TOXOPATHOLOGICAL EFFECTS OF PHENYLMETHANE ON MALE ALBINO MICE INTERNAL ORGANS

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ABSTRACT

This study was aimed to evaluate the toxic properties of phenylmethane on internal organs and liver enzymes (Alkaline phosphatase (ALP) and Alkaline serum transaminase (AST)changes in mice. Thirty albino mice randomly divided equally into 2 groups, each group contains (15) animals as following: 1st group was administrated orally (0.2 mg / kg B.w) of Phenylmethane daily for (8)weeks, 2nd group was considered as negative control group. Finally all animals euthanized and sample of blood were taken for biochemical examination and histopathological specimens were taken from liver, kidney and brain. Results revealed that phenylmethane pointedly enlarged in the level of liver enzyme after 8 weeks that ALP (88.50 ± 1.01), AST (250.20 ± 0.92) compare with control(50.00 ± 0.85), (231.80 ± 1.40). The histopathological lesions showed that the animals exposed to toxic dose of phenylmethane was characterized by inflammatory reaction, hemorrhage, congested blood vessels, necrosis, fibrosis and granuloma in internal organs. In conclusion, phenylmethane had possess toxic effects for internal organs in mice. To avoid its harm on human, the use of phenylmethane should be expertise.

Keywords: phenylmethane, histopathological changes, liver enzyme, albino mice.

التأثيرات المرضية السمية لمادة الفنيل ميثان على الاعضاء الداخلية لذكور الفئران البيضاء

بشرى ابراهيم القيسي سليمة لفتة حسن سرى عايد ردام
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المستخلص

الهدف من هذه الدراسة تقييم الاثر السمي للفنيل ميثان وما يحدثه من تغيرات نسيجية للأعضاء الداخلية و تأثيره على انزيمات الكبد في الفئران البيضاء ولهذا استخدامنا ثلاثون فأر قسمت عشوائيا الى مجموعتين: المجموعة الاولى جرعت بمادة الفنيل ميثان بتركيز(0,2ملغم/كغم. وزن الجسم) لمدة (8)اسابيع والمجموعة الثانية اعتبرت مجموعة سيطرة سالبة وتم سحب الدم من قلب الفئران مباشرة لدراسة مستويات انزيمات الكبد التي سجلت (250,20± 0,92) لأنزيم الAST و(88,50 ± 1,01) لأنزيم ال ALP مقارنة مع نتائج مجموعة السيطرة (50,00 ±0,85) لأنزيم ال ALP و (231,80± 1,40) لأنزيم الAST. تم اجراء الصفة التشريحية لكل من الكبد والكلية والدماغ لمعرفة التأثيرات المرضية الناتجة من التعرض المزمن للفنيل ميثان واوضحت نتائج الدراسة التأثير الكبير الذي سببته هذه المادة السامة مما ادت الى ارتفاع في مستوى انزيمات الكبد واوضح الفحص المجهري للأعضاء المذكورة تغيرات مرضية مثل التنكس الخلوي الحاد كذلك تلف ونخر في الاعضاء مع حدوث احتقان شديد في الكلية حتى يتطور الى حدوث الورم الحبيبي في الكبد كذلك تجمع الاوديما وتوسع الاوعية الدموية للدماغ .

الكلمات المفتاحية: الفنيل, التغيرات المرضية النسيجية, انزيمات الكبد, الفئران البيضاء المختبرية.

