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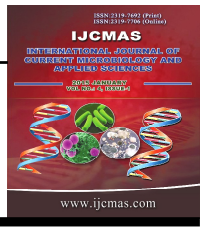

Original Research Article

Estimation of Cadmium and Cupper in Some Foods and Drinking Water in Iraqi Markets and Illustrated the Ultra Structural Alterations of its Ranged in Rats Spleen

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Keywords

Cadmium, Cupper, Foods, Drinking Water, Iraqi Markets, Rats Spleen

A B S T R A C T

This study was conducted to collection of five types of local foods samples which were Local Rashi (LR), Turkish Rashi (TR), Finger Pickle (FP), Acidic Pickle (AP), and Local Date syrup (LDS), at 15 trademarks for each types which package in polyethylene containers from Iraqi markets. and two types from drinking water ones as bottled water (BW) and the second was a home tap water (HTW), to estimate the concentrations of certain heavy metals Cadmium and Copper after three months of storage period of foods and drinking waters samples. Further to investigate the effect of orally dosage at contaminated levels from each cadmium at 0.0025, 0.005, and 0.01 µg/animal/day, and copper at 0.5, 1.0 and 2.5 µg/ animal/ day, on the ultrastructure changes in the Spleen tissues of rats groups, feeding 28 days. After processed the histological sections of the spleen tissues and examined under microscope. The results indicated that a significant ($p<0.05$) increases in concentrations of Cadmium and Copper, with increased in storage periods for three months, and were appear at last storage period at concentration ranged between 0.07 – 0.23, and 0.45 – 1.80 mg/l respectively in foods and drinking water samples. Also was shown from the histological of the spleen tissues image as the orally dosage concentrations of each cadmium and copper were caused a pathogenic effects in spleen, and the effects was increased with heavy metal concentrations increased, though they found a necrosis in the spleen cells, and the blood become congestion, furthermore, the lymphocytes cells was Infiltrated and occurred degeneration in spleen, in additions was showed to became swelling sinusoid, thickening reticulum and fibers of the spleen cells.

Introduction

Heavy metal pollution represent an important environmental problem due to the toxic effects of metal, and their accumulation throughout the food chain leads to serious ecological and health problem (Kadukova and Vircikova, 2005 and Jarup, 2003). Cadmium occurs naturally in ores together with zinc, lead and copper. Cadmium compounds are used as stabilizers in PVC products, color pigment, several

alloys and, now most commonly, in re-chargeable nickel– cadmium batteries. Metallic cadmium has mostly been used as an anticorrosion agent (cadmiation). Cadmium is also present as a pollutant in phosphate fertilizers, and sewage sludge, which, in all above contaminations sources the cadmium may be consumed directly by humans or transfer to farmland, may lead to contamination of soils, and to increased cadmium uptake by crops and vegetables, grown for human consumption. Therefor the food is was consider as the most important source of cadmium exposure (Kabata-pendias *et al.*, 1984). In addition, the cigarette smoking recently conducted as one of the major source of cadmium exposure, also, the industrial emissions (Staessen *et al*., 1999).

The adverse health effects of cadmium exposure may occur at lower exposure levels, primarily in the form of kidney as a tubular dysfunction and hepatic damage and anemia (Jeyaprakash and Chinnaswamy, 2005). The IARC has classified cadmium as a human carcinogen (group I) based on sufficient evidence in both humans and experimental animals (IARC, 1993).

Copper occurs naturally in the earth's crust in a variety of forms. It can be found in sulphide, carbonate, and silicate deposits and as pure 'native' copper (Ebong, 2008, Adoms, and Happiness, 2010). It as an essential nutrient that is incorporated into a number of metalloenzymes involved in hemoglobin formation, drug/xenobiotic metabolism, the cross-linking of collagen, and hair keratin, and the antioxidant defense mechanism. a recommended dietary allowance (RDA) of 0.9 mg/day (0.013 mg/kg/day) has recently been established. Exposure to excessive levels of copper can result in a number of adverse health effects including liver and kidney damage, anemia, immunotoxicity, and developmental toxicity (WHO, 1998 and Khan, *et al*., 2009). Recent studies in rabbits have suggested a link between copper in drinking water and Alzheimer disease (Guy, *et al*., 1999 and ATSDR, 2004).

