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NEUROTOXICITY OF URANYL ACETATE IN MALE RATS

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Abstract

The present investigation included the neurotoxic effect of different doses of uranyl acetate in male rats. Routine light and electron microscopic techniques were used in this study. The histological studies of cerebellum and cerebrum showed a significant increase of died and decrease of healthy pyramidal cells in the 3rd layer of cerebrum and Purkinje cells in the cerebellum. This may indicate the passage of uranium across the blood brain barrier or disruption of this barrier due to uranium exposure..

Keywords: **Cerebellum. Cerebrum, Purkinje cells, uranyl acetate**

Introduction

Uranium , a naturally occurring heavy metal, is found in all soils, water, and rocks (Craft *et al*.,2004).The release of uranium into the environment presents a threat to human and ecological health in many parts of the world (Murray *et al*.,2002). Its extensive use in the nuclear cycle and for military applications has focused attention on its potential health effects (Carriere*et al*.,2004). It penetrates through lung tissue and by ingestion to the blood stream and can be stored in the liver, kidney ,or other tissues for years (Patolka*et al*.,2004;Carriere*et al*., 2005; Li *et al* .,2005; Paquet*et al*.,2006).Other important possible targets of uranium exposure are bone (Kurttio*et al*.,2005) and brain in which it is transferred across the blood brain barrier (BBB) (Lemercier*et al*.,2003; Lestaevel*et al*., 2005; Paquet*et al*.,2006).Uranium was found to induce behavioral effects in rats(Belles *et al*.,2005).

Uranium given as uranyl acetate added to the mice chow showed highest accumulation in the brain compared to liver and kidney(Ozmen and Yurekli 1998). Genetic and histological effect of uranium in fish brain was detected by Lerebours*et al.*, (2010).

The aim of the current work was to study the neurotoxic effect of uranium as uranyl acetate in rat.

Material and Methods

Thirty male albino rats (***Rattusnorvegicus****)* weighing (**230 - 250 gm**) and 8-10 weeks old were used in the present study. The animals were bred and housed in plastic cages (56 x 39 x 19 cm) bedded with wooden chips in animal house / Biology Dept. /College of Science /Salahaddin University-Erbil. Temperature was set as (**22 ± 2 C**).Regular 12-hours diurnal cycles were kept using an automated light-switching devise. The animals were supplied with water and standard diet ***ad libitum.***

The studied rats were divided into 5 groups (six in each). Four groups were given daily oral aqueous doses (5 , 10,20,and 40 mg/kg b.w.) of uranyl acetate dehydrate (UAD) (Fluka AG , Buche SG)for 30 days against a control group which only received water.

Light microscopy

Samples of cerebellum and cerebrum were removed from the anesthetized animals . The samples were fixed in formol saline. All samples were processed for light microscopy by embedding in paraffin after dehydration and clearing. Four micrometers thick sections were stained by hematoxylin and eosin(Bancroft et al.,1977).

Electron microscopy

Tissue samples were fixed in 2.5% glutaraldehyde in 0.1 cacodylate buffer pH 7.2-7.4 for 24 h ,postfixed in 1% osmium tetroxide for 2 h , dehydrated in ethanol, cleared in propylene oxide , and embedded in araldite mixture. Plastic blocks were sectioned by ultramicrotome (Riechert co.) into 1µm and 600-900A° thick sections for light and electron microscopy respectively. The ultrathin sections were mounted on copper grids. One micrometer thick sections were stained by toluidine blue ,while the ultrathin sections were stained by uranyl acetate and lead citrate(Veeramachaneni*et al*., 1993) . Jeol TEM120 ll transmission electron microscopy (at Kuwait University/Medical College)at 80Kv was used for examining and photographing the ultrathin sections.

