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Histopathological changes of liver and kidney in rat following chronic exposure to butachlor

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Abstract
Introduction
Butachlor (2-chloro- 2, 6-diethyl-N-butoxymethylacetanilide)the isselective, systemic chloroacetamide herbicide which used extensively as pre-emergent and early post-emergent herbicide in Africa, South America, Asia and Iranespecially in Northern Province.Butachlor remains in soil and absorbs in ground waters and affect aquatics, animals and humans. Butachlor has toxic effect on different organs. In this research we evaluated chronic exposure to butachlor toxin and to observe histological changes of kidney and liver in rats.
Method and Material
A total of 16 male Wight Wistar rats, having almost similar age (about 10–12 weeks) and weighted 150-200 kg were divided randomly in two groups, each 8 rats. The control group consumed normal regime and water. The case group was administered with sublethal dosebutachlor 72.2 mg/kg equivalent ¼ LD_{50} for 3 months. After 12 weeks, the specimen of kidney and liver tissue was prepared and examined under lighten microscope.
Conclusion
Single cell necrosis, lobular hepatitis, interface hepatitis and bile duct proliferation were the histopathological changes in rats liver chronic exposure of butachlor and tubular necrosis and cast formation were the changes in kidney.

Key words: Butachlor, histopathology, kidney, liver

Introduction

Chloroacetamides are one of the most common used herbicides in the world which are known by excellent quality and low cost(1). Butachlor (2-chloro- 2, 6-diethyl-N-butoxymethylacetanilide) is one of the most important member of Chloroacetamides which used extensively as pre-emergent and early post-emergent herbicide in Africa, South America and Asia(2). Selective, systemic herbicide, absorbed primarily through germinating shoots and secondarily by roots(3). Trans located throughout the plant, with higher concentration in the vegetative parts than the reproductive parts.Butachlor control annual grasses and broad-leaf weeds and applied on wheat, rice, corn, tea, soybean and other crops(4). In Asia, 4.5×10^7 kg butachlor used annually to control weeds(2). Butachlor used in Iran especially in Northern Province widely(5). Butachlor remains in soil and absorbs in ground waters and affect aquatics, animals and humans(6). Butachlor affects lipids biosynthesis by inhibiting elongation of non sphingolipid very long chain fatty acids (VLCFAs) and cause lack of lipids, proteins, and lignin for the plant(7). This herbicide reach body through gastrointestinal system, respiratory system and skin contact(8). Approximately 85% of an orally administered dose is eliminated in 48 hours, 60% of the excreted herbicide is found in feces and 40% in urine(9). Butachlor metabolism occurred in liver and slightly in kidney(10). Butachlor has toxic effect on different organs like kidney, liver, stomach, reproductive system and brain. This herbicide and other chloroacetamides cause nausea and vomiting, blood pressure disturbance, bradycardia, tachycardia, seizure, hypoxemia dyspnea in humans(11). Toxic effect of butachlor have been reported on very animals for example growth retard in Eisenia fetida earthworm ,12) (13, genotoxicity in frog and fish(15 ,14), neurotoxicity in snails(16). Butachlor cause increase Glutathione peroxidase enzyme activity and oxidative stress, amino acid metabolism disorder and neurotransmitter disorder in fishes(17). Also through activating cell production, suppression of growth inhibitors and making changes in cell cycle cause gastric tumors in rats(18). Butachlor also makes histopathological changes in kidney and liver. Studies show butachlor cause histopathological alterations in kidney and liver tissue of rats like hepatocytes' degeneration

and fatty changes in liver and tubular degeneration in kidney(19). Also histopathological changes in fishes organ have been reported including sinusoidal dilation, hyperemia, hemorrhage, bile duct hyperplasia, mononuclear necrosis, hepatocytes' hypertrophy, interstitial edema and cytoplasm degeneration in liver and vacuolar degeneration of tubular epithelium, necrosis of tubular epithelium, granular cytoplasm in kidney(20) . Since that liver and kidney are two important organs in butachlor metabolism and exertion, in this study we intend to investigate chronic exposure to butachlor toxin and to observe histological changes of kidney and liver in rats. This research increases awareness of the destructive effects of herbicides due to the widespread use of pesticides in farms.

