

Benzene histotoxic and teratogenic Effects in exposed Mice

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Abstract:

Throughout the last two decades, benzene was a prominent source of an occupational and environmental pollution in Iraq. Especially from direct contact with private electric generators which were installed closer to homes or even inside every home in Iraq. As a result of handling without taking enough precautions, environmental threat has increased due to serious toxic, carcinogenic and teratogenic effects of benzene. Toxicity, carcinogenicity and teratogenicity of this chemical carcinogen were experimented in Swiss albino mice by intraperitoneal (i.p.) injection. Six-week-old male mice were used as a model for toxicity and carcinogenicity together with female mice were treated with benzene, and newborns were sacrificed to study the teratogenic impact. During a period of three months, twice per week, male mice were injected with two doses (0.1% and 0.2%) of benzene diluted with corn oil. Female mice were also treated with (0.1% and 0.2%) of benzene on day 7 prior to gestation at 72 hr intervals for one month and sacrificed 10 days after labor. At the end of experiment, sections of different organs were histopathologically observed and significant changes were noticed. Dose-related changes were detected during examination of males' liver showing hepatocyte swelling, degeneration and fibrosis and similarly glomerular enlargement and tubular necrosis of the kidney. New born mice liver sections showed a significant liver and kidney histological changes. These results may explain a correlation between tumor incidence and transplacental benzene exposure in mice and shade the light on the potential health risks that Iraqi community exposed to.

Conclusions: When the researchers take into account the degree of damage resulted from direct exposing Albino mice to benzene for a limited period of time, there will be a reasonable wariness because of the increasing level of toxicity and carcinogenicity in the Iraqi environment.

Key words: Benzene, teratogenicity, histotoxicity, carcinogenicity, in vivo.

التأثيرات النسيجية السمية والتشوهية في الفئران المعرضة للبنزين

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الخلاصة:

إن من أبرز المصادر المسببة للتلوث بيئياً و مهنيّاً خلال العقدين الماضيين في العراق هو البنزين. وخصوصاً بعد أن تم نصب العديد من مولدات الكهرباء الاهلية التي تعمل بالقرب من الدور السكنية بالإضافة الى داخل الدور السكنية نفسها. حيث إزداد التهديد البيئي الذي يسببه البنزين من خلال التأثيرات التشوهية والمسرطنة والسمية الناتجة من عدم أخذ الاحتياطات اللازمة أثناء الاستخدام. لقد تم حقن هذه المادة المسرطنة في الفئران البيضاء المختبرية داخل الصفاق لأختبار تأثير هذه المادة على المستوى التشوهي والتسرطني والسمي. إختبرت نماذج من ذكور الفئران بأعمار ستة أسابيع لدراسة الاثر السمي والتسرطني لمادة البنزين فضلاً عن دراسة التشوه الحاصل في الفئران المولودة حديثاً نتيجة معاملة الاناث اثناء فترة الحمل بهذه المادة. تم الحقن في ذكور الفئران بجرعتين (0,1% و 0,2%) من البنزين المخفف بزيت الذرة وعلى مدى ثلاثة شهور بمعدل حقنتين لكل اسبوع. أما الاناث فقد تم عوملت أيضاً بنفس الجرعة (0,1% و 0,2%) من البنزين قبيل الحمل لمدة اسبوع بمعدل حقنة واحدة لكل 72 ساعة وعلى مدى شهر كامل حيث تم تشريحها بعد مرور 10 أيام من الوضع. وبعد انتهاء مدة التعريض وإجراء فحوصات التشريح المرضي لمقاطع نسيجية مأخوذة من أعضاء مختلفة لوحظ وجود تغيرات مهمة. وخلال معاينة خلايا الكبد العائدة الى ذكور الفئران تم رصد تغيرات متعلقة بمستوى الجرعة تراوحت ما بين تورم وانحلال وتليف خلايا الكبد وفي نفس الوقت تضخم كببيبي وتكثُر انبوبي كلوي. كما أظهرت نتائج الفئران حديثة الولادة مساحة ضخمة من التكتُر فضلاً عن بؤرة من الخلايا المتحولة والمأخوذة من مقاطع نسيجية للكبد، إضافة الى تكثُر انبوبي واضح في خلايا الكلتيين. ربما يتضح من خلال هذه النتائج وجود ترابط بين ارتفاع نسبة حدوث الورم وتعرض الفئران للبنزين عبر المشيمة وكذلك تسليط الضوء على جزء من الاخطار الصحية الكامنة وراء تعرض المجتمع العراقي لهذه الملوثات.