For counting dying and healthy cells per millimeter square, five sections through each of the cerebellum and cerebrum were employed. All the measurements were performed using a Lietz microscope equipped with standardized square. The area of this square was calculated under oil immersion lens using stage micrometer and it was equal to 0.005184 $mm^{2.}$. The total number of pyramidal cells of the 3^{rd} layer of cerebral cortex and the Purkinje cells of cerebellum (healthy and died cells) were counted per standardized square and transformed to the number per square millimeter of counted region. The above procedure of counting cells was achieved according to the counting procedure of (Abdel-RahmanandZeki 1992).

Results

The total number of pyramidal cells of the second and third layer of cerebral cortex and Purkinje cells of the middle layer of cerebellum/mm^2 showed significant decrease ($p<0.05$) in the UA treated group compared to control. On the other hand, the number of died pyramidal and Purkinje cells/mm^2 was significantly increased ($p<0.05$) compared to control in a dose dependent manner (Fig 1 and 2respectively and Table 1).The mode of cell death of both cells was apoptosis-like mode of cell death(Fig 3) in which the cells appeared shrunken and nucleus condensed or fragmented.Electron microscopic figures revealed different degeneration levels of pyramidal and Purkinje cells (Fig 4& Fig 5 respectively). Some cells appeared died according to the programmed cell death in which the cell appeared shrunken and many apoptotic vacuoles were seen around the died cell (Fig 5).

Table(1): Effect of different doses of uranyl acetate on brain

Doses mg/kg/d	Total pyramidal cells/mm^2	Died pyramidal cells/mm^2	Total Purkinje cells/mm^2	Died Purkinje cells/mm^2
0	2228.33±3.59 ^{a}	35.33±2.91 ^{a}	689.00±4.90 ^{a}	4.33±0.13 ^{a}
5	2182.00±3.73^{b}	364.67±3.02 ^{b}	630.50±1.26 ^{b}	60.67±1.76 ^{b}
10	2109.00±5.91^{c}	465.20±5.20 ^{c}	578.80±2.81 ^{c}	90.40±1.21 ^{c}
20	1921.67±3.66^{d}	514.67±3.92 ^{d}	505.83±2.68 ^{d}	156.00±3.63 ^{d}
40	1730.29±3.97^{e}	704.29±4.06 ^{e}	464.14±3.79 ^{e}	201.86±2.57 ^{e}

Values in vertical columns followed by a different letter aresignificantly different at the 1% level. N=6 animals per group.

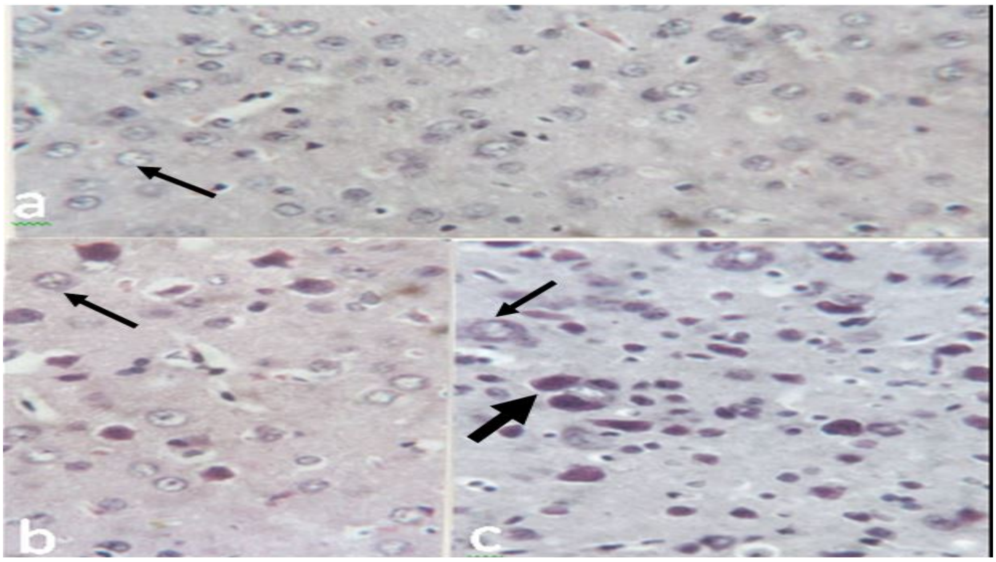

Fig 1: Plastic sections of rat cerebral cortex showing pramidal cells in the 3rd layer of the cortex , a)control section ,b)10mgUA/kg/d treated ,c) 40mg UA/kg/d treated rats (All mag.were 1000X),small arrow=healthy cells,large arrows =died cells

