Histological Changes of Liver, Kidney and Adrenal gland Tissues of Male Rats *(Rattus norvegicus)* Treated with Carbofuran.

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Abstract

The present study included the histological effects of the former daily orally intake from the carbofuran pesticides on liver, kidney and adrenal gland tissues of male albino rats. Thirty-two mature male rats were randomly divided into four equal groups; each group consists of eight males. First group was gives of normal saline for 30 consecutive days as control group, while second, third and fourth groups were given daily an oral dose equivalent to (2, 4, 8 mg/kg body Wight) of carbofuran pesticides for 30 days.

Histological examination that observed in the liver of rats received of carbofuran pesticides; including vacuolation of hepatocytes in centrilobular area and congestion of the central vein compared with the control group. The kidneys revealed that there was some deposition of amyloid, vacuolation of mesangial cells and cloudy swelling of renal tubular epithelial cells, while in the adrenal gland the changes including vacuolation and fatty changes in treated group compared with the control group.

Key words: carbofuran- liver- kidney - adrenal gland – histopathlogicl changes.

Introduction

The environmental pollution is one of the most serious problems that face mankind in this century; there are many types of pollutants that interfere with our-life both directly and indirectly. Furthermore, potential future hazards to human health and wildlife can be created by residues from some long-lived pesticides that may build up in the food chain and cause widespread contamination of the environment (Zaahkouk *et al*., 2000).

Pesticides are biological active chemicals used in agriculture to destroy or control weeds, insects, fungi and other pests (Chavarri *et al*., 2004). When pesticides are applied improperly, the resulting residues in the produce can pose significant health risks to consumers, who are increasingly aware of the potential for contamination of food and drinking water (Hotchkiss, 1992).

Carbofuran (2, 3-Dihydro-2, 2-dimethyl-7-benzofuranyl-N- methylcarbamate) is a broad spectrum systemic carbamate pesticide that kills insects, mites and nematodes on contact and after ingestion(Finlayson *et al*., 1979; Flickinger *et al.*, 1980; Kamboj *et al*.,2008). Carbofuran and other carbamate pesticides have widely replaced the more persistent and hazardous organophosphate pesticides used in agriculture due to its relatively short residual life in the environment and rapid degradation (Eisler,1985). As a result of its widespread use,

air, food, surface water and groundwater are contaminated with carbofuran and its metabolites which may affect the health of everyone(Aziz *et al.*, 2008). Serious threat exists to those who are in immediate contact with this substance during its use in manufacturing factories (Hamid *et al*., 2012). Various Experimental studies reported congenital malformation in chicken and duck embryos with carbaryl (Marliac *et al*., 1965) The histopathological changes were seen in various organs of Wistar rats like heart, liver, lung and brain on dermal exposure to carbaryl for four weeks (Toś-Luty *et al*.,2001). Carbofuran is highly toxic to fish and birds particularly in granular form, which birds often, eat mistaken for seeds, or through contaminated drinking water. Pesticides may induce oxidative stress, leading to generation of free radicals and alteration in antioxidants, oxygen free radicals, the scavenging enzyme system, and lipid peroxidation (Baron, 1991; Etemadi *et al*. 2002).

In view of the inconsistent findings, the present study was carried out to investigate the effect of Carbofuran on liver, kidney and adrenal gland by identifying the histopathological changes.

Material and methods

The experiment was conducted at the laboratory animal house of the Sciences College – University of Thi-Qar, where 32 males white laboratory rats (*R norvegicus*) sexually mature 16 weeks age and 250±50 gm weighing, were used. The animals were accommodated in the same laboratory condition by keeping them in special cages (acrylic cages) as (6 animals per cage) for about 30 days. The experiment conditions were unified for all animals, where the room temperature was set between 20-25 ^{O}C by the use of air conditioner, and the light period was 12 hours daily, by the use of two fluorescent lamps, and the humidity rate was about 50%. Food and water were provided daily (ad libitum).

Experimental Design

The animals of the experiment were divided into four groups with 8 male in each group. All groups were given daily oral single dose of treatment for 30 days as follows:

Group A: (Control): Administered orally 0.9 % normal saline (N.S) daily.