الإستنتاج: اوضحت هذه الدراسة خطورة التعرض للبنزين لكل من الذكور والحوامل والمولودون حديثاً وهذا قد يسلط الضوء على ازدياد نسب السمية والتسرطن في بيئة العراق بسبب التعرض المستمر لمادة البنزين عند الاخذ بنظر الاعتبار مستوى الضرر الذي سببته تلك المادة في الفئران المختبرية خلال تلك الفترة المحدودة من التعريض.

الكلمات المفتاحية: البنزين، التشوهات، السمية النسيجية، التسرطن، الفئران.

Introduction

Among the pollutant compounds frequently detected in the environment, benzene is a main source of concern (1). The simple aromatic hydrocarbon benzene (benzol; 1,3,5-cyclohexatriene; C₆H₆) occurs naturally in crude oil, petroleum and cigarette smoke (2). Benzene is a ubiquitous pollutant with known carcinogenic and hematotoxic effects in humans and mice (3). It is recognized internationally as an haematotoxin and carcinogen (4) (United Stated [US] National Toxicology Study Report, 1986) and exposure at high doses for extended periods indirectly causes myelotoxicity and leukaemogenicity in both animals and humans (5). Undoubtedly, exposure to potentially high levels of benzene continues to exist in both developing and developed countries (6).

It has been referred that chronic exposure to benzene typically causes bone marrow depression that often initially displays

clinically decreased peripheral blood cell counts (anemia, leukopenia, and thrombocytopenia), but may manifest pancytopenia, aplastic anemia, myelodysplasia, or myelogenous leukemia (7,8).

Transplacental genotoxicity of benzene at various concentrations was demonstrated in CD-1 mice by intraperitoneal injection (9). Mice appear to be more sensitive to hematotoxicity from benzene than rats or rabbits (10).

The present investigation was undertaken to elucidate the expected carcinogenic effect and histological changes resulted from benzene injection to adult male albino mice, and newborn mice that exposed to benzene during gestation period.

Material And Methods**Animals and experimental design**

Swiss albino mice of both sexes from the Iraqi Center for Cancer and Medical Genetic Researches (ICCMGR), animal house unite, 60 days old and weighing 30 ± 2 g, were

allocated randomly into three groups of six animals, including the commercial food and water that were given throughout the period of animal holding and experimentation that was according to the ICCMGR guidelines for animal handling. Mice were treated on the first day of experiment for a period of 30 days, and then killed 10 days following labor.

Chemicals and treatment

Benzene used in this experiment was made by Gainland Chemical Co. (GCC, U.K., 99%). The solvents and administration route followed in this study was benzene in corn oil, intraperitoneally; control animals were treated with corn oil only. Each five dams gathered with one male in a single cage were randomly selected and treated with each concentration of chemical (i.e., 0.1% and 0.2%) in the same manner.

Histological studies

Samples of organs were excised. They were extended, sliced, fixed in 10% buffered

formalin, embedded in paraffin, sectioned, stained with hematoxyline and eosin (H&E) and examined by light microscopy.

Results

Histopathological examination of new born mice:

New born mice which was exposed to benzene during gestation period showed pathological changes in the liver which showed massive area of necrosis (figure-1 and 2) as well as focal area of transformed cells which may develop to cancer as shown in the (figure-3 and 4). Control group showed no pathological changes (figure-5 and 6). Liver lesion was more severe in newborns than adult mice where liver sections showing wide area of necrosis in newborn while only degeneration in adult mice were seen. Moreover, there was focus of transformed cells in the liver of newborn which is showing the carcinogenic effect of benzene.

