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The Cytogenetic and pathological effects of toluene on female Mice

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Abstract

The study objective was to explore the effects of toluene on cytogenetic status incluin mitotic index, blast index, chromosomal aberration and DNA damaged, one hundred eighty adult female albino mice. They were selected randomly, equally, and then allocated into three bunches each was divided into three groups to represent control group, group 1 (G1) and group 2 (G2) administration from (LD50). These were administrated orally and continuously for 60 days with toluene at doses of 0.1 ml / kg B.W. ,and 0,2 ml / Kg B.W. Control group received olive oil and distilled water, G1 oral administration from LD50 daily for 30 days and G2 oral administration from LD50 daily for 60 days. Fasting blood samples collected by cardiac puncture technique at 0, 30 and 60 days of experimental. Blood drawn by cardiac puncture technique for measuring some parameters related to toluene toxicity on reproductive organ and cytogenetic status at 60 days. The occurrence of toxic effects of toluene manifested by central nervous sings including abnormal gait of animals in cage, tremor, ataxia and convulsion, rapid and shallow respiration, irritation of eye, as well as structural pathological changes in uterus and ovaries tissues sections including enlargement of ovaries with adhesion edematous, sloughing of endometrium, pyometria, necrosis, degeneration of ovaries cells with lymphocytic (infiltration and coughing) around blood vessels and lymphocytic granuloma with peritonitis , the results also showed toxicity effects of toluene on mitotic index , blast index , significant increase in chromosomal aberration mostly fragment and DNA damaged represented by (tail length ,tail intensity and tail movement).

المستخلص

صممت هذه الدراسه لمعرفه الدور السمي للتلوين في اناث الفئران البالغه على الجهاز التناسلي الانثوي (المبايض والرحم) والتغيرات الخلوية الوراثية مع دراسه التغيرات المرضيه النسيجيه (الرحم والمبيض) . تم استخدام (120) من اناث الفئران البيض البالغه وقسمت عشوائيا وبالتساوي الى ثلاث مجاميع : مجموعه السيطره ، المجموعه 1 (G1) ، المجموعه 2 (G2)تجرع من الجرعه النصف قاتله (LD50) جرعت المجموعتين يوميا ولمدة 60 يوميا عن طريق الفم من مادة التلوين وبتركيز (0.2 مل / كلغم من وزن الجسم) و كالتالي : مجموعه السيطرة جرعت عن طريق الفم وبزيت الزيتون والماء المقطر فقط ، في حين جرعت المجموعه 1 (G1) عن طريق الفم لمدة 30 يوما ، المجموعه 2(G2) عن طريق الفم لمدة 60 يوما . جمعت عينات الدم في الايام 0 ، 30 ، 60 عن طريق تقنيه ثقب القلب لغرض اجراء المعاير التعلقه بسميه التلوين على الجهاز التناسلي الانثوي والتي تشمل الانحرافات الكروموسوميه (اختبار دالة الانقسام mitotic index ، اختبار النوى الصغرى Micronuclei assay ، تقدير المذنب (الهالة) comet assay) والتغييرات المرضية للجهاز التناسلي الانثوي. اظهرت نتائج الدراسه الحاليه التغيرات المرضيه العيانيه والمجهريه للرحم والمبيض والتي اهمهاالتضخم مع التصاقات وانسلاخ بطانه الرحم ، والرحم القيحي والنخرمع ارتشاح بالخلايا اللمفيه في النسيج والتكفف حول الاوعيه الدمويه ، والورم الحبيبي اللمفي والتهاب البيرتون ، كما اظهرت الدراسه ارتفاع معنوي في الانحرافات الكروموسوميه معظمها التجزء وتقنيه تقدير المذنب (الهالة) . (طول وكثافه وحركة الذيل)