Group B: Administered orally 2 mg/kg B.W of Carbofuran daily.(low dose)

Group C: Administered orally 4 mg/kg B.W of Carbofuran daily.(intermediate dose)

Group D: Administered orally 8 mg/kg B.W of Carbofuran daily.(high dose)

Procedure of Tissue Processing

Histological examination carried out according to (Luna, 1968), liver, kidneys and adrenal glands were dissected out and fixed instantaneously in 10% formal saline for 24 hours. The specimens were washed in tap water, dehydrated in ascending grades of ethanol, cleared in xylene, embedded in paraffin wax. Paraffin sections were cut at 6µm thicknesses using a rotary microtome; the sections were stained with Harris haematoxylin and eosine. Observations were made using a light microscope and photographs were taken with an automatic photomicrographic system.

Results

Liver

Sections of control showed normal histological structures. (Figs.1). Furthermore, Group treated with a dose of (2) mg/kg.b.w of carbofuran for (30) days showed vacuolation of hepatocytes in centrilobular area (Fig.2).

While animals treated with a dose of (4) mg/kg. b.w of carbofuran orally for same time showed congestion of the central vein (Fig.3).. Moreover, group treated with a dose of (8) mg/kg. b.w of carbofuran for same time showed vacuolation of hepatocytes in centrilobular area , marked congestion of the central vein and marked sub capsular hemorrhage Fig (4,5).

Kidney

Sections of control rats showed normal histological structures (Fig.6). In addition further histological changes in kidney as:

Group treated with a dose of (2) mg/kg. b.w of carbofuran for (30) days showed slight deposition of amyloid , slight vacuolation of mesangial cells and cloudy swelling of renal tubular epithelial cells. (Figs.7and 8).

Also animals treated with a dose of (4) mg/kg. b.w of carbofuran orally for same time showed slight vacuolation of mesangial cells and cloudy swelling of renal tubular epithelial cells (Figs.9)and show amyloid infiltration in the renal tubular epithelium(Fig.10). Moreover, group treated with a dose of (8) mg/kg. b.w of carbofuran for same time showed amyloid infiltration in the renal tubular epithelium , vacuolation of epithelial cells in the cortical tubule Figs. (11,12 and 13).

Adrenal glands

Adrenal glands sections of control rats showed normal (Fig.14). Animals treated with a dose of (2) mg/kg. b.w of carbofuran for (30) days showed variable histological changes in structures and those changes were low vacuolation as in Fig (15). While animals treated with a dose of (4) mg/kg. b.w of carbofuran orally for same time showed clear vacuolaton as observed in (Fig.16). Moreover, exhibited sever vacuolation and fatty changes in treated group with (8) mg/kg. b.w. Fig(17).

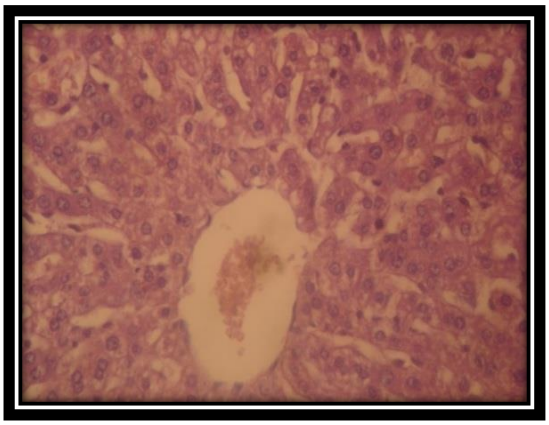

Fig (1): liver appear within normal limits 100X

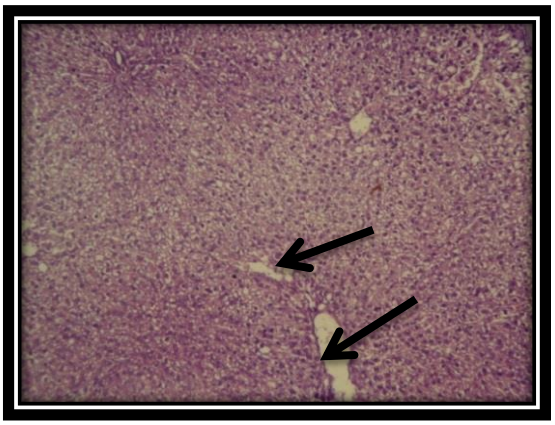

Fig (2): liver of low dose group show vacuolation of hepatocytes in centrilobular area. H&E(100X)