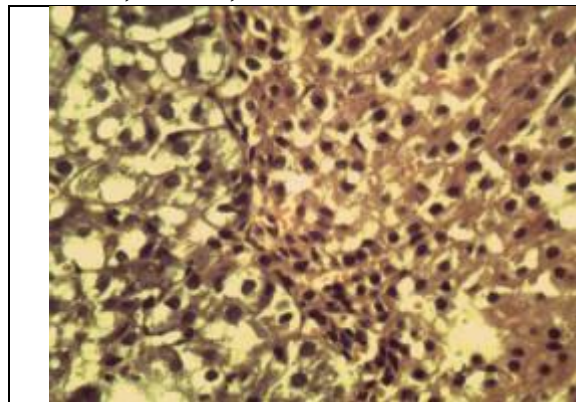


Figure-1 showed necrotic area adjacent to the normal hepatocyte 40x, H&E stain. .

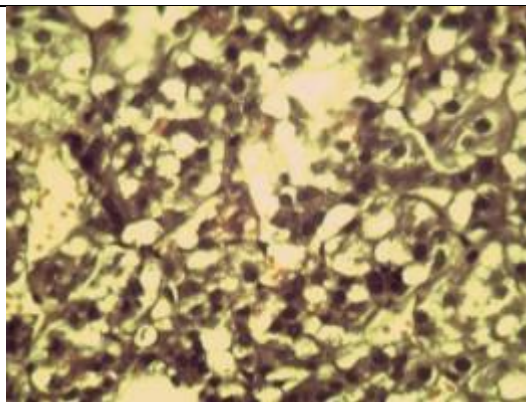
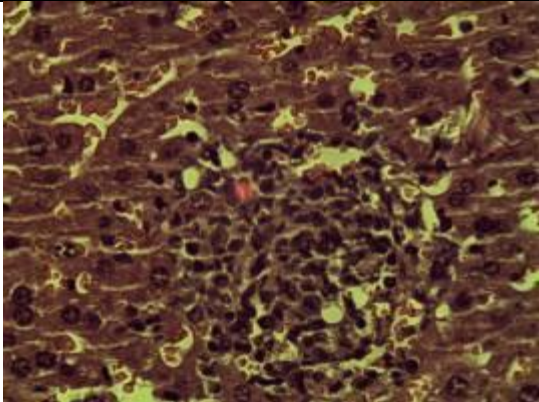
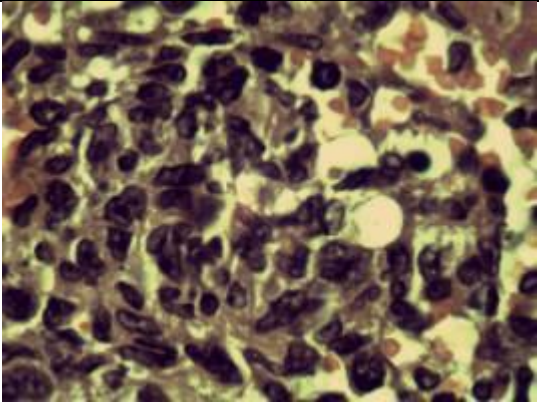
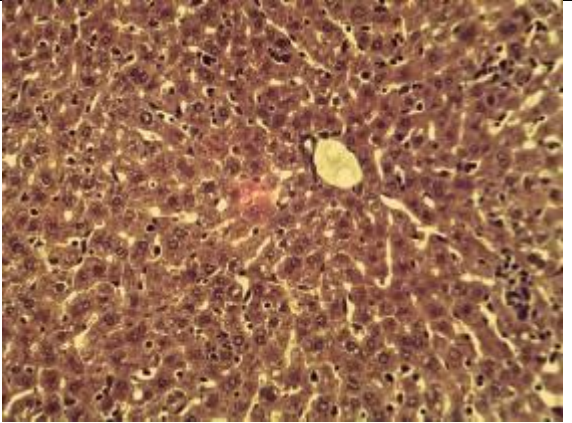
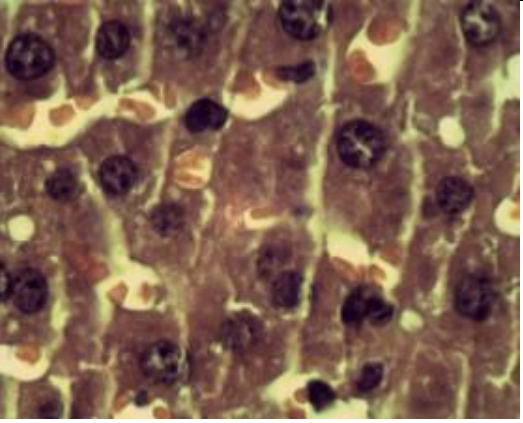


