

Pathological study of subchronic oral toxicity induced by Di (2- ethyl hexyl) phthalate in Japanese quails

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Abstract

In present study, we aimed to investigate the pathological changes induced by subchronic oral toxicity induced by sub lethal dose of Di (2-ethyl hexyl) phthalate (DEHP) in Japanese common quail. Forty male quails at one day old were divided randomly into two equal groups, the first group (treated group) was orally administrated by 10% of LD₅₀ of DEHP (557.41 mg/kg of body weight) for 42 days, while second group (control group) left without treatment throughout experiment period. The results of current study showed that there is congestion of kidney, microscopically they showed presence of hyaline degeneration in renal tubules, glomerulosclerosis, wide spread of degenerative and necrotic lesions in renal tubules, hemorrhage, infiltration of inflammatory cell especially macrophages and lymphocytes in interstitial tissue of kidney. While grossing of liver showed hepatomegaly with hemorrhage, microscopically the sections showed wide distributing of vacuolar degeneration (fatty change) in different size and shape of vacuoles in cytoplasm of affected hepatocytes, infiltration of inflammatory cells mainly macrophages and lymphocytes. In conclusion, the oral administration of DEHP at dose 557.41 mg/kg of body weight for 42 days induced degenerative and necrotic irreversible changes in both kidney and liver of Japanese quail that affect their health and production.

Keywords: Pathology, Subchronic toxicity, DEHP, Quails, Kidney, Liver

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دراسة مرضية للتسمم الفموي تحت المزمن المحدث بثنائي (٢- اثيل هكسيل) فثالات في طيور السمان الياباني

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الخلاصة

في الدراسة الحالية، هدفنا الى التحري عن التغيرات المرضية المحدثة بواسطة التسمم الفموي تحت المزمن بالجرعة دون المميتة الوسطية لثنائي (٢- اثيل هكسيل) فثالات في طيور السمان الشائع الياباني. أربعون ذكر من طيور السمان بعمر يوم واحد قسمت عشوائياً الى مجموعتان متساويتان، المجموعة الأولى (مجموعة المعاملة) جرعت فمويًا ١٠% من الجرعة المميتة الوسطية لمُلدن ثنائي (٢- اثيل هكسيل) فثالات (٥٥٧.٤١ ملغم / كغم من وزن الجسم) لمدة ٤٢ يوماً، اما المجموعة الثانية (مجموعة السيطرة) تركت بدون اية معاملة خلال فترة التجربة. اشارت نتائج الدراسة الحالية الى وجود احتقان في الكلى، ولوحظ نسجياً في مقاطع الكلى التتسكس الزجاجي hyaline cast في النبيبات الكلوية وانتشار واسع لآفات تنكسية وتنخرية في النبيبات الكلوية مع النزف فضلاً عن ارتشاح خلايا التهابية وخاصة البلعمات والمفوية في النسيج الخلائي للكلية. اما بالنسبة للفحص العياني للأكباد لوحظ ضخامة الكبد مع النزف، ولوحظ نسجياً في مقاطع الكبد انتشار واسع للتتسكس الفجوي (التغير الدهني) بأشكال واحجام مختلفة من الفجوات في هيولي الخلايا الكبدية، مع ارتشاح للخلايا الالتهابية والتي كانت بشكل أساسي الخلايا البلعمية والمفوية. نستنتج من هذه الدراسة ان التجريب الفموي لثنائي (٢- اثيل هكسيل) فثالات

بجرعة ٤١.٥٥٧ ملغم/كغم من وزن الجسم لمدة ٤٢ يوماً أحدثت تغيرات تنكسية وتخريرية لارجعية في كل من كلية وكبد السمان الياباني والتي تؤثر على صحة وانتاجية هذه الطيور.

Introduction

Phthalates are mostly plasticizers used to give flexibility and strength to polyvinyl chloride (PVC) plastics (1). Generally, phthalates used as additives in food and personal care products (2), paints, lacquers, adhesive and printing inks (3).

Di (2-ethylhexyl) phthalates (DEHP) a widely-used plasticizer of phthalate esters characterized by colorless oily liquid that easily dissolved in water, blood and other fluids containing lipoproteins (4). DEHP was first created chemically in Japan at 1939, now about 95% of DEHP is added to PVC as plasticizer (5). DEHP induced a global attention after many reports and studies about its toxicological potential and environment pollutants of both human and animals (6,7). DEHP have highly affinity to be dissolved in water and blood, therefore many countries prevented medical devices to be manufactured from DEHP plasticized PVC plastics (8-11). The environment of breeding of Japanese quail contained many sources of DEHP plasticized PVC devices that liberate these plasticizers in environment that cause serious disease in other animals (12), even more the toxic pathological effect of DEHP in Japanese quails not investigated before and even the subchronic exposure to sub lethal dose of DEHP not established previously. Therefore, the current work aimed to study the pathological changes induced by subchronic oral administration of sub lethal dose of DEHP in common Japanese Quails *Coturnix coturnix japonica*.