Materials and Methods

Samples Collection: Five food samples and tow drinking water samples were purchased randomly from selected markets in Tikrit city, Sallahalldin governorate, Iraq. The samples were contains fifteen trade marks from each ones. The food samples types were as Local Rashi (LR), Turkish Rashi (TR), Finger Pickle (FP), Acidic Pickle (AP), and Local Tricle (LD), while The drinking water samples were as Home tap water(HTW) and Water Bottled Packaged (WBP).The samples were coded with appropriate letters and numbers and considering to be not damage and not swollen of packages, furthermore installed the brand production history and access, and transported to the laboratory then storage at 25 º c for three months periods (APHA , 1998).

Estimation of heavy metals: A 2.0 ml homogenous representative from food and drinking water samples was obtained and placed in conical beakers, the solutions were then covered with watch glasses, heated to near boiling, and refluxed for 15 min. After refluxing, the solutions were cooled and then 5 ml of concentrated HNO3 were added and the solution was again allowed to reflux for an additional 30 min. This last step was repeated to ensure complete oxidation of the metals. After the third refluxing period, the sample was cooled to room temperature and 2 ml of deionized water and up to 10 ml of 30% hydrogen peroxide were added. The samples were then filtered to remove any particulates that might interfere with FAA

analysis. The filtrates were collected in 100ml volumetric flasks and were diluted with deionized water to volume (Viñas,et al., 2000 and Tuzen, and Soylak,2007]. Heavy metals including cadmium, zinc, copper and Tin have been estimated in food and drink water samples for each months at storage period, using a Perkin Elmer Atomic Absorption Spectrometer Model E LCO. The optimal wavelengths was used for the FAA analysis of each metals, Impact bead was utilized to improve the sensitivity, and each samples were read three times. Calibrations were performed in the range of analysis, and a correlation coefficient for the calibration curve of 0.98 or greater was obtained. The instrument response was periodically checked with known standards depended the method in (AOAC, 2004 and Soylak,2007). Data of results were analyzed by the ANOVA analysis, using the general linear model of the Statistical Analysis System (SAS, 2001). Significant treatment differences were evaluated using Duncan's multiple-range test (Duncan, 1955). All statements of significance are based on the 0.5 level of probability.

Laboratory Animals Initialization

Seventy, 23(±3) day-old male growth (Albino-Sprague Dawley Rats) were individually weighed, wing banded and housed in heated battery brooders under 12 hours fluorescent lighting daily with feed and water provided *ad libitum.* Rats were fed the optimal formula according to (NAS-NRC, 2002). The experimental design consisted of seven dietary treatments by the orally dosage from each Cd and Cu metal: 1) Control with 0.0 metal/animal/day; 2) 0.0025 µg Cd/animal/day ; 3) 0.005 µg Cd/animal/day; 4) 0.01 µg Cd/animal/day; 5) 0.5 µg Cu/animal/day; 6) 0.1 µg Cu/animal/day; and 7) 2.5 µg Cu/animal/day; These were two replicates of five rats per dietary treatment and the rats were maintained on these treatments to 4 wks. of age. At 4 wks. of age, six rats from each treatment (3 rats from each replicate) were bled by cardiac puncture for histological of spleen tissues of rats groups and the tissue were processed according to Bancroft and Stevens, (1982), immediately the spleen organ was taken and fixed in 10% neutral buffered formalin solution for 18-22 hours at room temperature, and Dehydration was made by passing the tissue through progressively graded concentration alcohol bathes (70, 80, 90 and 100%), and clearing of the tissue from alcohol by using xylene, after clearing, they were passed through a mixture of xylene and molten paraffin wax (melting 56-58 ^{o} C) for 30 minutes and embedding in special stainless steel containers (molds), the tissue were removed from the paraffin bathes to the molds, as well the tissues were sectioning by Rotary microtome and section thickness used 5 µm. the staining was made using Haematoxylin and Eosin stains . the end mounting of section was prepared by using DPX; when cover the section by the cover slips. The histological slides were examination used the Microscope. The microscopic images were takes used the digital camera (Sony Co., Japan).