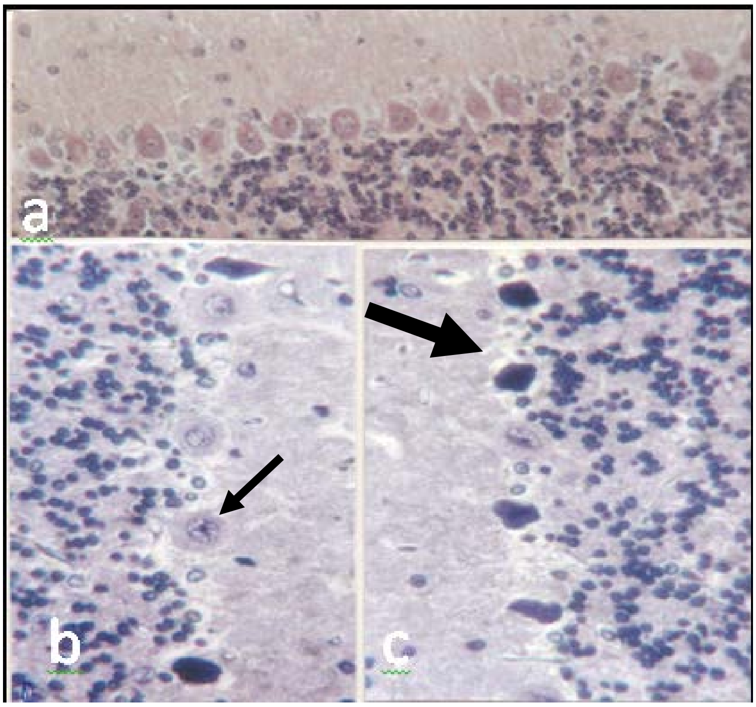

Fig 2 : Cerebellar cortex of UA treated rats a)Paraffin section of control b)5mgUA/kg/d treated group c)40mgUA/kg/d treated group.Allmag.were 400X.Notice healthy Purkinje cells(small arrows)and died Purkinje cells(large arrows)