Method and Material

Animals

A total of 16 male Wight Wistar rats, having almost similar age (about 10–12 weeks) and weighted 150-200 kg were procured from a Pastor Institute. All the rats were kept in wire cages under ambient temperature (24–27° C). All the standard protocols for maintaining rats were lied down. The institutional ethical committee of Alborz medical university approved the experimental protocols. The animals were divided into two groups randomly, each group contains eight rats. The experimental duration was 3 months. One group was control and consumed normal regime and water. The other group was case group and butachlor was administered with sublethal dose 72.2 mg/kg equivalent ¼ $LD_{50.}$ The toxicant butachlor was given to rats through oral gavage. The rats were kept in standard condition and sterile environment including 12 hours lightness and 12 hours darkness and 40-60% moisture. After 12 weeks, the rats were prepared for experiment. The rats anesthetized with 80mg/kg chlorate hydrate and kidney and liver tissues were extracted. The samples were stored in 10 a percent formalin solution for histopathological examination for 24 hours.

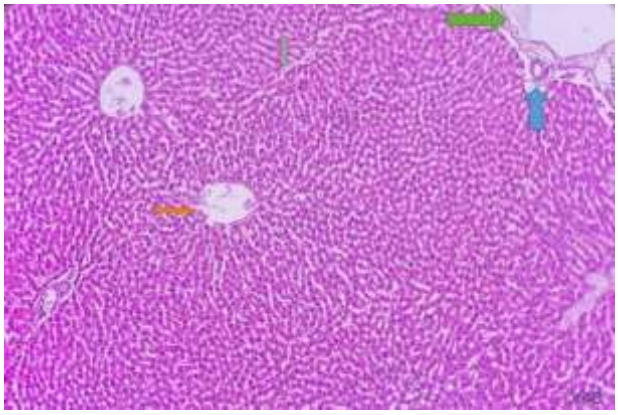


Fig 1. Transverse cut of liver tissue in case and control group (×40). In right picture (control group) and left one (case group) lobular structure of liver is intact. The red arrow is central vein. The gray arrow shows limiting plate. The green arrow shows portal vein and the blue one is bile duct.

Histopathological Technique

Representative sample piece of each organ were obtained and fixed in 10 a percent formalin fixative. After 72 hours fixation in fixator, the samples were kept in especial baskets in fresh 10 a percent formalin solution for 24 hours. Then samples were put in automatic tissue processor (autotechnicon). The tissue samples were immersing in ascending grades of ethanol in order to dehydrating. Ethanol is miscible with water in all proportions so that the water in the specimen is progressively replaced by the alcohol. Then the samples were cleared in xylene in order to remove additional fats around tissue specimen and prepare optimal transparency. Xylene also make possible the infiltration of paraffin wax to tissue samples. The samples were embedded with paraffin wax for block making. The specimens were cut at 5 μm thickness by rotary microtome. Finally the sections were stained by hematoxylin and eosin.

Histological parameters

After preparing the species, the tissue samples were observed from each rat under oil immersion lens (1000×) using computer-assisted light microscope. The hepatocytes were evaluated for fatty changes, cholestasis, necrosis and

inflammatory changes. The portal tracts for bile duct proliferation and infiltration of cells. The kidney tissue was evaluated for cell necrosis, size and cellularity of glomerulus and interstitial pathological changes.

Results

Liver histopathology

In histopathological study of liver in both case and control groups, hepatocytes, portal tracts and lobular structures of liver were examined. In both case and control groups, hepatic lobules were separated by narrow connective tissue and limiting plates were intact. Lobular structure of liver in both groups was intact. (Fig 1)

The green arrow shows portal vein and the blue one is bile duct.