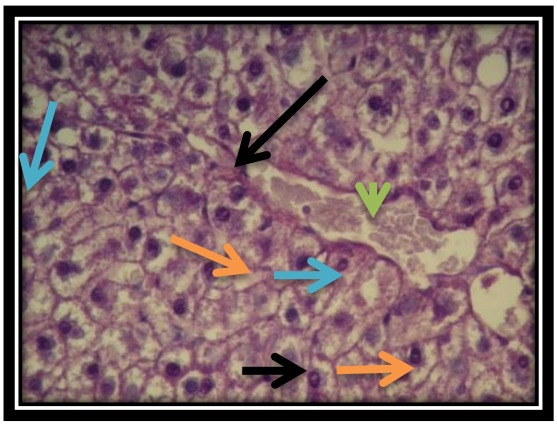

Fig (3): liver of intermediate dose group show vacuolation of hepatocytes in centrilobular area marked congestion of the central vein Pyknosis Referred to analysis of nucleus. H&E (500X).

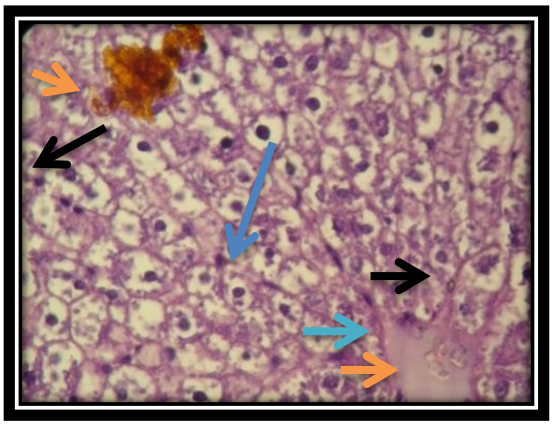

Fig (4): liver of high dose group show vacuolation of hepatocytes in centrilobular area marked congestion of the central vein Pyknosis H&E(500X).

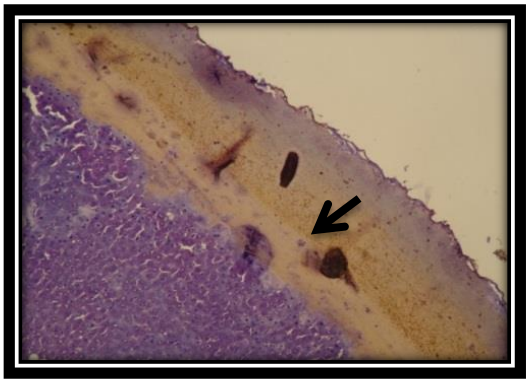

Fig (5): liver of high dose group show marked sub capsular hemorrhage. H&E

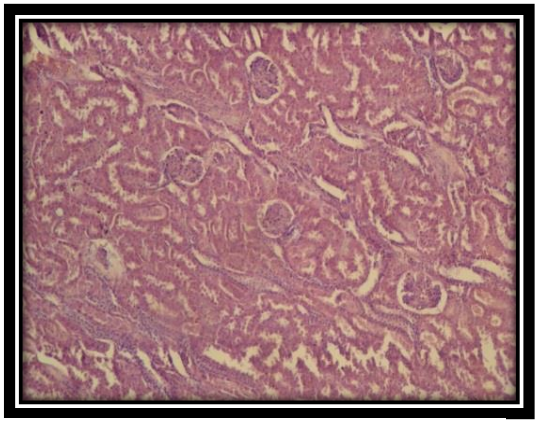

Fig (6): kidney appear within normal Limits (100X).

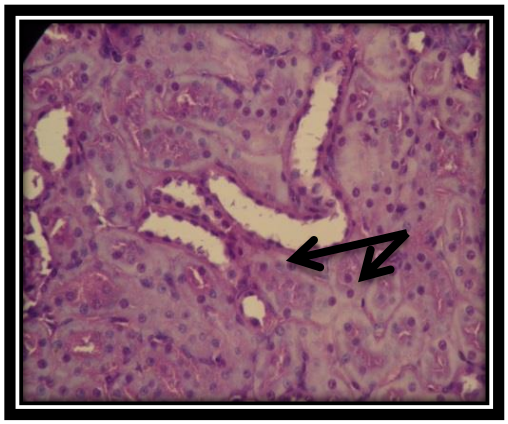

Fig (7): kidney of low dose group show slight deposition of amyloid H&E (200X).

