood sample was collected on heparin sol. for determination of blood picture (total erythrocytic count, haemoglobin content packed cell volume percent and total leukocytic count) that were performed according to technique described by Jain (1993).

Second sample was collected in clean, dry centrifuge tube without anticoagulant for obtaining clear serum. The separated sera were kept in deep freezer at-20C to be used for biochemical study.

Biochemical studies

Clear serum samples were used for determination of total proteins (Doumas, *et al.,* 1981), albumin (Drupt, 1974). Globulins was calculated as difference between total proteins and albumin. Serum transaminases activity levels (AST-ALT) were determined according to method described by Reitman and Frankel (1957), alkaline phosphatase activity levels (John, 1982), urea level (Fawcet and Scott, 1960) and creatinine (Husdan and Rapoport, 1968).

Statistical analysis

The obtained data were statistically analysed according to Petrie and Watson (1999).

RESULTS

The results of the present study were illustrated in Tables (1-5)

Table 1: Heavy metal concentration in the examined water samples (ppm) (n=5).

Groups \ Parameters	Lead	Cadmium	Mercury	Copper	Zinc
Fakous water (control)	0.90±0.11	0.74±0.02	0.092±0.01	1.07±0.03	3.34±0.05
Bahar El Bakarwater (tested)	2.89±0.09	0.023±0.01	0.81±0.02	2.95±0.08	4.72±0.42
Max.allowable level	0.05	0.005	0.001	1.000	5.00

* Significant at P < 0.05 ** Significant at P < 0.01

Table 2: Heavy metal residues in the control and tested raw milk samples (N=10).

Groups \ Parameters	Lead (ppm)	Cadmium (ppm)	Mercury (ppb	Copper (ppm)	Zinc (ppm)
control cattle	0.82±0.09	0.33±0.04	0.074±0.004	0.019±0.002	1.18±0.15
tested cattle	1.90±0.10*	0.69±0.02*	0.12±0.02*	0.036±0.001*	3.19±0.14*

*Significant at P < 0.05 ** Significant at P < 0.01

Table 3: Comparasion of the acceptable daily intake (ADI) values of the heavy metals with the calculated daily intake from raw milk of tested cattle (ppm).

Parameters / Groups	ADI(A) Ug/70kg person	Mean coc. of metals (ppm) in present study	Calc.daily of metal from consumption of 200 mil milk daily(B)	
			ug/day/person	%
Lead	500	1.90	380	76
Cadmium	700	0.69	138	19.71
Mercury	50	0.12	24	48
Copper	35000	0.036	7.2	0.0205
Zinc	70000	3.19	638	0.911

(A) FAO/WHO (1972) (B) Natrition Institute (1996)

Table 4: Erythrogram and total leukocytic count in control and tested cattle (n =10).

Parameters / Groups	T.R.B. Cs. (106/c.mm)	H.B (g m %)	P. C.V. (%)	T.WB.CS. (103/c.mm.)
Control cattle	8.9 ±0.60	11.63 ±0.38	37.412±0.89	9.85±1.06
Tested cattle	6.32±0.84*	7.5±0.81**	28.1±1.09**	7.52±0.53*

* Significant at $P < 0.05$ ** Significant at $P < 0.01$

Table 5: Some selectel liver and kidney function testsin control and tested cattle (n =10).

Parameters / Groups	Liver function			Kidney function	
	AST (U/L)	ALT (U/L)	Alk.ph. (I.U/ml)	Urea (mg/dl)	Creatinine (mg/dl)
control cattle	51.04±1.73	10 .71±1.42	52.61±1.53	18.32±2.75	2.05±0.41
tested cattle	59.21±2.41**	19.27±2.4**	61.24±2.31**	25.82±1.20*	3.71±0.24**

*Significant at $P < 0.05$ ** Significanhi $P< 0.01$

Table 6: Mean value of glucose, Proteinogram in control and tested Cattle (n=10).

Parameters / Groups	Glucose (gm/dl)	T.Protein (gm/dl)	Albumin (gm/dl)	Globulins (gm/dl)	A/G ratio
control cattle	61.83±1.42	7.60 ±0.54	3.85 ±0.42	3.75±0.36	1.03±0.23
tested cattle	54.79±2.84	5.91 ±0.60*	2.70 ±0.41*	3.21±0.21*	0.84±0.20*

*Significant at $P < 0.05$ ** Significant at $P < 0.01$

DISCUSSION

This study was undertaken to investigate mainly serious toxic effects of heavy metals, with paying interest to the haemato-biochemical, liver and kidney effects that may arise as a consequence of long term exposure to such harmful chemicals as well as its residues in milk.

Clinical symptos appeared in cattle present in Bahar El Bakar region were anemia, abdominal colic, liver dysfunction, renal damage, skeletal disorder with symptoms resemble osteomalachia. Nearly samilar result were recorded by (Donaldson, 1980 and Gossel and Bricker, 1990)

In the current work, it has been found that the heavy metals (lead, cadmium, mercury, copper and zinc) concentration in examined fresh water samples from Fakous City were 0.90, 0.74, 0.092, 1.07, 3.34 ppm respectevely. The above mentioned results were supported by previous result recorded by Abd El-Kader, *et al.* (1993) 0.74, 0.04, 0.9 and 0.29 ppm for Lead, cadmium, copper and Zinc respectevely. Our results go hand in hand with those reported by Abd El Nasser, *et al.* (1996) in river nile water in Assiut region. Water from Bahar El Baker region in our study contain more heavy metals (lead, cadmium mercury, copper and Zinc) as 2.89, 0.023, 0.81, 2.95 and 4.72. This increase in heavy metals in water of Bahar El Baker water may be attributed to the level of pollution of water with agriculture drain, sewage discharge, waste water and industrial effluents.