Lobular hepatitis were observed in case group. In lobular hepatitis, infiltration of mononuclear cells into hepatocytes' parenchymal and hepatocytes necrosis is seen. (Fig 2)

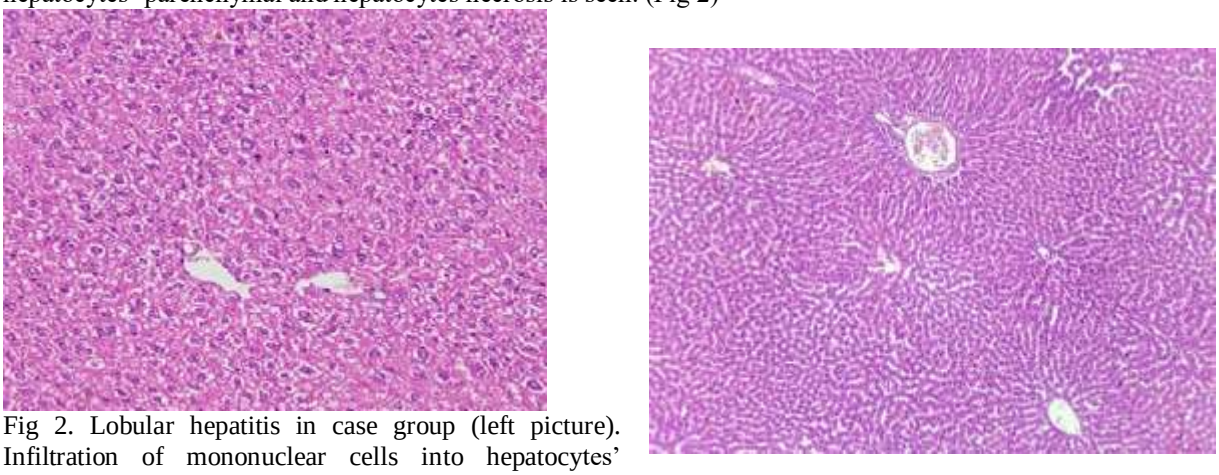
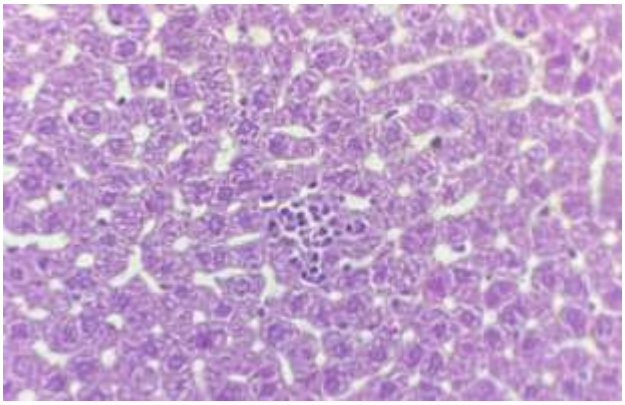
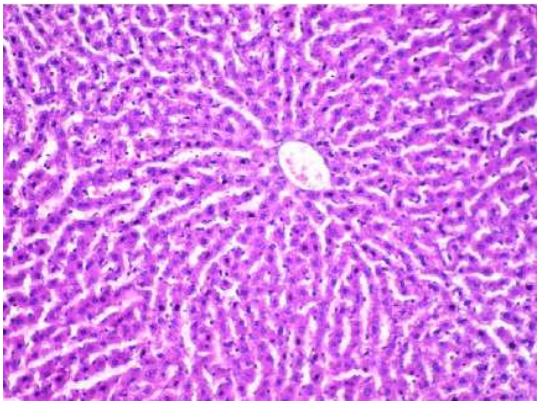


Fig 2. Lobular hepatitis in case group (left picture). Infiltration of mononuclear cells into hepatocytes' parenchymal is seen in control group (left picture), but control group (right picture) is intact.