Figure-2 showed cells in different stages of degeneration and liver cells underwent necrosis 40x, H&E stain..

	
Figure-3 Represent focal of transformed cells inside the hepatic tissue 40x, H&E stain.	Figure-4 Transformed focal of hepatocyte showing characters of transformation as increase nucleus-cytoplasm ratio and morphological changes. 100x, H&E stain.
	
Figure-5 Section in control group liver showed no lesion in hepatic parenchyma. 20x, H&E stain.	Figure-6 Normal hepatocyte in control group. 100x, H&E stain.

Kidney of treated group lesion was tubular necrosis with glomerular enlargement (figure-7), control group showed no pathological changes (figure-8). There was no histological lesion in lung tissue (figure-9).

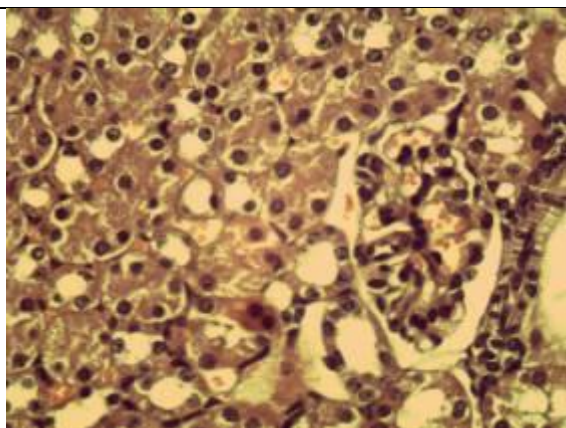


Figure-7 Treated group with tubular necrosis and glomerular enlargement. 40x, H&E stain.

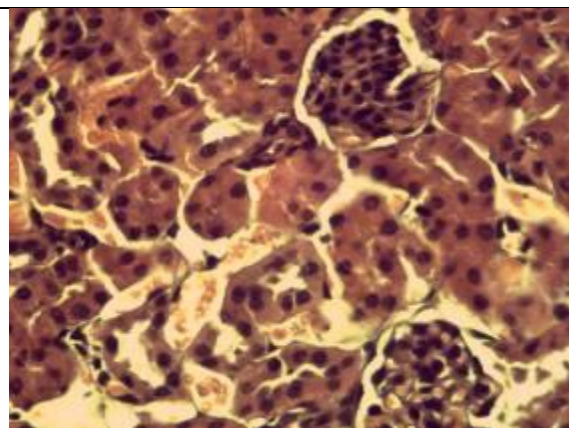


Figure-8 Control group 40x, H&E stain.

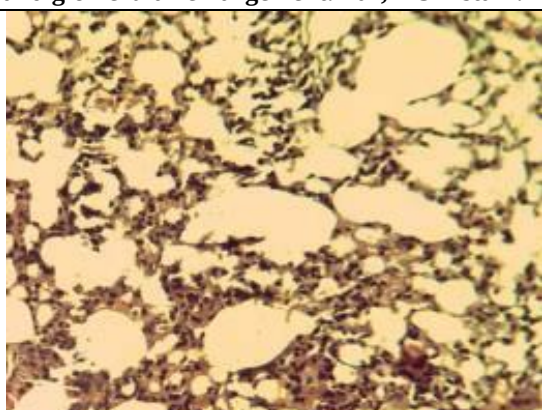


Figure-9 Lung tissue showed emphysema. 10x, H&E stain.