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Introduction

Toluene has hazard toxic air pollutant when inhaled in higher concentration of it from car fumes , factory fumes , pesticide or

cigarette fumes or dermal exposure to it or any other exposure source that lead to central nervous system (CNS) depression and cardiovascular disease (Xu et al ., 2009) , however toluene are widely used in veterinary medicine such as in production of pesticides, insecticide and also enter in the production of veterinary dipping that used for skin infection or parasitic infection , also toluene enter in the production of veterinary pharmaceuticals (inhalant drug for its intoxicating properties or spray parasitic drug and disinfectant solution or drug like halamide, Toluene have other uses away from veterinary uses like used for commercial and industrial purposes as solvent and constitute raw materials for the production of polymers , paints , glues, rubber dyes , varnish and Petroleum products such as gasoline , diesel fuel, and lubricating, and also used in the manufacture of cigarettes (Mehmet et al ., 2011), also these aromatic hydrocarbon compounds have carcinogenesis effects especially on lung carcinoma (Faye et al ., 2014) to human and animals when chronic and occupational environmental exposures to these compound will cause also chromosomal aberration and DNA damage by induced oxidative stress and inflaminflamotry process (Lotte et al ., 2008 ; Ian et al ., 2013 ; Alessandra *et al*., 2014) and that lead to causes tumor , also chronic exposures are associated with problems in the kidney, liver, and blood systems (Howard et al ., 2007)

Materials and methods

Experimental animals

One hundred twenty Female albino mice (*Musmus culus*), weighted about (35 to 40 g.) were used , they were 4 - 6 months of age which obtained from animal house at Institute of Embryo Research and Infertility Treatment/Al-Nahrain University . Animal were housed plastic cages in condition room (22 – 25 °C) , the experiment of this study were conducted in the animal house of the Pathology Department, Collage of Veterinary medicine , Baghdad University , with controlled lightening using automatic electrical timer providing daily light of twelve hours (7 .00 Am to 19 . 00 Pm) and twelve hours night cycle, Mice let for two weeks for adaption and they were supplied with normal mice food (pellet diet) and distal water. Albino female mice were selected randomly and divided into two experimental groups as following: 1st experimental (LD50) group: contain 30 female albino mice 2nd experimental groups: Contain 90 female albino mice were randomly selected for 3 equal subgroups as following: 1stgroup (control group): contain 30 female albino mice 2nd group: contain 30 female albino mice treated orally from LD 50 of toluene for 30 days 3rd group : contain 30 female albino mice treated orally from LD 50 of toluene for 60 days cytogenetic techniques were done at day 60 in ministry of science and technology /Iraq . Cytogenetic study performed according to the method of Al-Sudany, 2005. Parameter which are

studied were Mitotic index (MI), Blast index (BI),chromosomal aberration and micronuclei examination, single cell gel electrophoresis (commet assay) were performed for genotoxicity and were made for bone marrow cells commet assay protocol was performed according to the ITRC (Dhawan et al, 2010). To study Histopathological studies, Mice were anesthetized by chloroform via inhalation route and scarified by withdrawal of blood from heart instantly , After scarification, uterus and ovaries were excised blotted opened longitudinally and the macroscopic appearances were recorded to detect any abnormal gross changes in internal organs including location, color, size, shape, consistency and appearance of cut section. Then the tissue preserved in 10 % neutral formalin puffer solution till the planning of histological system, after 48 hrs. of the fixation, processing was routinely done with a set of increasing alcohol concentrations, tissues section were embedded in paraffin blocks, and sectioned by microtome at 5µm, After that All tissues of uterus and ovaries were stained with hematoxylin and eosin stain and the histopathalogcal changes were observed under light microscope (Lee and Luna ., 1968).

Statistical Analysis

Data were analyzed by using complete random design (C.R.D) to study the effect of level of toluene on cytogenetic parameter, Micronuclei and DNA damage, to analysis data were used packages of SPSS (Version 14). to study the significant differences between means using Least significance differences.