Materials and methods

Ethical statement

All of the experimental procedures were conducted in accordance with the regulations of the College of Veterinary Medicine, University of Mosul, concerning protection of animals against cruelty.

Experimental animals

A total of 40 males of Japanese quails at one day old weighing 10 - 12 gm were used in this work, they are maintained at 24 hours day at first three days of life then reduced to 20 hours day and 4 hours night cycle for the first week of age, later the day hours decreased by two hours weekly until fifth week of age were the light cycle is maintained at 12 hours day 12 hours night cycle. Quails were housed in clean properly ventilated caged free of DEHP plasticized PVC devices with clean litter made of wood with free access to food and water throughout the experiment period.

Study groups

At first day of age animals were divided into two groups as followed: Group I (DEHP treated group): a 10% of LD₅₀ of DEHP were given via orogastric intubation once daily for 42 days from one day old of quails. Group II (Control group): left without any treatment throughout the whole period of experiment.

At the end of experiment time, all quails were euthanized using sharp knife by decapitation, after death specimens from tissue of kidney and liver were obtained for paraffin embedding with light microscopic examination.

Histopathological study

Samples of kidney and liver tissue were fixed in 10% neutral buffered formalin for 72 hours, dehydrated by descending concentration of ethyl alcohol, cleared by chloroform and xylene, infiltrated by hot paraffin wax at 58- 60 °C, later these tissue samples were embedded in paraffin, sectioned at 4-6 µm and stained by routine Harris hematoxylin and eosin stain (H&E) (13). Stained sections were examined with light microscopy at 40x and 400x magnification power (14).

Chemical supply

Di (2-ethylhexyl) Phthalate from Sigma - Aldrich company were used. DEHP were supplied as oily liquid of odor even colorless at purity of 99.50% with concentration of 0.995 gram / ml of supplied solution.

Oral administrated dose

A 10% of LD₅₀ of DEHP were have been used, the LD₅₀ were calculated by (15) at same age and weight, which is 5574.1 mg/kg of body weight and the toxic dose used in current work was 557.41 mg/kg of body weight in Japanese quails, and the size of administrating dose 0.250 ml / quail. This dose calculated by dilution one milliliter of DEHP (contain 995 mg/ml) in 10 ml of distilled water.

Results

The results of current study showed that the gross examination of kidney reveled congestion of both kidneys which appears as red to black in color (figure 1-A), microscopically the kidney sections showed presence of hyaline degeneration in renal tubules with glomerulosclerosis (figure 1-B), also wide spread of degenerative and necrotic lesions in renal tubules hemorrhage (figure 1-C), these all these lesions caused loss of normal architecture of kidney tissue with infiltration of inflammatory cell especially macrophages and lymphocytes

in interstitial tissue of kidney around affect renal tubules (figure 1-D).

While grossing of liver showed hepatomegaly with sub capsular hemorrhage (figure 2-A), microscopically the liver sections showed wide distributing vacuolar degeneration (fatty degeneration) in affected hepatocytes (figure 2-B), these lesions appear in different size and shape of well

bounded washed vacuoles in cytoplasm of affected hepatocytes leading to pushing nucleus at cell edge given it ring like shape similar to fatty tissue (figure 2-C), these lesions observed with infiltration of inflammatory cells at centrilobular region (around central vein) mainly macrophages and lymphocytes (figure 2-D).