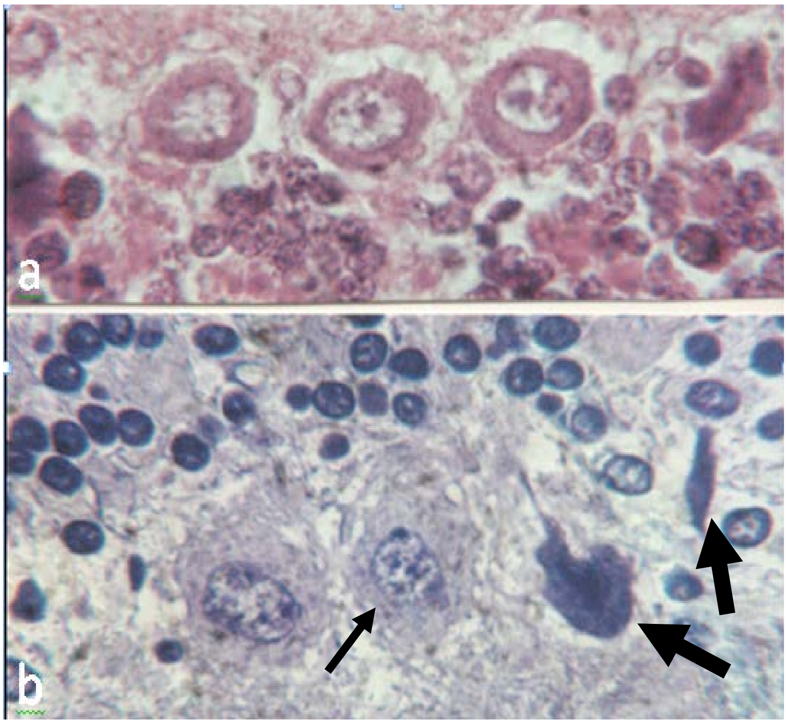

Fig 3: High magnification of Purkinje cells in the a)control,b)10mgUA/kg/d treatedrats showing healthy (small arrows)and died Purkinje cells(large arrows) ,both are 1000X.

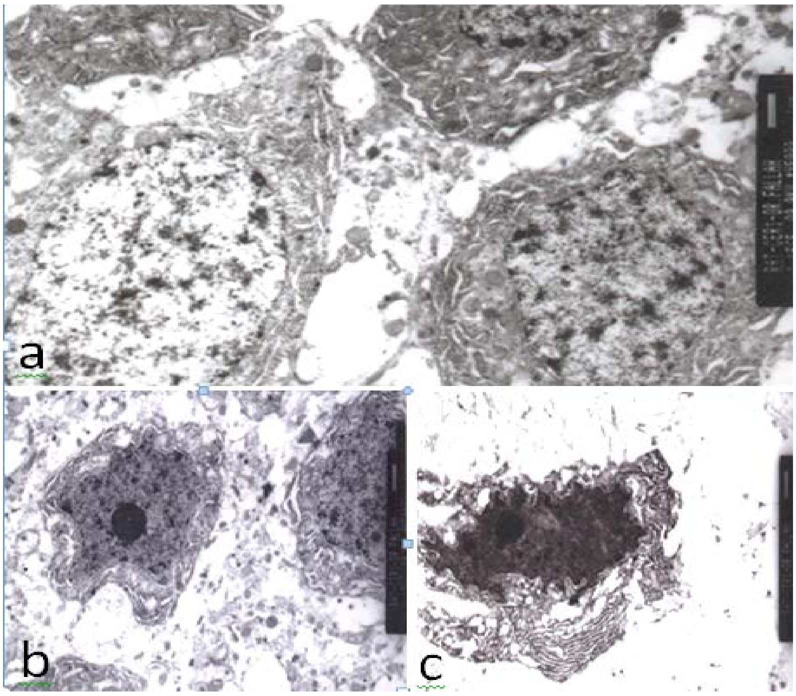

Fig 4: Electron micrographs of pyramidal cells in the UA treatedrat cerebral cortex showing a)normal appearance bar=1µm , b)bar=1µm & c) degenerated shrunken cell .bar=1µm