Results

Effects of toluene on cytogenetic status

The results in table (1) showed that there were a significant differences between G1 and G2 administration of toluene as comparing with control especially gaps , breaks , deletion, rings, central fusion and fragment while significant difference between G1 and G2 administration of toluene break and fragment and compared with control respectively: (2,3± 0,6), (2,6 ± 0,8), (2,6 ± 0.8), (1,26 ± 0,5) , (1,72 ± 0,3), (0,1 ± 0,01). The table (2) showed there is significant increase ($p \leq 0,05$) in Micronuclei between G1 and G2 at days 60 as comparing with control group respectively (1, 25 ± 0,09), (1,45 ± 0,10) , (1,52 ± 0,12) . Table (3) reported significant (tail length) increased between G2 and G1 groups of toluene administration as comparing with control group respectively (54 . 25 ± 1,56) , (36 .24 ± 2. 14), (12 .18 ± 0.28), also (tail movement) showed significant increased between G2 and G1 groups of toluene administration as comparing with control group (56. 0 ± 3.58) , (54.0 ± 3.28), (2.86 ± 0.63), no significant differences in (tail intensity) between G2 and G1 as compared with control respectively. The table (4) showed there is significant increased ($p \leq 0.05$) in mitotic index between `G2 administration toluene group and G1 administration group comparing with control respectively (4.05 ± 0.50) , (3.4 ± 0.51),(3. 35 ± 0.52). While there were no significant difference in blast index between G2 and G1 group of toluene administration as comparing with control.

Table (1). The toxicity effects of toluene on chromosomal aberration at 60 days

treatment	Gap	Break	Deletion	Ring	Central fusion	Fragment	Total
Control	1,0 ± 0,2 b	1.2 ± 0 c	0 ± 0 b	0 ± 0 b	0 ± 0 b	0,1 ± 0,01 c	2,3 ± 0,2 c
G 1	3,4 ± 0,7 a	2,3± 0,6 b	3,4 ±0,9 a	1,25± 0,10 a	1,15 ±0,28 a	1,26 ± 0,5 b	12,7 ± 0,8 b
G2	3,82± 0,90 a	2,6 ± 0,8 a	3,45± 0,72 a	1,2 ± 0,12 a	1,28 ± 0,15 a	1,72 ± 0,3 a	14,07 ± 0,9a

Means bring different letters in the same column denoted significant differences between administration groups at ($p \leq 0,05$)

Table (2).The toxicity effects of toluene on Micronuclei at days 60

Groups	Mn means
Control	1 , 25 ± 0,09 b
G1	1,45 ± 0,10 a
G2	1,52 ± 0,12 a

Mean bring of different letters in the same column denoted significant differences between groups as comparing with control

Table (3). The toxicity effects of toluene on DNA damaged (using comet assay) on day 60

Treatment	Tail length	Tail intensity	Tail movement
Control	12 .18 ± 0.28 c	2.86 ± 0.63 b	0.36 ± 0.08 c
G1	36 .24 ± 2. 14 b	54.0 ± 3.28 a	27 . 0 ± 2.2.35 b
G2	54. 25 ± 1,56 a	56. 0 ± 3.58 a	43 .5 ± 4.5 a

Mean bring of different letters in the same column denoted significant differences between groups as comparing with control.

Table (4). The toxicity effects of toluene on mitotic index and blast index

Group	Mitotic index	Blast index
Control	3. 35 ± 0.52 c	14. 2 ± 1.3 b
G1	3.4 ± 0.51 b	32. 6 ± 1.6 a
G2	4.05 ± 0.50 a	32.8 ± 1. 62 a

Mean bring of different letters in the same column denoted significant differences between groups as comparing with control.

Effects of toluene on ovaries and uterus tissue of mice:

1. Ovary

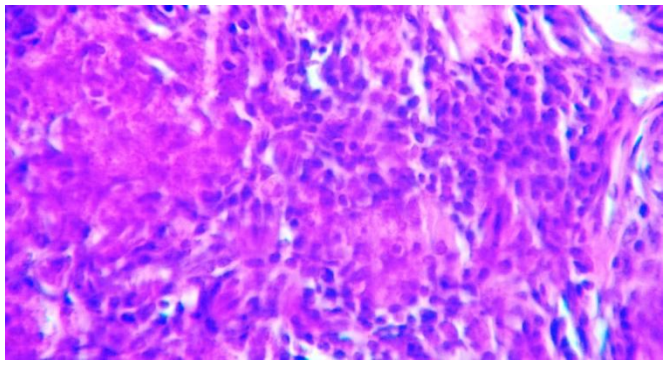

Fig (1). Microscopic changes in mice ovary at 30 days of (G1) administration of toluene : A - hemosiderin pigment free in the medulla , B - necrotic area , C- hemosiderin engulf by macrophages (X40 , H and E stain).