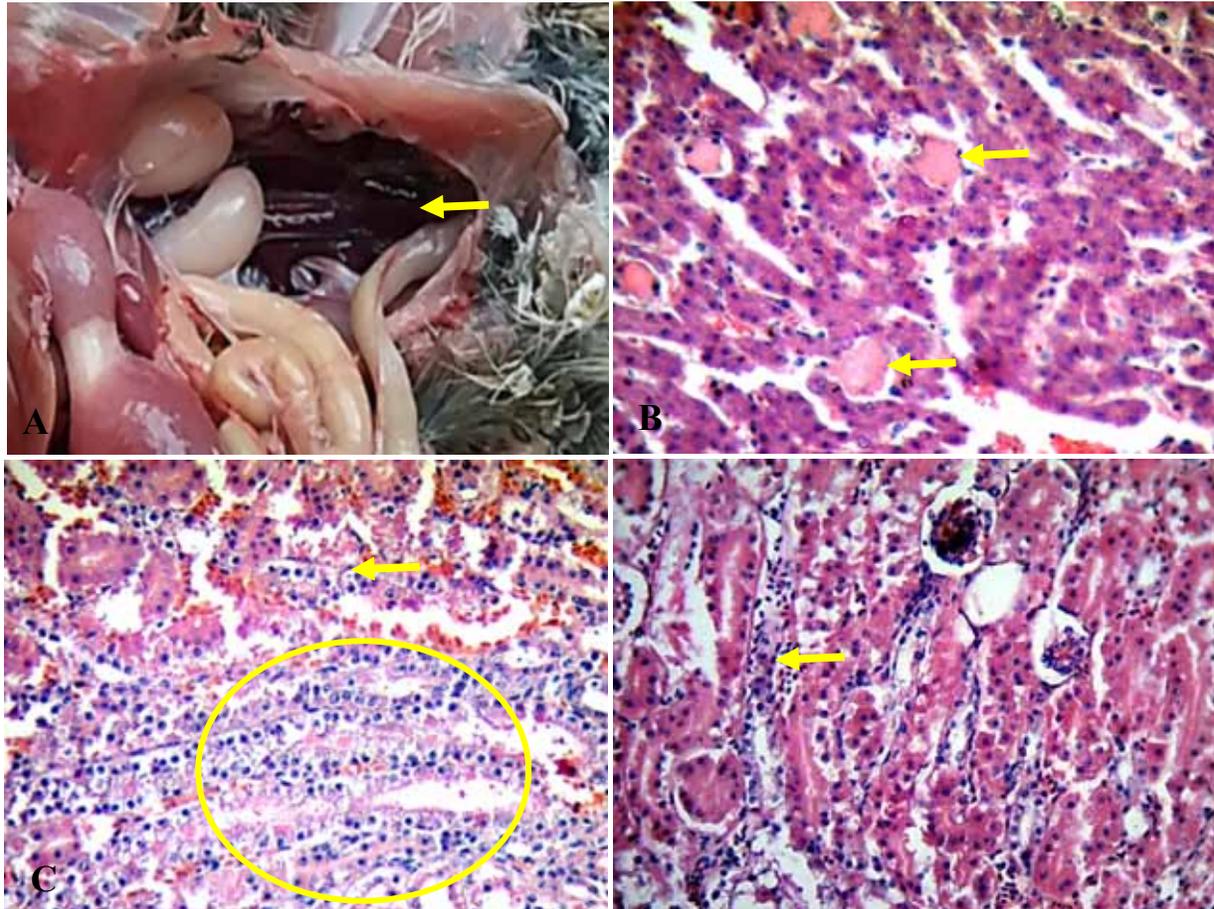


Figure 1: Quail kidney, after 42 day of oral administration of DEHP at dose of 557.41 mg/kg. (A) Congestion of kidney (arrow). (B) Hyaline cast in lumen of renal tubules (arrow). 40x. HE. (C) Coagulative necrosis of renal epithelial cells (arrow) with vacuolar degeneration (circle). 400x. HE. (D) Infiltration of inflammatory cells especially macrophages and lymphocytes around affected renal tubules. 400x. HE.

Discussion

Kidney consider the main site for DEHP excretion while liver the site of metabolism after absorption from intestines (16), which explain the severity of pathological changes in these two tissues. in liver DEHP converted to mono (2-ethylhexyl) Phthalate (MEHP) which is more toxic and more oxidative metabolite of DEHP (7). The result of current histopathological study was in agreement with other studies conducted in mice, rats and poultry exposed to

DEHP, in which there is degenerative and necrotic changes in renal tubules and hepatocytes (12,17).

Glomerulosclerosis present in DEHP treated quails section occur due to continuous irritation by oxidative stress produced by both DEHP and MEHP that lead to inflammatory reaction with tissue destruction using deposition and formation of scar tissue in affected glomeruli with fibrosis lead to loss of function of these secretory units (18).