Interface hepatitis were observed in case group. Interface hepatitis, formerly named piecemeal necrosis, is an inflammatory process which is defined by death of hepatocytes at the interface of parenchyma and the connective tissue of the portal zone, accompanied by a variable degree of inflammation and fibrosis. This term was used because the death of hepatocytes involve apoptosis as well as necrosis. (Fig 3)





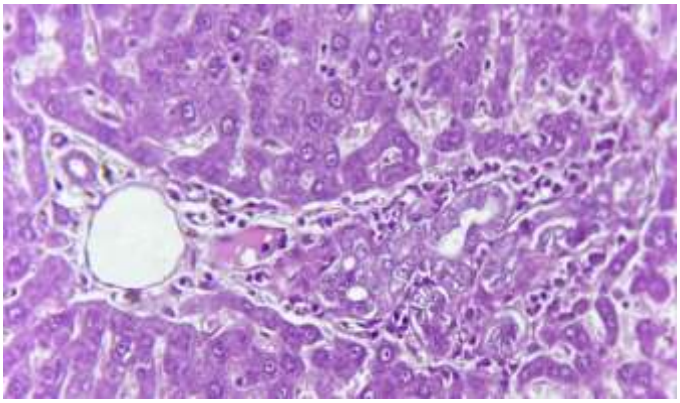
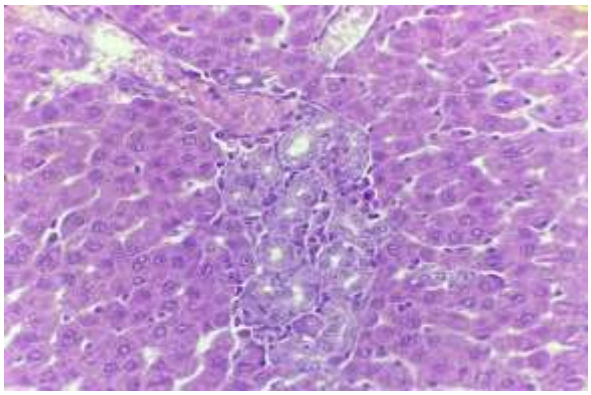


Fig 3. Interface hepatitis in case group (left picture).Infiltration of mononuclear cells, hepatocytes necrosis and fibrosis are seen in case group (left picture), but control group (right picture) is intact.

In vertices of adjacent lobules, triangular portal space is visible. These spaces contain bile duct, portal vein and hepatic artery. Bile ducts was seen in form of small circles which their walls was covered by a layer of cuboidal epithelium with round nucleus. Evidence of bile ducts proliferation in case group was obtained. (Fig 4)



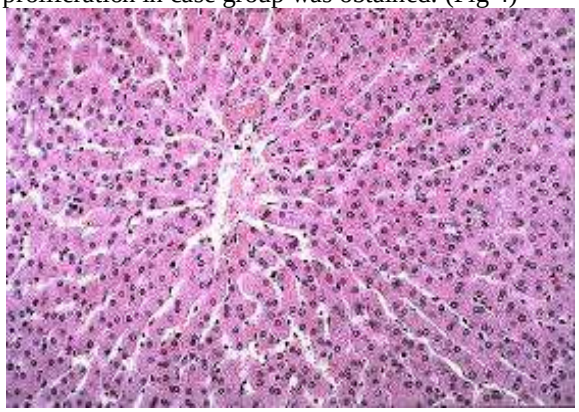


Fig 4. Transverse cut of liver tissue in case and control group (×100). In case group (left picture), bile ducts proliferation is seen, but control group (right picture) is intact.

Single cell necrosis or individual necrosis can be both apoptotic and oncotic. This process usually seen in different kinds of hepatitis and accompanied with other processes such as inflammation and degeneration. (Fig 5)