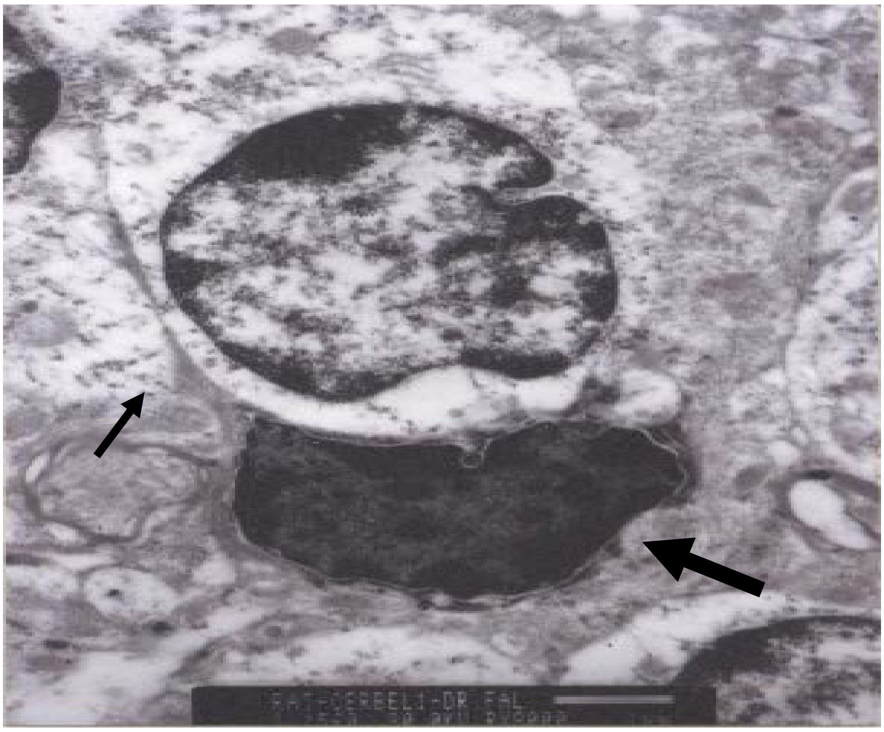

Fig 5: Electron micrograph of UA treated rat cerebellar cortex showing the healthy (small arrow)and died (large arrow) Purkinje cells bar=5µm.

Discussion

The present work detected a significant decrease of healthy pyramidal and Purkinje cells/mm^2 which may be due to the degenerative effect of uranium on these cells. This degenerative effect was reflected by the significant increase of died neuronal cells. The mode of cell death of these cells was apoptotic-like cell death. These effects indicated that this metal may cross the blood-brain barrier(BBB).This crossing was suggested by several studies which confirmed the accumulation of uranium in the brain of animals after uranium ingestion through natural uranium ingestion (Lemercier*et al*.,2003) , uranyl acetate uptake(Ozmen and Yurekli, 1998; Barber *et al*.,2005;Briner and Murray 2005) , uranyl nitrate ingestion (Paquet*et al*.,2006)implanted uranium pellets (Pellmar*et al*.,1999)or injected depleted uranium (DU) (Lestaevel*et al*.,2005). The latter paper referred to a dose dependent increase fashion of uranium in the rat brain with the number of implanted DU pellets and this may explain our results with respect to the dose dependent decrease of pyramidal and Purkinje cells/mm^2 and increase of died cells.The latter effect may also be due to the oxidative stress caused by uranyl acetate treatment.Such oxidative stress through free radicals production has been reported in mammals and other vertebrates such as fishes (Gagnaire*et al*.,2013). Oxidative stress can induce neuronal cell death in a variety of circumstances (Halliwell, 1992).On the other hand ,Abou-Donia*et al*.,(2002) suggested that DU could induce an increase in the generation of nitric oxide in the cortex of the animals treated with 0.1 mg/kg uranyl acetate. The latter compound produced in the CNS is a highly reactive species that has been implicated in a variety of neurodegenerative diseases (Gobbel*et al*.,1997; Squadrito and Pryor,1998;Bogdan, 2001). Furthermore, it is also possible that the long-term health effects following exposure with uranyl acetate may be a consequence of changes in the BBB permeability(Abuo-Donia*et al*.,2002). Briner and Murray (2005)detected behavioral effect and lipid oxidation in the brain of rats related to the amount of DU exposure. Lipid oxidation may alter ionic conductance, cell membrane fluidity, or a number of other cellular functions(Schaich 1992). As a conclusion, uranyl acetate may cause severaldose dependent neurotoxic effectas a result of neurocytes degeneration in the exposed rats.

References

Abdel-Rahman M., andZeki TZ, (1992). Cytotoxic action of malathion on renal and hepatic tissues of mice.*J.Egypt.Germ.Soc.Zool*.8B:105-114.

Abou-Donia M.,Dechkovskia A. M.,GolsteinL.B.,Shah D.U.,Bullman S.L., and Khan W.A. (2002):Uranyl acetate-induced sensorimotor deficit and increased nitric oxide generation in the central nervous system in rats, *Pharmacology,Biochemistry Behavior*,72:881-890

Bancroft JD, Stevens A, and Dawson IMS. (1977):Theory and Practice of Histological Techniques. Edinburgh, London, New York. Churchill-Livingstone.