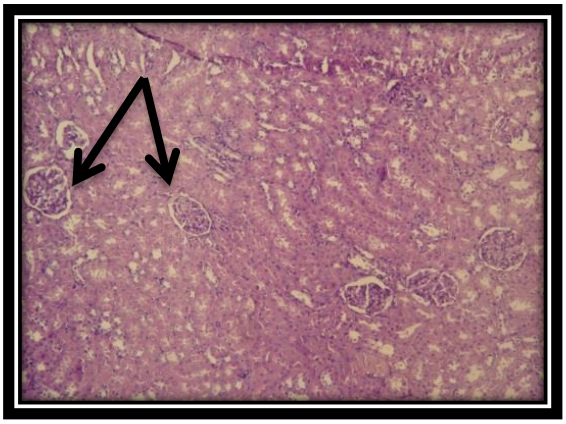

Fig (8): kidney of low dose group show cloudy swelling of renal tubular epithelial cells. H&E.100X

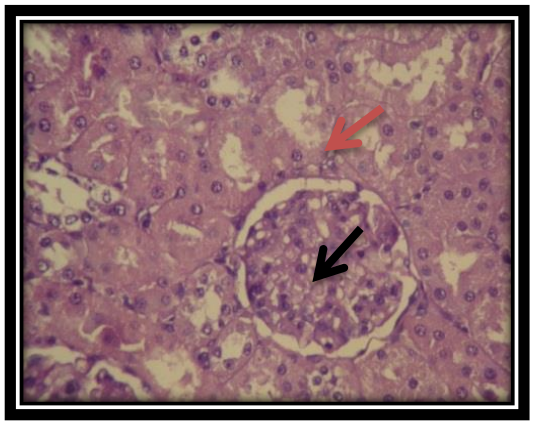

Fig (9): kidney of intermediate dose group show ( ) slight vacuolation of mesangial cells ( ) cloudy swelling of renal tubular epithelial cells . H&E (500X)

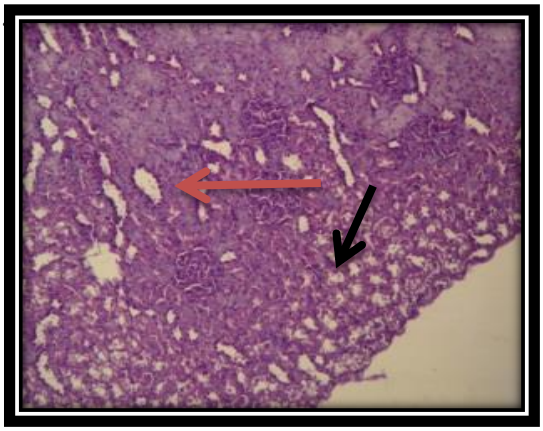

Fig (10): kidney of intermediate dose group show amyloid infiltration in the renal tubular epithelium

H&E. 500X

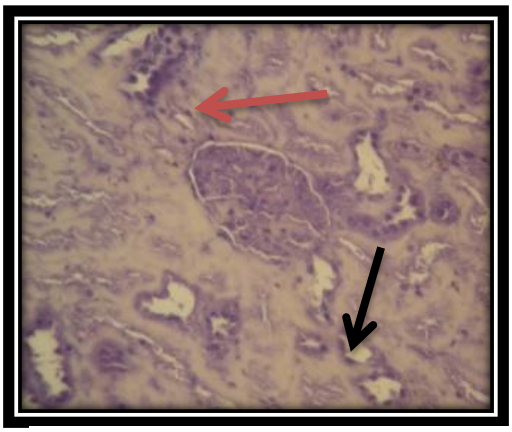

Fig (11): kidney of high dose group show (⟶) amyloid infiltration in the renal tubular epithelium ( ) vacuolation of epithelial cells in the cortical tubule. H&E(100X)