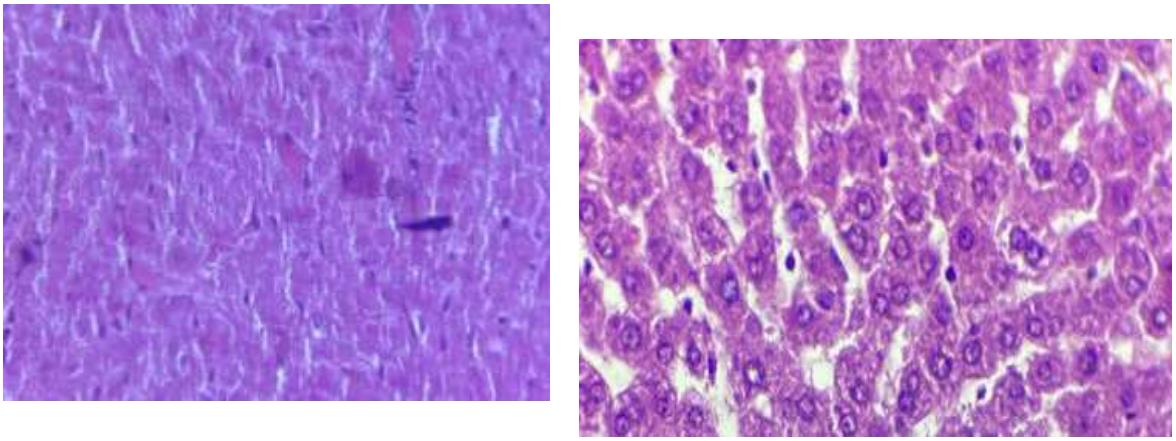


Fig 5. Transverse cut of liver tissue in case and control group (×100). In case group (left picture), Single cell necrosis is seen, but control group (right picture) is intact.

Kidney histopathology

In histopathological study of liver in both case and control groups, glomerulus, distal and proximal tubules and interstetium were examined. Glomerulus of both groups were observed. Glomerulus have 2 layers bowman's capsule: parietal layer consist of wide cells with elongated nuclei and visceral layerconsist of large cell with purple nuclei or podocytes. No pathological changes have been seen in each layer or bowman's space in both groups. The size of glomerulus and cellularity in both groups was normal. Base membrane in both group was intact. (Fig 6

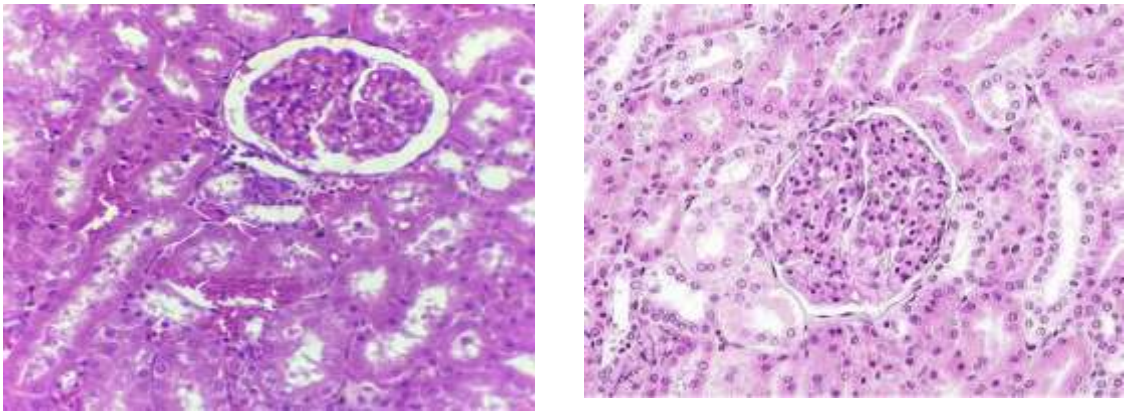


Fig 6. Transverse cut of kidney tissue in case and control group (×100). No histopathological changes is seen in both case (left picture) and control (right pictures) glomerulus.

