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Pathological and Biochemical Methods for Detection of Uranium Pollution in the Diyala-bridge Region, Iraq

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Abstract

Background: The present study comprised two distinct experiments. The first experiment involved a field survey conducted in the Diyala Bridge area of Baghdad, Iraq, spanning from December 2021 to May 2022. Soil, milk, and blood samples were collected from locally grazed cattle to ascertain uranium concentrations. In the second experiment, rats were subjected to poisoning with Uranyl acetate. Upon completion of the experiment, samples of liver, kidneys, and blood were collected for histopathological and biochemical analyses.

Methods: Twenty-four albino male rats were divided equally and randomly into two groups i.e., control group and intoxicated group. Uranyl acetate was given daily for 120 days by 0.05 mg/kg to the latter group. At the end of the experiment liver and kidney sample were taken for histopathological examination and stained by Masson-trichrome stain.

Results: The finding of eosin and hematoxylin stain and biochemical assay for CuZn-SOD U/mg and catalase K/mg protein were showed significantly elevated while liver tissue showed necrosis atrophy atherosclerosis cirrhosis and carcinoma characterized by irregular cell mitotic figure extensive fibrous tissue, tissue kidney presented interstitial hemorrhage and multiple granuloma.

Conclusion: Intoxicant by Uranyl acetate showed significant elevating for CuZn-SOD U/mg and catalase K/mg protein, in contrast liver tissue showed necrosis atrophy atherosclerosis cirrhosis and carcinoma, tissue kidney presented interstitial hemorrhage and multiple granulomas.



Introduction

Pollution has been a global problem for a long time yet just began to be critical after the biotransformation of chemical material like dioxin radioactive pollutant, etc. [1]. Uranium occurs naturally in very small amounts in rocks, soils, water bodies, plants and animals. The history and use of uranium reflect advances in mining, chemistry and physics, and in our understanding of radioactive substances and their potential applications [2]. Earth's crust contains Uranium as a natural component, and it exists naturally in oxidative state [3]. Uranium reach human via food like table salt vegetables cereals which transfer from soil and pollutant soil to plant and human or animals' product mostly (milk, Eggs, meat and fish meat) [4, 5]. Uranium forms are soluble complexes with bicarbonate, citrate or proteins in the plasma when immediately absorbed from tissue and bone [6]. And is directly harmful to the lungs and kidneys [7] and its compounds cause changes in the kidney, lung, liver, nerves, circulation, reproductive system, hemopoietic, DNA deformation and decrease bone development and its known carcinogenic and genotoxic agents [8, 9]. Uranyl nitrate molecular formula $UO_2(NO_3)_2 \cdot 6H_2O$ with molecular weight 502.13 is a water soluble salt it's a radioactive and oxidizing compound composed of yellow bipyramidal rhombic crystals it easily absorbed by human pulmonary, cutaneous and GIT routes [10&11].

One of the major sites of Uranium pollution in Iraq is AL-Tuwaitha nuclear research site which is located [18] km South west Baghdad and in the Diyala Bridge areas (AL-Arifi and Altaamim Distract) and at Gulf War in 1981 was destroyed and mostly Nowruz reactor in AL-Tuwaitha nuclear research [12].

Methods

Sample collection

Collection of the soil samples

Randomly 100 soil samples were collected from the lands of Diyala Bridge area like (AL-Tuwaitha, AL-Arifi and Altaamim Distract) Soil samples about 500gm were collected by cutting out the soil (50-100)cm depth then the samples saved in glass containers for laboratory test. Soil samples in laboratory were grinding in mortar and Sieve in fine mesh about 75 μ m then the soil powder after homogenized about 1 gm mixed with 0.2 gm. of methylcellulose then pressed using a piston into a pellet with dimension of 1 cm. diameter and 1.5 mm thickness these pellet to make a pair were covered with CR-39 debtor on both sides [13, 14].

Cattle blood collection

Hundred blood samples were collected from both sexes randomly from local grazing cattle in Diyala Bridge area and blood about 10 CC collected from cattle

jugular vein, labeled and saved in heparinized tube in cold box for Uranium detection [13, 14].