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INTRODUCTION

phenyl methane (C6H5CH3) is a clear, colorless liquid with aromatic odor, its manufactured from binary chief sources: catalytic conversion of petroleum and aliphatic aromatized hydrocarbons also by product of the coke oven industry**(**3**).** Phenyl methane widespread in the ecological due to wide variety used of commercial and household products(7). Its solubility in water is 535 mg/liter**(**20**).** Because its better solvent material can dissolve other substances, its naturally occured in crude oil and made in process gasoline production and other petrol as of crude oil and in making coke from coal **(**21**).** Phenylmethane is breakdown by serial hydroxylation and oxidation to benzoic acid, glycine and benzoic acid conjugated form hippuric acid constitutes, the main direction of phenylmethane detoxification and elimination, phenylmethane breakdown by cytochrome P-450 (CYP) enzymes that occurrence in liver (5**).** Inflammation and degeneration of respirational epithelium and pulmonic injuries have been detected in rodents exposed to great side by side of phenylmethane by breathing also slight adverse effects such as heart muscles fibers necrosis, hepatic swelling, lung congested and hemorrhage and necrosis of renal tubules were conveyed and great-frequency range loss have been informed **(**20**).** Hazardous materials pollution and chemicals caused direct or indirect toxic effect and pathological lesion in organ exposure to these compound has long been a very serious environmental issue and a major public health problem (1) The aims of this study evaluated biochemical and histopathological changes in the mice treated with the Phenylmethane.

MATERIALS AND METHODS

Experimental design

Thirty male albino mice distributed equally into two individuals each one contains (15) animals as following: 1st group was administrated Phenylmethane orally (0. 2) mg / kg B.w) daily for(8) weeks, 2nd group was considered as negative control group.

Preparation of phenylmethane

Phenylmethane (Sigma- Nether Lands) is present in the form of powder(0.2 mg / kg B.w) given by fine plastic stomach tube to 1st group for (8)weeks

Biochemical analysis: Blood samples were collected directly from heart of mice by using syringe(1) ml in period at (8)week after treatment, blood samples were transported in epindorf and saved in freezer overnight then centrifugation (1500 rpm) for (3)minutes, finally the serum put in storage in the frozen(-20 C°) until biochemical analysis, biochemical analysis of Alkaline phosphatase (ALP) and Alkaline serum transaminase (AST) were measured using kits-linear chemical- Spanish. And examined in Pharmacology Department, Faculty of Veterinary Medicine, Muthana University.

Histopathological examinations: Specimens taken from internal organs including: liver, kidney and brain were kept in 10% formaldehyde solution(72) hours for fixation and then processed routinely by using the histokinete, tissue sections were embedded in paraffin blocks, and section by microtome and staining with hematoxyline and eosin stain according to(11, 12), then examined by using light microscope in Pathology Department, Faculty of veterinary Medicine, Bagdad University.

Statitical analysis: The data were analyzed by ANOVA one way. The significance level was designated at $P < 0.05$.

RESULTS AND DISCUSSION

Significantly ($P \leq 0.05$) increased (ALP) and (AST) were experiential in animals of 1st group compared with 2nd group respectively (table, 1).

Table1. Alkaline phosphatase (ALP) and Alkaline serum transaminase (AST) in mice at(4,8)weeks post treatment with phenylmethane.

Groups	ALP/ units	AST/ units
1st group treated with phenylmethane 8 weeks after administration	A 88.50 ± 1.01	A 250.20 ± 0.92
2nd group control negative 8weeks after administration	B 50.00 ± 0.85	B 231.80 ± 1.40

Data expressed as mean and standard error refer to insignificant variances between groups (P≤0.05), different between capital letter mean significant in Colum

Clinical signs and symptoms: All treated animals were depressed and showed decrease appetite for food consumption during the study period, emaciated and anorexia were due to

pathological lesions which lead to indigestion of stomach and mal absorption of intestine (5).

Histopathological examination

The histopathological results of 1st group treated with Phenylmethane showed mononuclear cells(MNCs) aggregation in liver parenchyma Figure 1 accompanied with granuloma Figure2, also narrowing of sinusoid with deposition of hemosiderin Figure 3, and necrotic of liver parenchyma with fibrosis Figure 4 cellular swelling degeneration and MNCs infiltration Figure5. While the main lesion in kidney showed congestion and MNCs infiltration with dilatation of renal tubules Figure 6. with acute cellular swelling and congested of glomeruli and renal tubules Figure 7, The brain showed odema and dilatation of meningeal blood vessels and necrosis Figure 8.