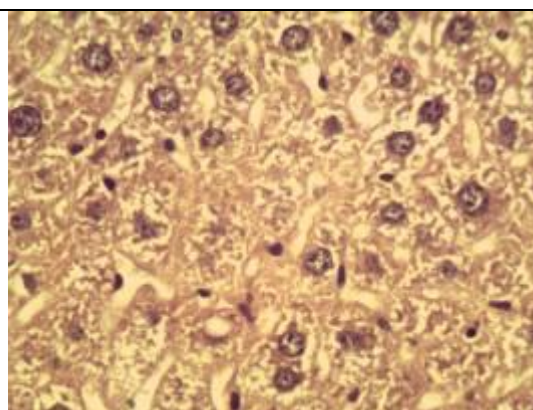


Figure-10 Showed swelling and degenerated hepatocyte in 0.1ml benzene 20x, H&E stain.

Histopathological examination in male mice:

Male mice which treated with 0.1ml of benzene for 30 days showed pathological changes in the liver which represented by hepatocyte swelling and degeneration (figure-11). Liver of mice treated with 0.2ml of benzene showed the same lesion (figure-12). Control group showed no pathological changes (figure-3).

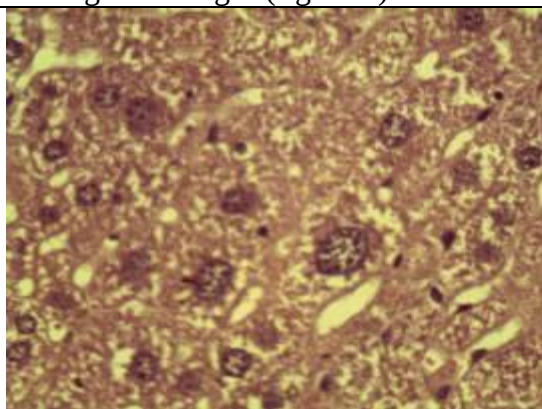


Figure-11 Showed swelling and degenerated hepatocyte in 0.1ml benzene 20x, H&E stain.

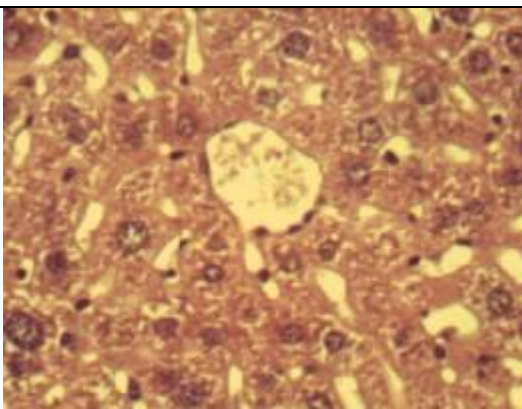


Figure-12 Represent control hepatic tissue 20x, H&E stain.

Kidney of 0.1ml benzene treated group lesion was mild tubular necrosis (figure-13), while 0.2ml treated group had more sever lesions as increase in interstitial tissue and cellularity as a pre-neoplastic lesion, furthermore there was fibrosis, glomerular enlargement and tubular necrosis (figure-14, 15 and 16). Control group showed no pathological changes (figure-17). There was no histological lesion in lung tissue (figure-18).

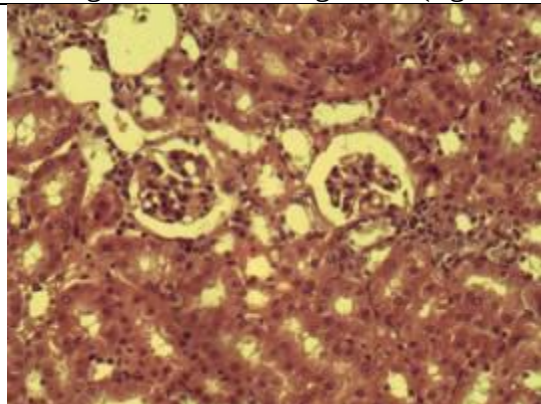


Figure-13 Revealed mild tubular necrosis in male mice injected with repeated does of benzene (0.1ml). 20x H&E stain.