Collection milk sample

Collection from female cattle 100 milk samples were collected daily from the cattle above and area two drops of milk by drying milk and left square piece of CR-39 detector at room temperature for Uranium detection sample save in glass container [13, 14]. Natural Uranium stander samples were prepared according to (IAEA) International Atomic Energy Agency [13, 14]. The experiment analysis above was done in the Ministry of Science and Technology in Baghdad, Iraq.

Experimental animals

The doses of uranyl acetate (0.05) mg/kg body weight were determined. of uranyl acetate according to [15] and is administration orally. Twenty - four albino male rats aged (120) days and body weight (200-250) gm. were housed in sterilized polypropylene cages (120*120*30) cm^3 in the animal house of the department of Pathology, Collage of Veterinary Medicine, Baghdad University, Iraq, in twelve hours dark light cycle with (21 $^{\circ}$ C) ambient Celsius degree temperature. All rats were given normal rat pellet and drinking water ad libitum [16]. All animals were under identical management protocol and guide line of Institutional Animal Ethics Committee.

Intoxication of rats

Twenty - four albino male rates were divided randomly and equally into two groups: -1st group (12) animals act as control group (C) received normal rat diet and water ad libitum while 2nd group (12) albino male rats daily and orally administration (0.05)mg/kg B.W. of Uranyl acetate for 120 days. Uranyl acetate dihydrate $UO_2(CH_3COO)_2 \cdot 2H_2O$ (Purity 99%) was obtained from AccuStandard Inc (New Haven, Connecticut, USA). All rats scarified under ethics protocol under ether anesthesia at 120 days for biochemical and pathological examination.

Blood collection

Cardiac puncture technique on fasting blood were collected (3 ml) and then blood left (15) minutes to stand and coagulate in refrigerator then for (15) minutes at (500) rpm in centrifuge. Serum was separated and kept at (-20) $^{\circ}$ C till assay.

Biochemical assay

All below chemicals (purity \geq 99%) were obtain from Sigma chemical Co.(St. Louis, Missouri, USA) and were of analytical grade or of the highest grade available and were done in laboratory of Science and Technology Ministry, Baghdad, Iraq.

- a. **CuZn-SOD (Iu/mg) activity** [17] was measured by inhibition of nitro blue tetrazolium (NBT) reduction due to O₂ generation by the xanthine / xanthine oxidase system and marked by spectrophotometer at (560nm).
- b. **Catalase (CAT) activity (k/mg)** [18]. The enzymatic decomposition of H₂O₂ followed by decrease in absorbance at (240nm).
- c. **Serum creatinine (mg/dl)** [19].

Histopathological change

At days (120) all animals groups were sacrificed and liver and kidney were carefully dissected from abdominal cavity then immediately fixed in 10% neutral buffered formalin for routine histopathological technique [20]. All tissue stained with Masson trichrome stain for fibrous connective tissue detection and eosin and hematoxylin stain.

Statistical analysis

All the groups' data were statistically read by SPSS program, version 17 software (2010). Including one way ANOVA for comparisons among groups. P value of less than ≤ 0.05 were considered statistically significance, all data were expressed as means \pm standard error (SE) [21].

Results

Determination of Uranium soil (mg/kg), blood and milk (mg/L)

Uranium concentration in milk (0.43 ± 4.22) mg/L, in blood (0.55 ± 4.22) Mg/L and in soil (6.23 ± 0.54) mg/kg samples were showed significant increased ($P \leq 0.05$) when compare to minimum acceptable Uranium concentration in milk (1.2 ± 0.00) Mg/L, in blood (1.8 ± 0.00) Mg/L and in soil (2.8 ± 0.00) mg/kg

Biochemical assay

In current study the level of SOD, CAT and protein in 2nd group which administrated (0.05) mg/kg B.W showed significant decreased $p \leq 0.05$ in CuZn-SOD U/mg protein (255.0 ± 16.2) when compared with control group (370.0 ± 12.0) U/mg protein.

Catalase k/mg protein in 2nd group decreased significantly ($p \leq 0.05$) (4.50 ± 0.3) when compared with control group (8.60 ± 0.4) k/mg.