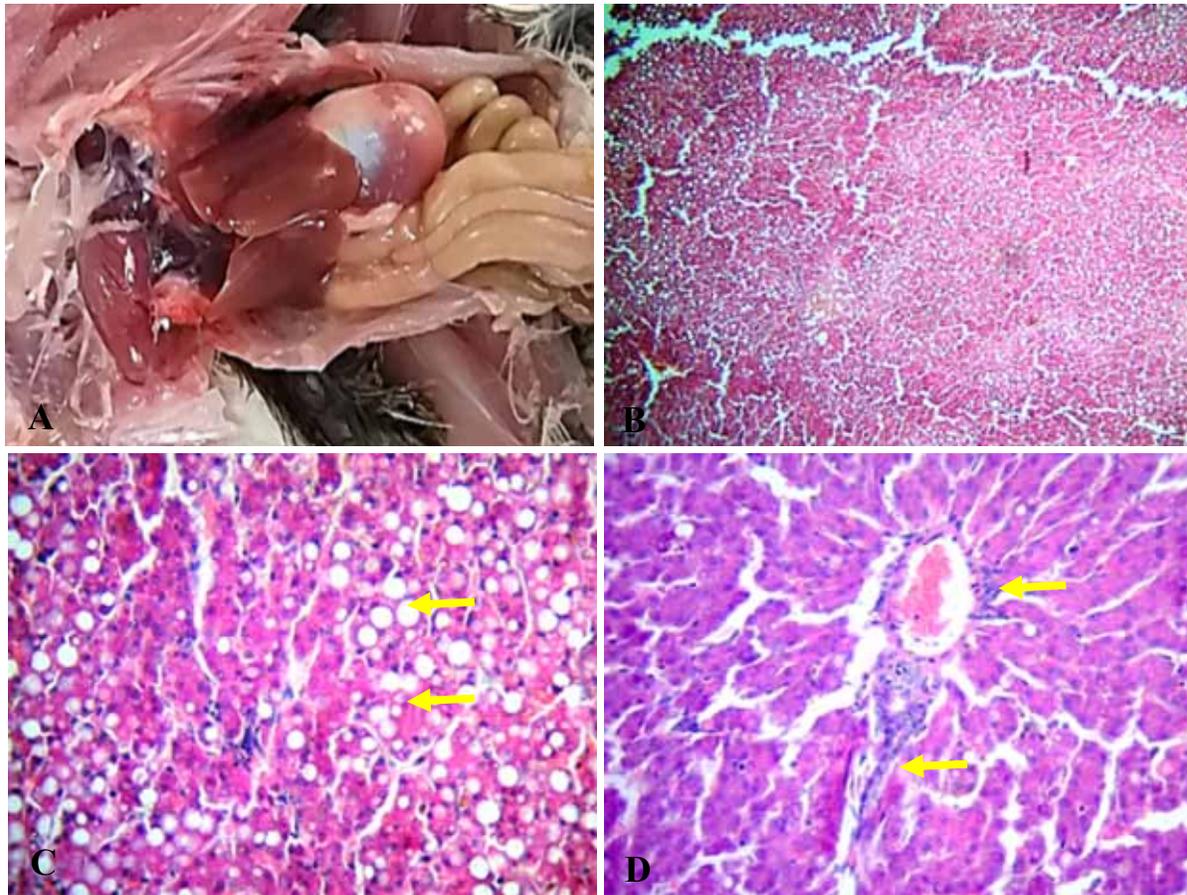


Figure 2: Quail liver, after 42 day of oral administration of DEHP at dose of 557.41 mg/kg. (A) Hepatomegaly with hemorrhage. (B) Wide distribution of vacuolar degeneration in hepatocytes. 40x. HE. (C) High magnification of previous image (arrow). 400x. HE. (D) Infiltration of inflammatory cells at centrilobular (arrow). 400x. HE.

The vacuolation lesions observed in hepatocytes cytoplasm in quails administrated DEHP occur due to increase in lipid peroxidation that take place in hepatocytes, in which the DEHP and MEHP produce abundant generation of superoxide anion causing increase in lipid peroxidation, with time continuous elevated lipid peroxidation lead to cellular injury and changes in cell membrane permeability lead to failure of $\text{Na}^+ - \text{K}^+$ pump lead to influx of fluid inside hepatocytes which accumulated in form of vacuoles in addition to lipid that accumulated in vacuoles of cisterna from endoplasmic reticulum (19).

In addition to previous pathophysiological mechanism in hepatocytes, the exposure to DEHP lead to activation Peroxisomes Proliferating Activating Receptors (PPARs) that lead to increase in expression of gens that coding to different types of enzymes involve in fatty acids metabolism as palmitoyl- CoA oxidase, enoyl-CoA hydratase, carnitine acyltransferase and α -glycerophosphate

dehydrogenase, all these enzymes that increase in concentration in hepatocytes caused alteration in β oxidation that lead to increase in fatty acid metabolism especially large and medium in hepatocytes that not coordinated with increase in conjugation with proteins to form lipoproteins that excreted from hepatocytes which accumulate in endoplasmic reticulum that secreted in well bounded vacuoles, also the increase of CoA above elimination level of free radicals by catalase lead to damages in hepatocytes DNA (20,21).

The infiltration of inflammatory cells in both renal and hepatic tissue were noticed in current work due to production of IL-8 and IL-6 cytokines that released from exposed cells to DEHP and activated monocytes lead to chemical attraction of lymphocytes and macrophages (22).

In conclusion, the present study revealed that the subchronic oral toxicity of plasticizer DEHP in common Japanese Quails induced important degenerative and

necrotic changes in both kidney and liver that interfere with health and production of these birds.

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