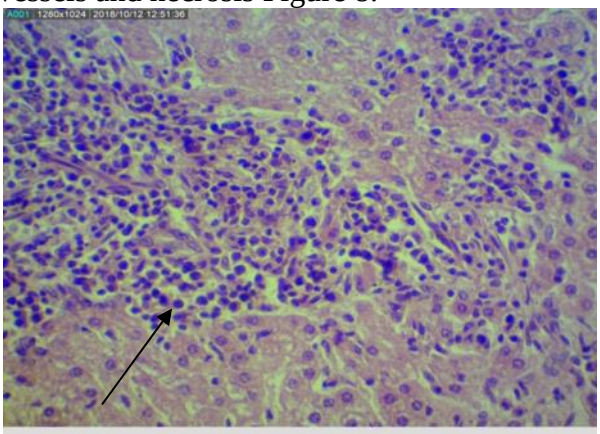

Figure1. Histopathological section in the liver of 1st group showed MNCs aggregation in liver parenchyma⟶(H&E stainX40).

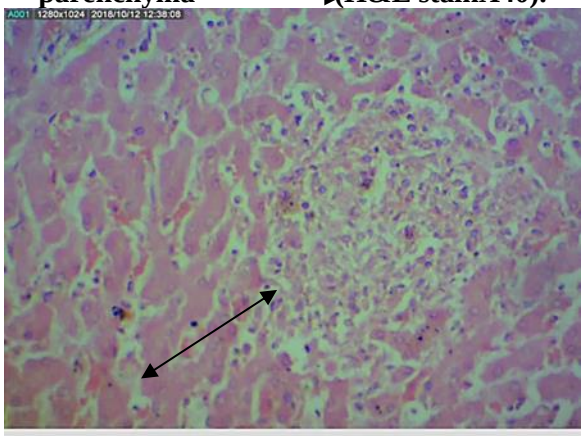

Figure2. Histopathological section in the liver of 1st group showed granuloma in the liver parenchyma⟷(H&E stainX40)

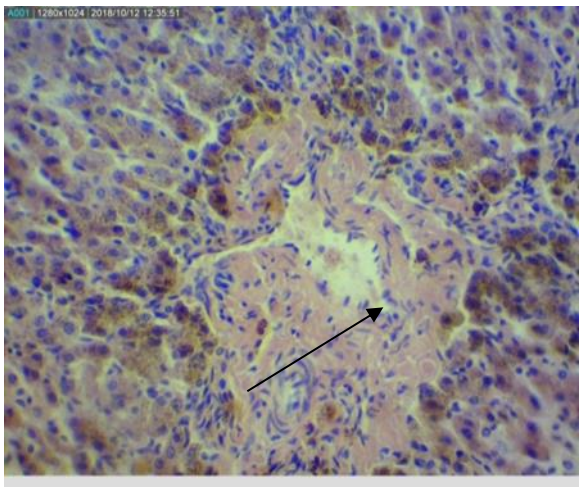

Figure 3. Histopathological section in the liver of 1st group showed sinusoid narrowing with hemosiderin deposition ⟶ (H&E stainX40).

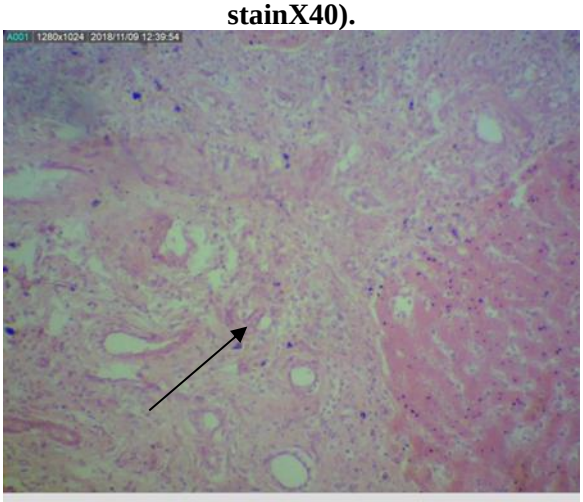

Figure 4. Histopathological section in the liver of 1st group showed necrosis of liver parenchyma with fibrosis⟶ (H&E stainX20).