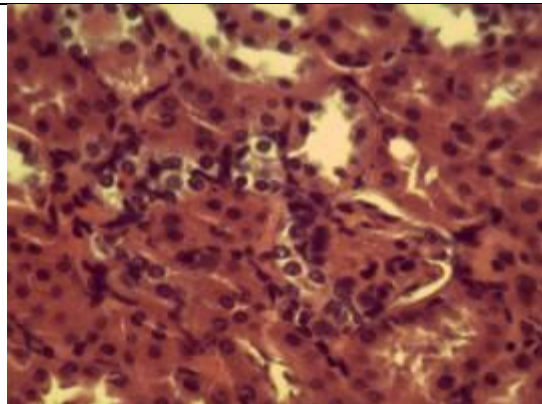


Figure-14 0.2ml treated group, tubular lumen blockage and glomerular enlargement. 40x H&E stain.

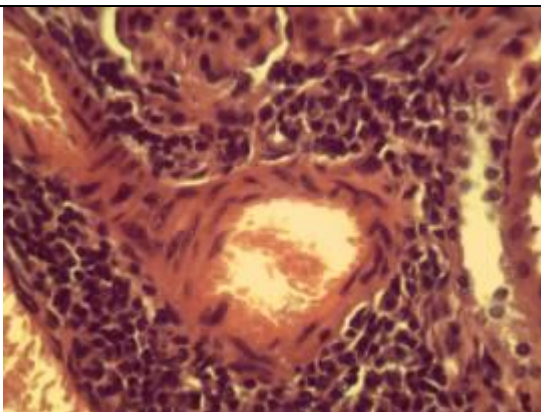


Figure-15 0.2ml treated group, fibrosis and pre-neoplastic lesion with increase in cellularity. 40x H&E stain.

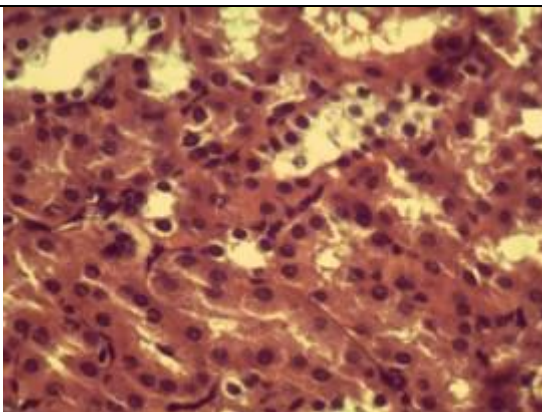
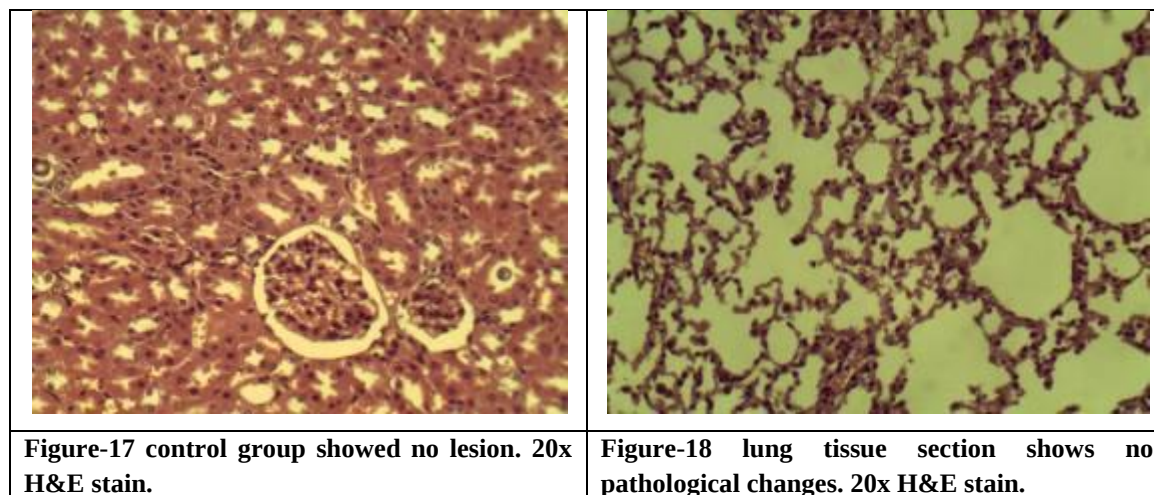


Figure-16 Tubular damage and necrosis with cell swelling. 40x H&E stain.