Barber DS,Ehrich MF, and Jortner BS.(2005):The effect of stress on the temporal and regional distribution of uranium in rat brain after acute uranium acetate exposure.*Toxicol.Environ.HealthA*.68(2):99-111.

BellesM.,AlbinaM.L.,LinaresV.,GomezM.,Sanchez D.J., and Domingo J. (2005): Combined action of uranium and stress in the rat I.Behavioral H effects, *Toxicology Letters*,158:176-185

Bogdan C. (2001):Nitric oxide and the regulation of gene expression. *Trends Cell Bio*.(11):66-75

Briner W., and Murray J. (2005):Effects of short-term and long-term depleted uranium exposure on open-field behavioral and brain lipid oxidation in rats,*Neurotoxicol. and Teratol*.27:135-144.

Carriere M,Gouget B.,Gallien J.,Avoscan L.,Gobin R.,Verbavatz J. and Khodja H. (2005):Cellular distribution of uranium after acute exposure of renal epithelial cells:SEM,TEM and nuclear microscopy analysis.*Nuclear*

Instrument and Methods in Physics Res.Sec.B.231(1-4):268- 72.

Carriere M.,AvoscanL.,CollinsR.,Carrot F.,Khodja H.,Ansoborio E., and Gouget B. (2004):Influence of uranium speciation on normal rat kidney (NRK-52E) proximal cell cytotoxicity,*Chem Res Toxicol*.17(3):446- 452.

Craft E.S.,Abu-Qare A.W.,Flahert M.M.,Garofolo M.C., and Rincavage H.L.(2004): Abou-DoniaM.B.Depleted and natural uranium :Chemistry and toxicological effects.*J. Toxicolo. Environ. Health. Part* B.7:297-317

Gagnaire B, Cavalie I, Camilleri V, and Adam-Guillermin C. (2013): Effects of depleted uranium on oxidative stress, detoxification, and defence parameters of zebrafish*Daniorerio*. *Arch Environ ContamToxicol*. 64(1):140-50.

GobbelGT,Chan TYY, and Chan PK, (1997):Nitric oxide- and superoxide-mediated toxicity in cerebral endothelial cells.*J.Pharmacol Exp. Ther*.,282:1600-1607.

Halliwell B. (1992):Reactive oxygen species and central nervous system.*J. Neurochem*.(59):1609-1623.

Kurttio P.,Komulainen H.,Leino A., Salonen L., Auvinen A., and Saha H. (2005): Bone as a possible target of chemical toxicity of natural uranium in drinking water.*Environ.Health prospect*,113(1):68-72.

Lemercier V.,Millot X.,Ansoborio E.,MenetrierF.Flury-Herard A.,RousselleCh.,andScherrmann J.M. (2003):Study of uranium transfer across the blood-brain barrier.*Radiat. Protect. Dosim.* 105:243-245.

Lerebours A, Bourdineaud JP, van der Ven K, Vandenbrouck T, Gonzalez P, CamilleriV, Floriani M, Garnier-Laplace J, and Adam-Guillermin C.(2010): Sublethaleffects of waterborne uranium exposures on the zebrafish brain: transcriptional responses and alterations of the olfactory bulb ultrastructure. *Environ Sci Technol.* 15;44(4):1438-43.

LestaevelHoupert P.,Bussy C.,Dhieux B.,GoumelonP.,andPaquet F. (2005:The brain is atarget organ after acute exposure to depleted uranium*Toxicol*. 212(2-3):219-226

Li WB.,RothP.,Wahl W.,Oeh U.,Hollriegl V., and Paretzke Hg(2005):Biokineticmodeling of uranium in man after injection and ingestion,*RadiatEnviron. Biophysic*,44(1)29-40.

Murray VSG,BailelMR,Spratt BG. (2002): Depleted uranium:a new battlefield hazard.*Lancet*360:31-32.