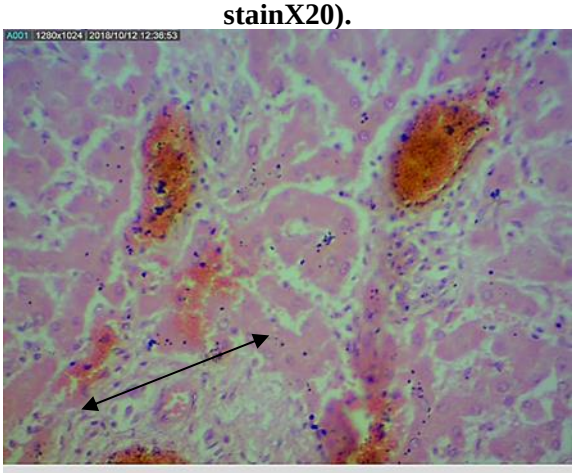

Figure 5. Histopathological section in the liver of 1st group Showed cellular swelling degeneration and MNCs infiltration ⟷ (H&E stainX20)

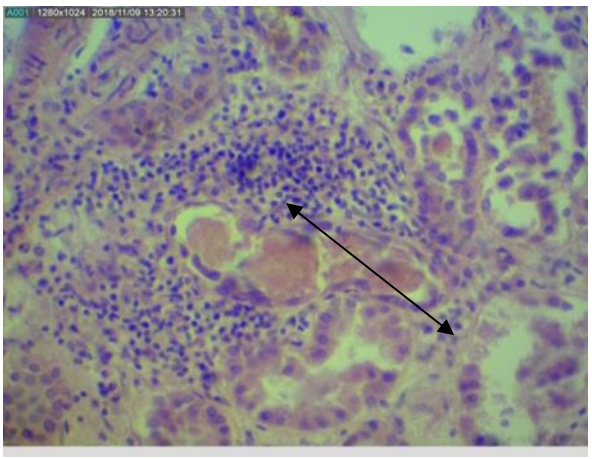

Figure 6. Histopathological section in the kidney of 1st group showed congestion and MNCs infiltration with renal tubules dilatation (H&E stainX40).

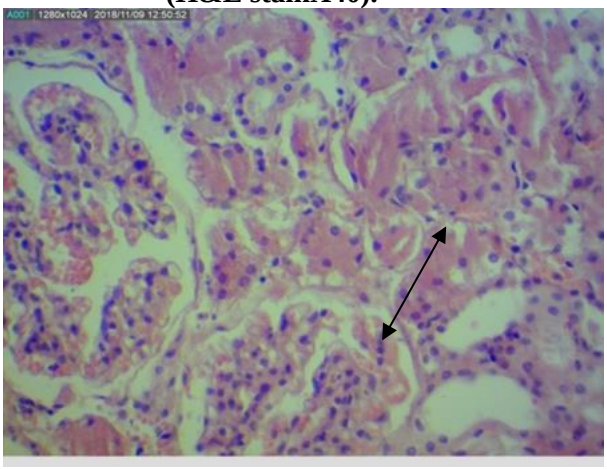

Figure 7. Histopathological section in the kidney of 1st group showed acute cellular degeneration with congested of glomeruli and renal tubules (H&E stainX40).

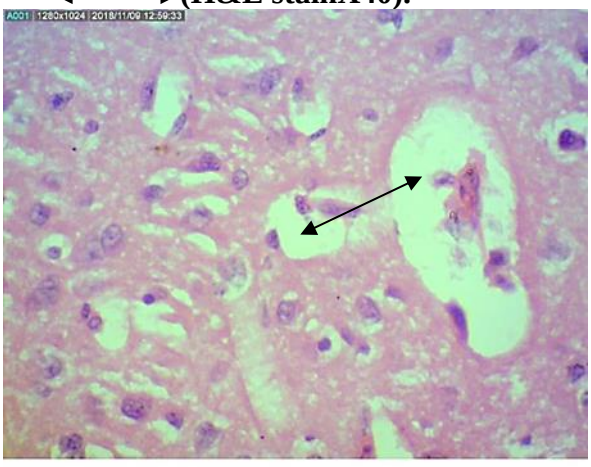

Figure 8. Histopathological section in the brain of 1st group showed odema and dilatation of meningeal blood vessels and necrosis of neurons (H&E stainX40).