Result and Discussion

Estimations heavy metals contaminations of food and drinking water samples: The results in table 1.was illustrated the mean concentration of each Cd and Cu in locally food and drinking water samples after storage at 25 ^{o} c for three months period. The results were show that the Cd was non found in all samples in the first month, except in the Local Debbs which was appear at 0.01(mg/ml), while the Cu metal was founds in all samples in the first month storage period at concentration ranged

between 0.06 to 0.95 (mg/ml). The concentrations of each Cd and Cu in the second month periods was founds from Cd at 0.04 to 0.08 mg/ml while from the Cu at 0.09 to 0.85 mg/ml. The results in the third month storage period was shown the Cd and Cu concentrations were be increased significantly in all foods and drinking water samples, and the range of Cd concentration became at 0.19 to 0.23 mg/ml while for Cu at 0.37 to 1.64 mg/ml. The results were agreed with (Khansari, *et al*., 2005), whom presence of cadmium in canned tuna at 0.022 µg/g, and with (Suppin,*et al*., 2005), whom noted the cadmium at 0.014 mg/kg in canned and fresh fish consumed in Australia, and with (Kim, *et al*., 2008), who reported that the concentration of copper in some foods was greater than 0.1 µg/g, The amounts of the Cd and Cu in foods and drinking water samples were appear at high levels and don't agreed with the standard limited guide which at not accede to 0.005 and 0.02 to 0.1 mg/l respectively according to (WHO, 2003). The reason for increased heavy metals contaminations levels can be attributed to one or more factors which relation with transferring of metals from plastic toys structures to the foods or water samples, and the rate of transfer may depended on the environmental storage and foods and water samples compounds specially in the foods were the moisture percentage which was the most carrier of metals (ATSDR,1999, Onianwa, *et al*., 2001 and Aarnisalo, *et al*., 2005).

Histological studies: The microscopic examination results were showed the normal structure of spleen form weight pith, red pith, thickening reticulum and sinusoids (Fig. 1). The orally administration of 0.0025 µg/animal/day from cadmium were notes the difference effects at histological section spleen in rats as causes swelling sinusoid and necrosis , Degeneration and showed signals fiber (fig.2). While with the administrated of 0.005 µg/animal/day from cadmium were obtain the swelling, sinusoid, degeneration, congestion, infiltration lymphocyte as a lotus (fig. 3). When increased the orally dosage of Cd to 0.01 µg/animal/day they show as increased in thickness wall of blood vessels and there is congestion, swelling, sinusoid, necrosis and Degeneration appearance. The copper effects when orally dosage to rats at 0.5 µg/animal/day, was found swelling, sinusoid, congestion and increase in thickness of muscles septa or trabecular (Fig.5).The 0.1 and 1.0 µg/animal/day from Cu was caused to increase in thickness wall of in vessels blood wall, infiltration lymphocyte, necrosis and fimbriation of cells tissues of rats (Fig.6, 7).

The sign of the renal lesion is usually a tubular dysfunction, showed by an increased excretion of low molecular weight proteins [such as β2-microglobulin and α1-microglobulin (protein HC)] or enzymes [such as N-Acetyl-β-D-glucosaminidase (NAG)] (Jarup, *et al*., 1998 and Hotz, *et al*., 1999). These dysfunction was related for negative effects on the others organs specially the spleen which was related with the immunological functions. While the copper effects may be due to the analyze of proteins in response to the impact of copper to metabolism of proteins in the liver because of the most important functions of the liver's ability to manufacture some of the amino acids that necessary for building protein, and the spleen dysfunction was occur as results for these effects (Kadukova, and Vircikova, 2005).

Table.1 Mean cadmium and copper concentration in food and drinking water samples in selected Iraqi markets