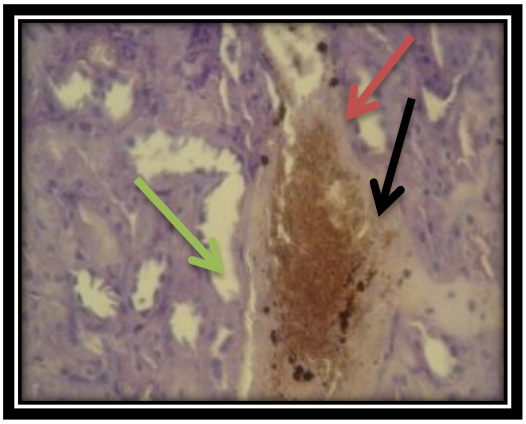

Fig (12) kidney of high dose group show (→) marked deposition of amyloid (⟶) narrowing of tubular lumen. H&E (125X)

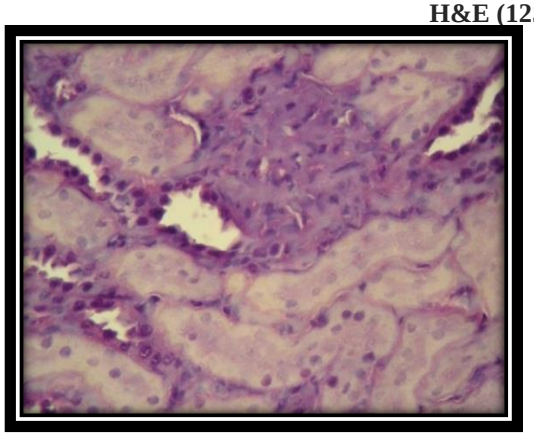

Fig (13): kidney of intermediate dose group show (marked deposition of amyloid ( ) narrowing of tubular lumen ( ) renal vascular congestion . H&E (125X)

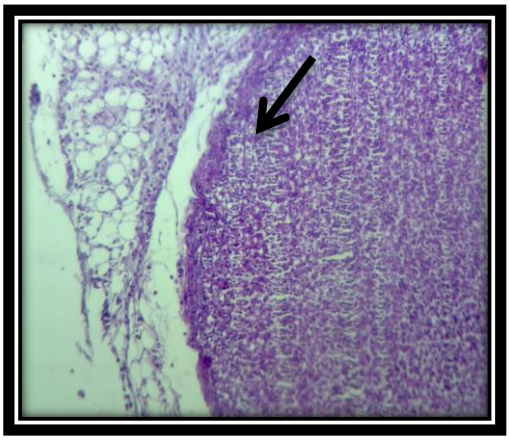

Fig (14): Adrenal gland appear within normal limits (100X)

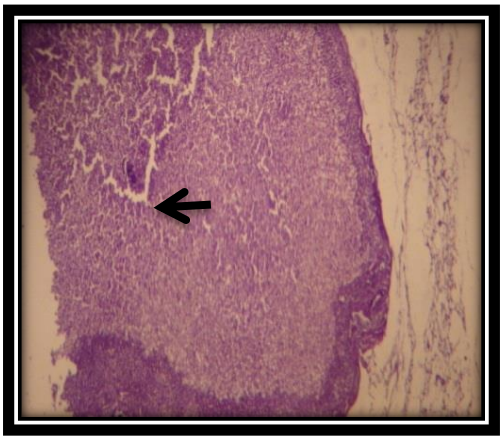

Fig (15): Adrenal gland of low dose group show Low vacuolation. H&E (100X)

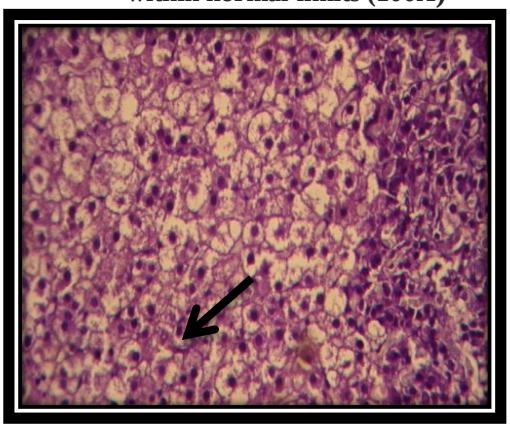

Fig (16): Adrenal gland of intermediate dose group show increase vacuolation. H&E(200X).