Serum concentration of creatinine (mg/dL) indicated significant increased $p \leq 0.05$ in Uranium group when compared to control group.

Histopathological Examination

No important histopathological lesions were observed in livers and kidney of 1st group (control group), while

2nd group at (120) days livers showed hepatic cells atrophied with increased width of sinusoids figure (1).

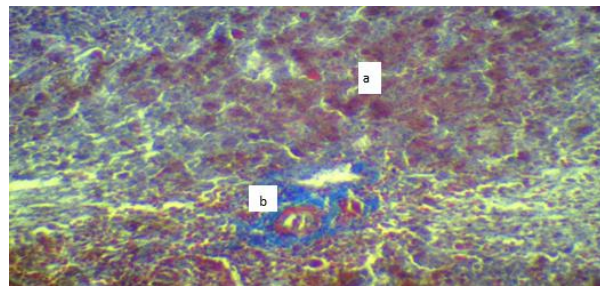


Figure 1: Histopathological lesion of liver (uranyl acetate 0.05mg/kg B.W group) at day (120) showed: a-hepatic cells atrophied b-mononuclear cells aggregation around central vein. X400 (Masson- trichrome stain).

In some areas multiple irregular nodules consist of center of necrosis and hemorrhage surrounded by different types of mononuclear cells mostly macrophages, lymphocytes also surrounded by giant cells and fibrous connective tissue causing damage most layers of hepatocytes with dense blue fibrous connective tissue figure(2).

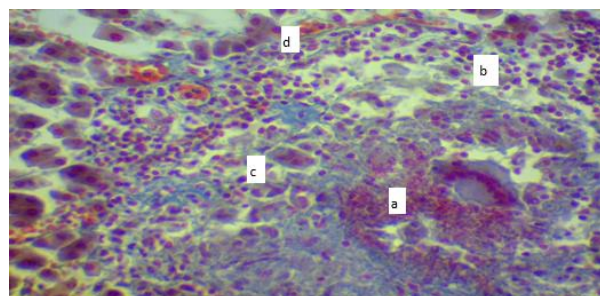


Figure 2: Histopathological lesion of liver (uranyl acetate 0.05mg/kg B.W group) at day (120) showed: a-irregular nodules of necrosis and hemorrhage b-different types of mononuclear cells c- giant cell d-fibrous connective tissue X400 (Masson- trichrome stain).

Granulomatous areas showed increase in the fibrous connective tissue with blue coloration, other liver cells consist a huge amount of leukocytes mostly eosinophil's and lymphocytes, collapsed of all arteries with sever atherosclerosis figure (3). Liver cirrhosis characterized by hyperplastic parenchymal nodules separated by fibrous bands with distortion of the normal liver architecture with moderate infiltration of mononuclear cells with newly bile duct fig(4). Kidney section in 2nd group presented sever acute cellular swelling, sever interstitial hemorrhagic with granuloma formation consist of (necrotic center surrounded by foreign body giant cells) Fig (5).

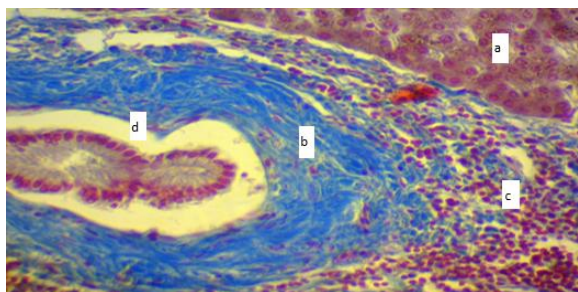


Figure 3: Histopathological lesion of liver (uranyl acetate 0.05mg/kg B.W group) at day (120) showed: a-liver necrosis b-thick fibrous connective tissue c-extensive leukocytes mostly eosinophil and lymphocytes d-atherosclerosis X400 (Masson-trichrome stain).