Heavy metals types	Types of Local food samples	Concentration of Cd and Cuin food and drinking water samples (mg/ml)		
		First month	Second month	Third month
Cadmium	Local Rashi	ND	0.04^{a} ±0.001	0.22^{b} ±0.02
	Turkish Rashi	ND	0.02^{a} ±0.001	0.23^{b} ±0.02
	Finger Pickle	ND	0.08^{a} ±0.01	0.22^{b} ±0.002
	Acidic Pickle	ND	0.08^{a} ±0.01	0.22^{b} ±0.01
	Local Debbs	0.01^{a} ±0.001	0.06^{a} ±0.01	0.19^{b} ±0.01
	Home Tap Water	ND	0.04^{a}±0.001	0.21^{b} ±0.01
	Bottled water	ND	0.06^{a} ±0.01	0.20^{b} ±0.01
Copper	Local Rashi	0.06^{a}±0.001	0.09^{a} ±0.02	0.60^{b} ±0.01
	Turkish Rashi	0.36^{a} ±0.001	0.53^{b} ±0.01	1.64^{c} ±0.03
	Finger Pickle	0.31^{a} ±0.001	0.85^{b} ±0.01	0.85^{b} ±0.06
	Pickle Acidic	0.29^{a}±0.01	0.33^{a} ±0.01	1.30^{b}±0.01
	Local Debbs	0.21^{a}±0.001	0.54^{b} ±0.01	0.68^{c} ±0.01
	Home Tap Water	0.25^{a} ±0.01	0.29^{a} ±0.01	0.37^{b} ±0.01
	Bottled water	0.12^{a} ±0.01	0.16^{a} ±0.01	0.95^{b}±0.01

a-c: Values within rows with no common superscript differ significantly at 0.05.
ND: Mean not detected.

Fig.1 Spleen tissue cells of control group showed the weight pith (WP), red pith(RP) , thickening reticulum (Tr) and sinusoid (S) (H&E 400X)

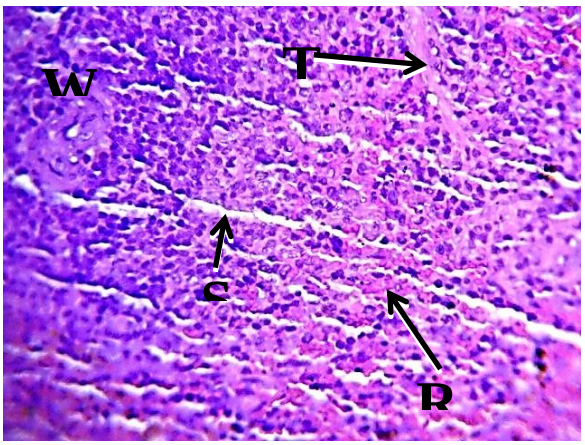


Fig 2 Spleen rat administrated of cadmium at concentration 0.0025 µg showed the Necrosis (N)&Degeneration (D) and Swelling the Sinusoids (SS) (H&E 400X)

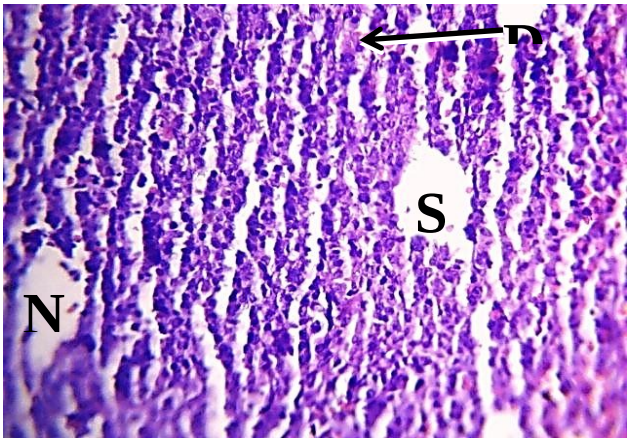


Fig.3 Spleen rat administrated of cadmium at concentration 0.005 µg showed congestion (CON) and Degenerations (D) (H&E 400X)

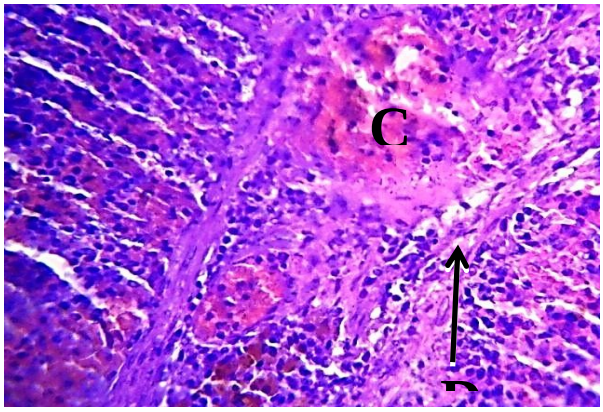


Fig.4 Spleen rat administrated of cadmium at concentration 0.001 µg showed Congestion (CON) & Necrosis(N) and Thickening the Wells (TW)