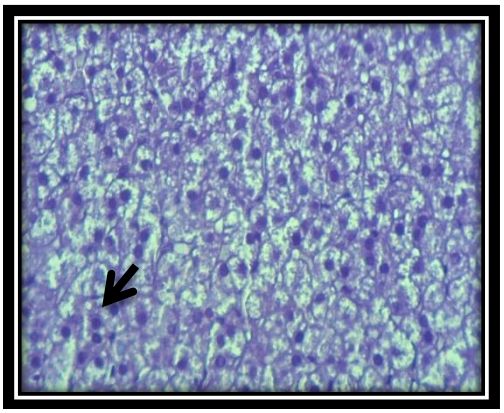

Fig (17) Adrenal gland of high dose group show Enlarge zona facuolated cell with fatty droplet. H&E (200X)

Discussion

The present study demonstrated that 30-days the oral intake exposure of rats to carbofuran at was caused the liver appeared show vacuolation of hepatocytes in centrilobular area, marked congestion of the central vein and sub capsular hemorrhage.

The results are in agreement with many authors (El-Damaty *et al*.,2012;Hamed *et al*.,2012; Ksheerasagar and Kaliwal,2006; Selmanoglu and Akay, 2000) they reported similar

histopathological changes including mononuclear cell infiltration, congestion, hydropic degeneration and hepatocellular damage in the liver of male rats treated with dimethoate, endosulfan and carbaryl. Also, Benjamin *et al*. (2005) who found that a 60 day exposure of male rats to pesticides mixture of monocrophos (0.156 mg/day),endosulfan (0.2 mg/day) and HCH (1.3 mg/day) caused fatty change with necrosis in the liver of rats that suggest this change indicated that some toxic metabolites may be transported from intestine to liver ,resulting in these changes. Similar studies showed the synergistic toxic effect of an organophosphate pesticide Dimecron and a carbamate fungicide on liver and brain, that reported the histopathological changes like the destruction of hepatocytes and extrusion of nuclei(Thangavel et al.,1994)

The deposition of amyloid, slight vacuolation of mesangial cells , cloudy swelling of renal tubular epithelial cells, narrowing of tubular lumen and renal vascular congestion. Also, different other studies, agreement with this present study , showed that carbofuran and other pesticides induced liver and kidney in experimental animals (Attia and Naser,2009;Uboh *et al*.,2011; El-Damaty et al.,2012) .They reported similar histopathological changes including some blood congestion between tubules and small area of hemorrhage in the interstitium of blood vessels . Thus, observing changes in kidney may be thought that oxidative stress may play a role to the mediator in changing configuration of cell membrane and seem to account for the morphologic alteration of kidney (Saadi et al.,2008).

In the present study, we observed that carbofuran induced a Vacuolation and Enlarge zona facuolated cell with fatty droplet in adrenal gland .the results agree with (Zayed, 2002 and Akhtar *et al.,* 2009) that showing the administration of chlorpyrifos pesticedes reduced weight gain and histopathological changes in adrenal gland with significant brain and plasma AChE inhibition.

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التغيرات النسيجية المرضية لكبد,كلى والغدة الكظرية لذكور الفئران *(Rattus norvegicus)* المعاملة بمبيد الكلورفوران

رشا صالح نهير

علوم الحياة, كلية العلوم, جامعة ذي قار

الخلاصة

اجريت الدراسة الحالية لمعرفة تأثيرسمية مبيد الكلوفوران على انسجة الكبد ,الكلى والغدة الكظرية لذكور الجرذان المختبرية.

استخدم في التجربة الحالية (32) ذكرا بالغا, قسمت عشوائيا الى اربعة مجاميع متساوية بواقع 8 لكل مجموعة اعطيت المجموعة الاولى المحلول الفسيولجي لمدة 30 يوم واعتبرت كمجموعة سيطرة, جرعت المجموعة الثانية والثالثة والرابعة ب(2- 4- 8 ملغم/كغم) على التوالي بمبيد الكلورفوران ولمدة 30 يوما.

بينت نتائج الفحص المجهري للأنسجة أن هناك تغيرات في كبد الفئران المعاملة بمبيد الكربوفوران, اذ شملت ظهور فجوات في خلايا الكبد و احتقان الوريد المركزي مقارنة مع مجموعة السيطرة, كما بين فحص الكلى أن هناك بعض الترسب للاميلويد مع تفجي الخلايا الظهارية للأنبوب الكلوي كما بينت النتائج وجود تغيرات في انسجة الغدة الكظرية متمثلة بظهور التفجي بالإضافة الى التغيرات الدهنية في المجاميع المعاملة مقارنة مع مجموعة السيطرة.