Discussion:

The outcome of this study was illustrated as a histopathological change after treatment of mice with benzene for a limited period of time, revealing a significant change within the tissue sections of liver, lung and kidney. Data presented in this study has involved an assessment of benzene carcinogenicity expressed in term of pathological lesions occurred during intraperitoneal route of administration, which has been approved to be the most acute route of treatment.

And the reason for dealing with mice at the above-mentioned doses of benzene, because it has been shown that proportionally more benzene will be converted to toxic metabolites at low doses than at high doses. Furthermore, mice metabolize benzene faster and convert more of the benzene to toxic metabolites than rats (11). Some studies have shown that benzene can cause transplacental cytogenetic effects in new born mice (12). Similar results were obtained by Xing et al. (13). Moreover, Benzene can cross the placenta of experimental animals, and haematopoietic changes have been observed in the fetuses and offspring of mice exposed to concentrations of 16–65 mg/m³ during days 6–15 of gestation (14). At this stage of gestation, the metabolic capacity of the fetal

liver exceeds that of adult bone marrow. (23 and 24).

Benzene is quickly distributed through the blood to utmost, if not all, tissues. (25, 26, and 27). In the present work there were significant histological changes identified in liver, kidney, and lung. These changes were obvious in liver indicated by massive area of necrosis (Fig. 1 and 2) and a focal area of transformed cells (Fig. 3 and 4). Previous study mentioned that liver is the main site for the metabolism of benzene, while the bone marrow was the minor site (15). Ghantous and Danielsson, 1986 found that in pregnant mice exposed to benzene concentrations of 2,000 ppm for 10 minutes, the parent compound and its metabolites were found in lipid-rich tissues, such as brain and fat, and in well-perfused tissues such as liver and kidney, as well as in the fetuses and placenta. Au et al. (16) were able to detect chromosome aberrations in lung macrophages after prolonged exposure (6 weeks) at concentrations as low as 0.32 mg/m³, and in lymphocytes from the spleen of mice at 0.13 mg/m³ (17). That chromosomal aberration may explain the appearance of tubular necrosis and glomerular enlargement in kidney tissue (Fig. 7) as compared with control (Fig. 8).

The severity of lesions noticed in kidney was proportional to the concentration of benzene showed as increase in interstitial tissue and cellularity as a pre-neoplastic lesion and tubular necrosis (figure-14, 15 and 16). Variation of severity of benzene on kidney between newborn and male mice may be due to the differences of exposure periods. Previous study indicated that there were no increases in kidney tumors, following ethyl benzene exposure (18).

Lung tissue of newborn had showed an emphysema (Fig. 9) in contrast with lung tissue of male mice (Fig. 18) indicated by no significant impact on lung tissue, which was confirmed by the absence of lesions. It has also been evidenced that due to benzene inhalation in mice, increased lymphomas, leukemias, and lung adenomas have been observed, although not all were observed in all strains tested (19). Also in a separate carcinogenicity study in mice B6C3F₁, doses of benzene ranging from 25 to 200 mg/kg were administered 5 days/week for 103 weeks, resulted in increased incidence of the alveolar/bronchiolar carcinomas (20). These differences in administration route, duration, age, sex and species may explain these variations among results.

From the above results it can be seen that both doses of benzene have led to significant toxicities in newborn mice. Measuring these effects by histopathological manifestations. Results indicated the possibility of histological lesions and necrosis found in kidney and liver tissues due to this short-time exposure along with existence of a severely polluted environment with war associated chemicals in many of the Iraqi cities (21). This requests more attention and enormous effort to decrease the correlated health complications mainly on human and even animals which being consumed by human as well.

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