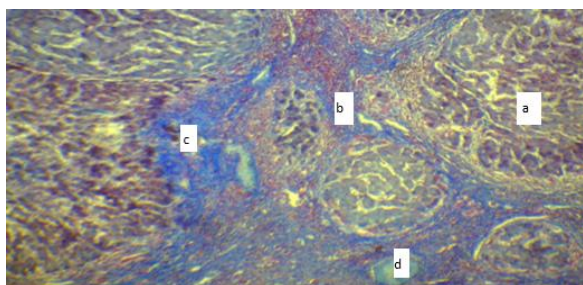


Figure 4: Histopathological lesion of liver (uranyl acetate 0.05mg/kg B.W group) at day (120) showed a-hyperplastic parenchymal nodules b-thick fibrous tissue c-mononuclear cell infiltration d-newly bile duct X400 (Masson-trichrome stain).

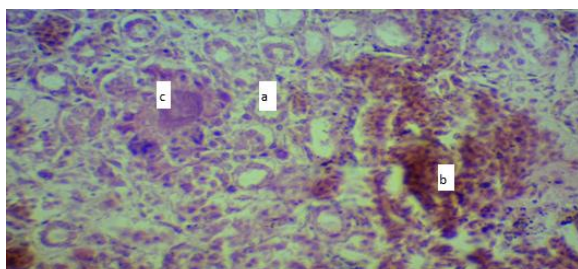


Figure 5: Histopathological lesion of kidney (uranyl acetate 0.05mg/kg B.W group) at day (120) showed: a-acute cellular swelling b-sever interstitial hemorrhage c- foreign body giant cell granuloma X 200 (H&E stain).

Discussion

Our results showed significant increase in Uranium concentration in soil, milk and blood of cattle when compared to minimum accepted uranium concentration. Uranium accumulation in Diyala bridge area like (AL-Tuwaitha), AL-Arifi and Altaamim) District due to war (1981) and terrorism [22] also gulf war resulting in presence radioactive of Uranium in soil surface and the invade the deeper layer of ground [23]. The bioaccumulation of soil uranium in the above areas that act as potentially long-term source of food contamination because Uranium half-life(4.5×10^9) years [24], when ingested randomly by cattle which grazing in these contaminated areas accumulate in

milk, blood, meat and different tissue like kidney, bone, brain and mostly liver [25]. The current study showed significant decreased in CuZn-SOD and catalase with in creatinine which induction of oxidative stress in tissue and may be due to lipid peroxidation causing increase cell fluidity and cell death due to oxidative stress and free radical [26]. Liver is often show by toxic effects of xenobiotic due to its ability to accumulate xenobiotic by active transport [27]. Uranium has ability to accumulate in hepatocytes and cause toxicity [28 and 29], by induce oxidative stress in mitochondria or lysosome [30]. In rats hepatocytes uranium is potential oxidative marker including reactive oxygen species (ROS) formation and generation excessive (ROS) in liver. Liver cirrhosis is the global lesion occurs due to liver damage from toxin which causes losses of liver function in human and animals [31]. Formation of pseudolobules without central vein surrounding by fibrous connective tissue (cirrhosis) to maintain regeneration of hepatocyte due to Uranium mutagenicity effect of uranium genetic mutation causing liver carcinoma and such effects can include genetic mutations generation of (ROS) lead to liver carcinoma [32]. Chronic toxicity due to Uranium exposure experimental due to excreted via kidneys especially in proximal convoluted tubules causing renal tubular epithelial degeneration, necrosis with inflammatory cells infiltration and increased serum creatinine due to brush cell injury and alteration in cells and lysosome membrane with injury to mitochondria leading to impaired energy and altered calcium ion hemostasis, uranium induction cellular oxidative stress [33], by increased lipid peroxidation and depletion of antioxidant enzyme[34], causing vacuolation mesangial and epithelial cells of glomeruli, dilated renal tubules, interstitial fibrosis with mononuclear cells infiltration[35], with fibroblastic proliferative by produce protein like collagen I, IV and fibronectin-growth- factor β (TGF- β) and platelet-derived growth factor (PDGF) [36].

Author Contributions

Hashim M. Obaid: Research article, funding the acquisition and experiment design.

Bushra. I. al. Kaisi: Preparing materials, explain the finding, statistical analysis, review and editing.

Conflict of Interest

The authors declare that there is no conflict of interest.

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