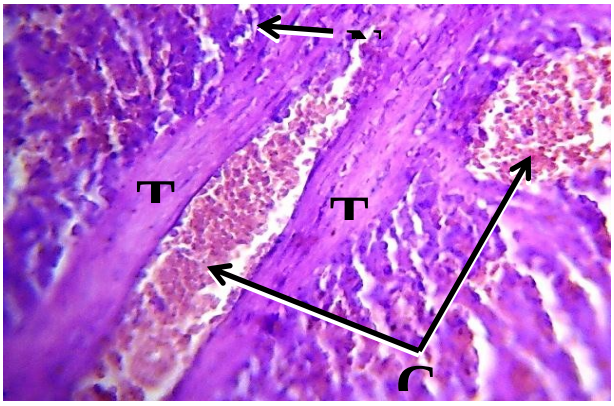


Fig.5 spleen rat administrated of copper at concentration 0. 5 µg showed the Necrosis (N) & Congestion (CON) and Swelling the Sinusoids(SS) (H&E 400X)

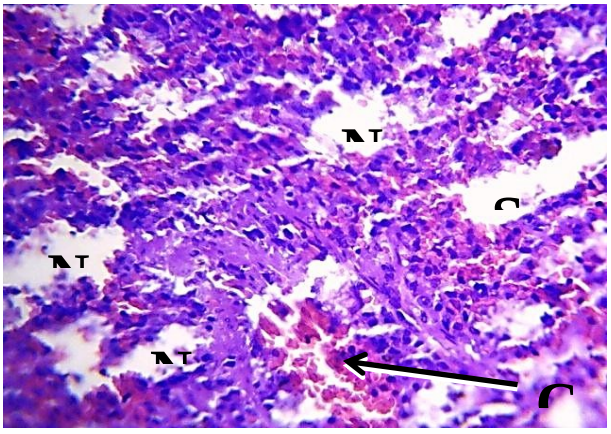


Fig.6 Spleen rat administrated of copper at concentration 1 µg showed the Degeneration (D) & Congestion (CON) and Thickening the Wells (TW) (H&E 400X).

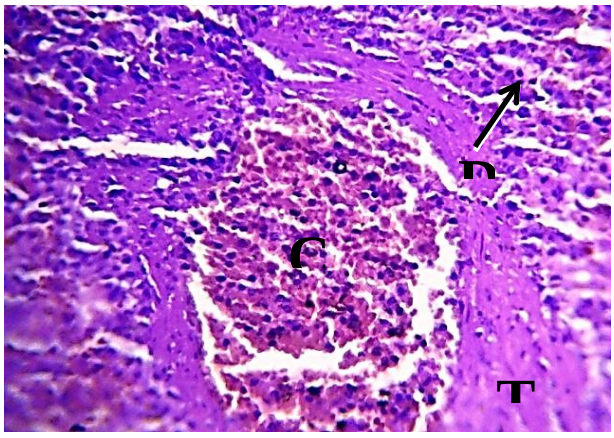
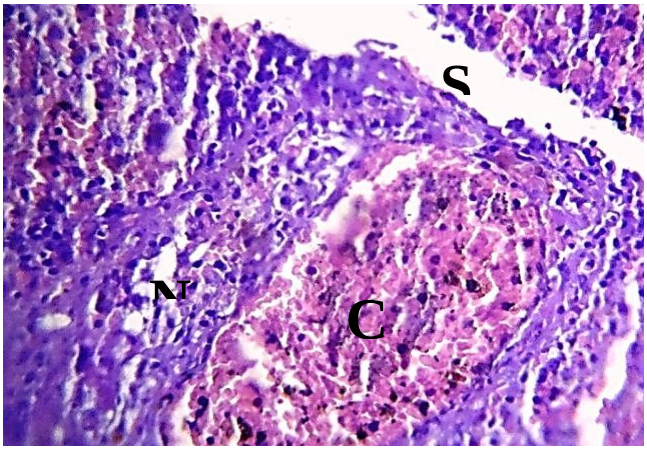


Fig.7 Spleen rat administrated of copper at concentration 2.5 µg showed the Necrosis (N)& Congestion (CON) and Swelling the Sinusoids(SS) (H&E 400X)



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