The present result showed that animals treated with phenylmethane for 8 weeks expressed significant(0.05) of ALP and AST as comparing to control negative group, these finding may indicated that the phenylmethane causes damage of the hepatocytes by phenylmethane, this evidence was in agreement with Summaedaey, A.S. 1989 who explained that the phenylmethane induce necrosis of liver cells and this coincident with our results of histopathology. The pathological lesions in examined organs in 1st group treated with phenylmethane may be attributed to oxidative stress is importantly convoluted in the pathophysiology of different hepatic lesion due to treatment with phenylmethane which lead to reactive oxygen species such as malondialdehyde, 4-hydroxynonenal and declined anti oxidative defense systems, inflammation and injury Kim, J.W. *et.al* 2015**.** The histopathological change in the liver showed necrotic of liver parenchyma with fibrosis ,cellular swelling degeneration and MNCs infiltration with narrowing of sinusoid with deposition of hemosiderin these changes agree with Arauz, J. E. *et.al* 2016 who found that the lipids, hepatocytes proteins and deoxyribonucleic acid are amid the cell buildings that chiefly pretentious by reactive oxidative stress and reacted species of nitrogen, this process disrupted at cellular and molecular level the structural-function association on hepatic cells at diverse places, the lesions agreement were recorded by Gislaine, CR. *et.al* 2012 who study that the liver cells of mice treated with Permethrin caused austere changes in the hepatic cell, decreasing of nuclei size and causing vacuolar changes in the hepatocytes, exciting multiplying of Kupffer cells, changed the amount of proteins, polysaccharides, lipids, and vacuoles in the cytoplasm. Outcome of reactive oxidative stress on mitochondrial proteins influence which would weaken function of mitochondrial and cause some toxic effects to hepatic cell at last Bailey, S.M. *et.al* 2002. ROS and RNS generation activated by oxidative stress signals that induced by cytokine in liver parenchymal and and inflammatory cells induction, the shift in the stability of cytokines in hepatocytes including TNF-α, IL-1β and IL-6 also gives to liver destruction in intoxicating hepatitis Hoek, J.B. *et.al*.2002 The other pathological lesions hemorrhage in the liver and spleen parenchyma attributed to toxic effects of

organic compounded on endothelial cells that lead to rupture of blood vessels that agree with Szretter, K.J. *et.al*. 2007 who found that pesticides such as (IC) can induce rupture in the wall of central arteries of the spleen. The pathological lesions in the kidney may be linked with enlarged levels of ROS/RNS and reduced antioxidant ranks that agree with Alwan, MJ. 1996 who found that impairment of post proximal nephron function, with subsequent disturbance of cellular acid-base homeostasis when exposure to toxin. The present study showed narrow glomerular space due to hypercellularity of the glomerular at Gislaine, CR. *et.al* 2012 weeks post-treatment, this result was similar to that reported by (Saleh, A. A. 1993 and Shalaby, S. M. *et.al*. 2010 who recounted that treated rats (with cypermethrin, permethrin and fenvalerate) kidneys showed diverse forms of degenerative changes varied from of acute cellular swelling, to vacular degeneration. Numerous variations of kidneys cause by reactive oxidative stress via production of oxidant greater than before and reduced antioxidant defense system Palipoch, S. 2013. According to Robbins, M.E.C. *et.al*. 2002 irradiation induced ROS generation and lead to nephritic disease in rats via oxidative stress generation, specific DNA oxidative stress marker detected and local renal irradiation explains a marked, dose-self-determining rise in glomerular and tubular cell nuclear DNA oxidation complementarily with determined and chronic oxidative stress.

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