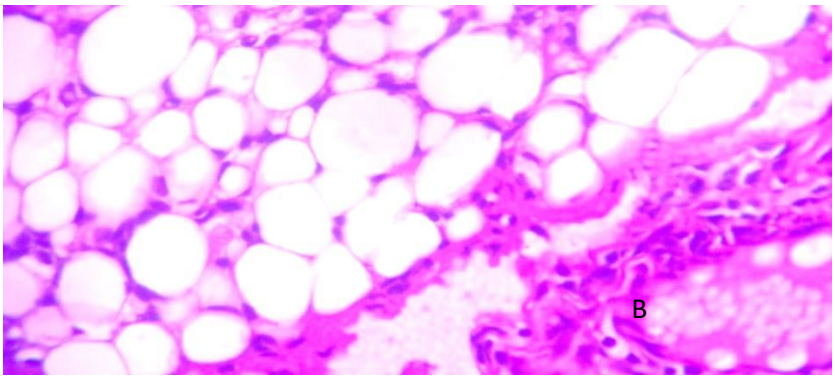

Fig (2). Microscopic changes in mice ovary at 60 days of (G1) administration of toluene : A- fibrin exudate , B – infiltration of tissue by macrophages (X40 , H and E stain)

2. Uterus

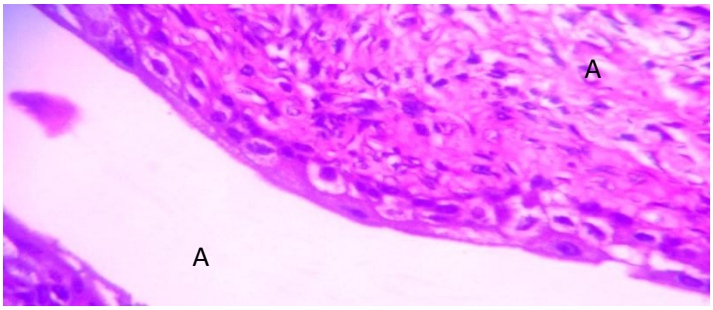

Fig (3). Microscopic changes in mice uterus at 30 days of (G2) administration of toluene : a- multiple areas of vacuolation and necrosis , b – sloughing of endothelial cells (x40 , H and E stain)

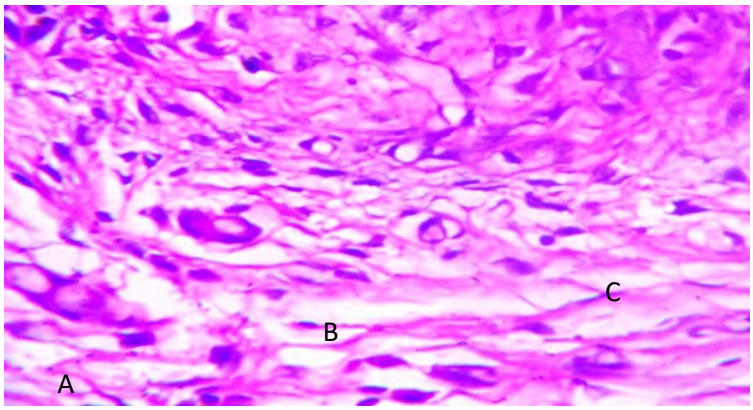

Fig (4) Microscopic changes in mice uterus at 60 days of (G1) administration of toluene: :A - Hyperplasia of endothelial cells , B- vacuolation and hemorrhage of endometrium , C- fibrinous infiltration with mononuclear cell (X40 , H and E stain)

Discussion:

LD50 was calculated Through (The up and Down procedure), and according to the toxicity rate of toluene depend on Taş et al. (2013) , the LD50 of Toluene which given to rats through oral administration was fall under toxicity rate which since LD50 was nearly (6 ml / kg B.W.) so the LD50 was calculated through (UDP) to mice is (0.1 ml / kg B.W. , 0.2 ml / Kg B.W.) (Shetty et al., 2007 ; Lipnick et al., 1995)

Effects of toluene on cytogenetic status (MI, BI, MN, Chromosomal aberration and damaged DNA).