Statistical analysis of the obtained data revaeled that the concentrations of heavy metals(lead,cadmium,mercury,copper and zinc) in raw milk samples from Fakous City in the present work was 0.82, 0.033, 0.0074, 0.19 and 1.18 respectively. Nearly similar findings were reported by Ibrahim (2005). They found the concentrations of the trace elements (lead, cadmium, mercury, copper and zinc), that in raw milk collected from cattle in sharkia governorate may be 1.970, 0.353, 0.088, 0.024 and 1.266 respectively. Higher levels of this trace elements (lead, cadmium, mercury, copper and zinc) were found in raw milk collected from cattle in bahar el baker region 1.90, 0.69, 0.12, 0.036 and 3.19 respectively. The higher trace elements concen-trations in the milk may be attributed to the excessive exposure of the lactating cows to environmental trace elements contamination of the water, soil and feeding stuffs (Goyer, 1992).

The present study illustrated that the heavy metals induced sgnificant change in erythrogram and total leukocytic count represented by significant reductions in total erythrocytic and leukocytic count, haemoglobin concentration and packed cell volume. This observation was previously recorded by EL-Sebai, *et al.* (1994) in rabbit, Naser, *et al.* (1996) in cow and (Ibrahim, 2000) in mice. Anaemia is a common feature of cadmium intoxication in animals (Prigge, *et al.* 1977). Coles (1986) mentioned that one of the specific metabolic activity of lead is reduction oh haemoglobin synthesis. Reduction observed in erythrogram and total leukocytic count in our study may be explained on the bases of a fact reported by Polprasert (1982) that copper is very toxic at higher concentrations, abnormally high copper levels cause anaemia. Also the long term exposure to cadmium may lead to toxic effects which induce slight anaemia (WHO, 1980). Also this probably results from impaired intestinal absorption of iron due to competition of cadmium with iron transfer system from the intestinal mucosa and the resultant iron deficiency (Fox, *et al.* 1971) and destruction of erythropiotein producing cells in the kidneys due to renal dysfunction as a result of severe damage of tubular and peritubular cells (Horiguchi, *et al.*, 1996). While, lead has a large affinity for the thiol and phosphate containing ligands, inhibiting the biosynthesis of heme (Forstner and Wittman, 1983). Therefore, the hematopoietic system important targets of lead toxicosis and cause anaemia.

From the present study it has been observed that haevy metals induced deleterious effect on biochemical constituents and elicited a significant increase in (AST-ALP), alkaline phasphatase activity levels, urea and creatinine levels in cattle reared in Bahr El Bakar region. This result may be due to toxic effects of lead on the liver tissue and that induced increase in liver enzyemes (Forstner and Wittmann, 1983). Cadmium accumulates mainly in the liver and kidneys (Goyer, 1989) and the chronic cadmium toxicosis included kidney damage with increase in urea and creatinine (Donaldson, 1980). Saygy, *et al.* (1991) recorded that cadmium induced damage in the liver as fibrosis in portal area. Also mercury causes severe kidney damage with increase in urea and creatinine (Manahan, 1989). Our results came in agreement with Novelli, *et al.* (1998) in rats supplemented with cadmium.

The obtained results for some serum parameters were summerized in Table (6). The results revealed a significant decrease in serum glucose of cattle in tested cattle than control one.Same result was recorded by Naser, *et al.* (1996) in cow suffering from lead toxicity and he attributed to a state of inappetence. Hypoproteinemia, hypoalbuminemia and hypoglobulinemia were observed in cattle reared in Bahar El Baker region. This may be explained on the bases of a fact reported by Sallam, *et al.* (2000). Chronic exposure to copper causes liver cirrhosis that led to degenerative changes in the liver and kidney. Furthurmore, lead toxicity induced liver dysfunction and have toxic effects on sulfhydrylcarboxy and imidazole contaning protein, membran protein and globulin (Fell, 1984). Also chronic cadmium toxicity produces nephrotoxicity and proteinuria (Friberg and Elinder, 1985). Coles (1986) mentioned mentioned that the liver is a primary organ which is responsible for biosynthesis of albumin. Hypoproteinemia and hypoalbuminemia due to chronic toxicity by heavy metals are also reported (Gossel and Bricker, 1990). The reduction in the concentrations of blood total protein and albumin indicates the impaired protein synthesis in the liver (Khan, *et al.* 1993).

From the previously mentioned results it can be concluded that, water in Bahar El Baker and milk obtained from cattle reared in Bahar El Baker contain high concentrations of heavy metals and induce adverse effect in biochemical parameters So, it is recommended that the government planing should include sanitary protection of surface water against heavy metal pollution including hygienic disposal of agricultural discharges, sewage and industrial effluents in Bahar El Baker region.

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