OzmenM.,andYurekli M. (1998):Subacute toxicity of uranyl acetate in Swiss-Albino mice.*Environ. Toxicol.Pharma*.6:111-115.

PaquetF.,HoupertP,BlanchardonE,DelissenO, MaubertC,Dhieux B, MoreelsAM,Frelon S, and Gourmelon P. (2006): Accumulation and distribution of uranium in rats after chronic exposure by ingestion, *Health physics*, 90(2)139-147.

PatollkaJ,KassaJ,StilltinaR,Safr G, and Havei J. (2004):Toxicological aspects of depleted uranium, *J App.Biomed*.2:37-42.

PellmarTC,FuciarelliAF,EjnikJW,HamiltonM ,HoganJ,Strocko S, EmondC,Monttaz HM, and Landauer MR. (1999):Distribution of uranium in rats implanted with depleted uranium pellets.*Toxicol Sci*.(49):29-39.

Schaich K.M. (1992):Metals and lipid oxidation.Contemporaryissues,*Lipids* 27(3):209-218.

SquadritoGL., and Pryor WA. (1998):Oxidative chemistry of nitric oxide:the roles of superoxide,peroxyntrite,carbondioxide. *Free Rad. Biol.Med*, 25:392-403.

Veeramachaneni, D. N. R., Moeller, C. L., Pickett, B. W., Shiner, K. A., and Sawyer, H. R. (1993):On processing and evaluation of equine seminal samples for cytopathology and fertility assessment: The utility of electron microscopy. *J. Equine Vet. Sci*.**13,** 207–215.

التاثيرات السمية العصبية لخلات اليورانيوم في ذكور الجرذان

الخلاصة

تضمن البحث الحالي دراسة التاثير العصبي للجرعات المختلفة لخلات اليورانيوم في ذكور الجرذان. واستخدمت طرائق تقليدية مجهرية ضوئية والكترونية لدراسة الخلايا العصبية في المخ والمخيخ. أظهرت الدراسات النسيجية زيادة ملحوظة في عدد الخلايا العصبية الهرمية الميتة في الطبقة الثانية والثالثة لقشرة المخ في المجاميع المعاملة بخلات اليورانيوم، بينما لوحظ انخفاض في عدد الخلايا السليمة من النوع نفسه. ولوحظت النتيجة نفسها فيما يخص خلايا بركنجي الموجودة في المخيخ وكانت تلك التغييرات في كلا النوعين من الخلايا طردية مع زيادة جرعة خلات اليورانيوم. قد تشير هذه النتيجة الى اختراق عنصر اليورانيوم الحاجز الدموي الدماغي.

کاریگەری `ژەهراوی دەماری سرکەی یۆرانیۆم لە جرجي سپیدا

پوختە

ئەم توێژینەوەیە لێکۆڵینەوەی کاریگەری دەماری ژەمی جۆراوجۆری سرکەی یورانیۆم لە جرجي نێر. لەم توێژینەوەیەدا، رێگای پشکنینی شانەزانی میکرۆسکۆبي رووناکی وئەلەکترۆنی بەکارهێنرا بۆ دیراسەتکردنی دەمارخانەکانی مێشك و مێشکۆڵە. توێژینەوەکە دەریخست کە ژمارەی دەمارەخانە مردووەکانی توێژی مێشك لە زیادبووندابوو لەو گروپانەی کە سرکەی یۆرانیۆمی پێدرا، بەڵام خانە ساغەکان بەرەو کەمبوون چوون. هەمان ئەنجام بۆ خانەکانی پرکنجی مێشکۆڵە دەست کەوت. ئەو گۆڕنکاریانە لەگەڵ زیادکردنی ژەمەکانی سرکەی یۆرانیۆم تەریب بوو. ئەم ئەنجامە لەوانەیە ئەوە دەربخات کە توخمی یۆرانیوم بە بەربەستی خوێن ومێشك تێپەڕ بێت.