The results suggested that the causes of chromosomal aberration may be due to cytotoxic effects of toluene on the cell of bone marrow , and this agreed with Baunchinger et al. (1982), Nise et al., (1991), Hammer , (2002) were reported that in increase in the chromatid break and gaps . also are in agreement with Haley, (1987) reported increased incidents of chromosomal aberrations in the bone marrow cells of rats exposed to 112 ppm toluene by

Determination of LD50

inhalation , Dobrokhotov et al. (1977) were reported toluene was induce chromosomal aberrations in the bone marrow cells of rats following chronic inhalation exposure to 610 mg/m^3 toluene for 4 hours/day for 4 months , Roh et al., (1987) were reported also there is increase in chromosomal aberration when exposure of mice and rat to toluene . however , our result disagreed with Forni et al., (1971) , Funes et al., (1977), Gerner and Friedrich., (1978) , Maki et al., (1980); were reported that the toluene not induced effects on the chromosomal aberration , have reported no differences in chromosomal aberrations between control subjects and toluene-exposed workers and this disagreed with our result The higher percentage of Micronuclei may be related to sever destruction of the body tissue that induced by toluene toxicity and these lead to formation higher number of pro – inflammatory cytokines that facilitated extravasation of the inflammatory cell from blood vessels to the site of

inflammation as well as stimulated bone marrow to produced large number of immune inflammatory cell , and according to sever neutrophils and macrophages infiltration in the bone marrow , according to the above the result may indicated that these inflammatory cells also play essential role in the chromosomal aberration due to the toluene induced (ROS) and these may be destruction the basis of chromosomal strands these evidence are in agreement with Pitarque et al ., (2002) were reported in a population of shoe factory workers exposed to solvents (including toluene, gasoline, and acetone), increase in micronuclei induction was observed in peripheral lymphocytes , However, increased micronuclei induction was not associated with solvent exposure in Brazilian shoe makers exposed to solvent-based adhesive (Heuser et al., 2005, 2007) or Mexican painters exposed to unreported concentrations of toluene (Moro et al ., 2012).

On this base of these observation the result suggested that the higher percentage of micronuclei in our experimental was in consistent with other cytogenetic of result of chromosomal aberration mitotic index , and blast index , moreover , the higher percentage of DNA damaged indicated by comet assay (SCGE), since SCGE is a test for genotoxicity and can used to identified possible mutagens and carcinogens (Anderson et al., 1998), so from the result showed the toluene have the ability to induced DNA damaged in the bone marrow of female mice , since there is significant increased ($p \leq 0.05$) in the animal that administration with toluene as compared with control and these damaged observation with increased the dose of administration and this result agreement with Fan Wang et al. (2013) were reported volatile organic compound (toluene one of these compound) induced the oxidative stress and genotoxicity response due to (ROS) formation and these are cytotoxic agents causing oxidative damage by attacking cell membrane and DNA due to if excessively produced and not (ROS) timely scavenged, oxidative stress results in the accumulation of dysfunctional proteins, lipid peroxidation products and damaged nuclear or mitochondrial DNA , Also our result agreement with Heuser et al. (2005, 2007); Moro et al. (2012) were reported in Mexican painters exposed to unreported concentrations of toluene there is DNA damaged assay by SGCE .

The current result agreement with Martinez et al., (2009) were reported toluene induce excess of basal DNA damage in lymphocytes of rats exposed to thinner inhalation in comparison may be due to there is Interference with cellular redox regulation and induction of oxidative stress cause oxidative DNA damage and also agreement with Martinez *et al*. (2006) were showed Thinner (Toluene was the most abundant compounds in commercial thinner) causes toxic effects on brain and other organs and induce oxidative stress is a well-documented mechanism for DNA oxidation has potentially genotoxic consequences , however , our result disagreed with Dobrokhotov et al. (1977) Gerner-Smidt and Friedrich, (1978); Richer et al. (1993); Pitarque et al. (1999) have reported no increase in SCGE.

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