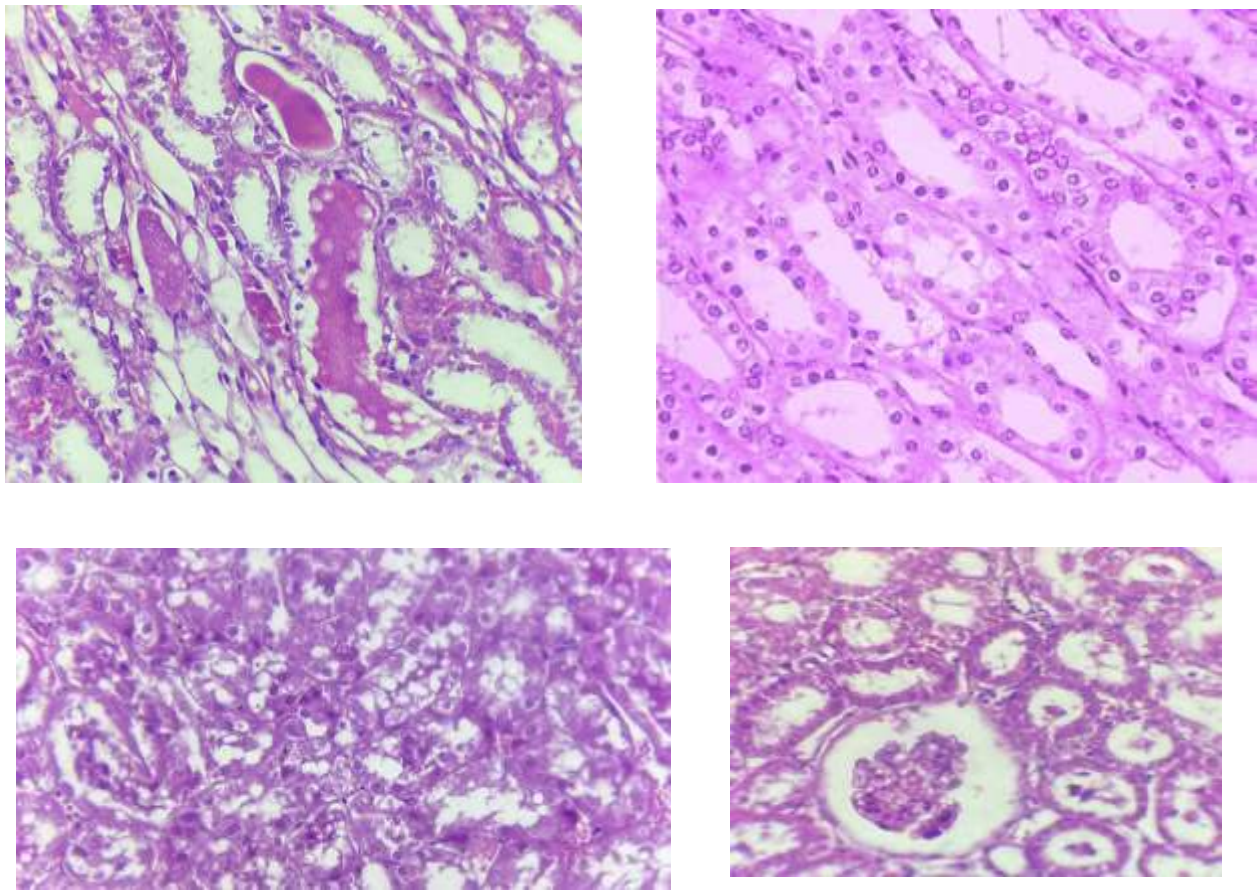


Fig 7. Transverse cut of kidney tissue in case group (×100). In right picture, early tubular necrosis is seen. In left picture, tubular necrosis dilation of tubular lumen and loss of nuclei in tubular epithelium is seen.
In medulla the section of urinary collecting tubules was seen. Among these tubules, Henle loop section covered by simple squamous epithelium was seen. Colloidization and casts in collecting tubules in case group was seen. (Fig 8)
Fig 8. Transverse cut of kidney tissue in case and control group (×100). Colloidization and casts in collecting tubules in case group (left picture) is seen. Control group (right picture) is intact.

In urinary pole of glomerulus, proximal tubules with simple cuboidal epithelium were evaluated. Among proximal tubules, in transvers cut, distal tubules were located. These tubu les were like circular lumen covered by cuboidal cells on base membrane. Tubular necrosis in form of simplification of tubular epithelium with loss of brush border, loss of nuclei in tubular epithelium, interstitial edema in early stages and dilation of tubular lumen and accumulation of necrotic tubular epithelial cells were seen in case group. (Fig7)

Discussion

Butachlor is a chloroacetamide herbicide which use extensively as a pre-emergent and early post emergent herbicide for eliminating broad leaf weeds and grasses(21). This herbicide use in rice, wheat, corn, soybean field. Butachlor use widely in South America, Africa and Asia. Due to low price and easy accessibility this is common in Iran especially in north of Iran(5). Unfortunately this herbicide remains in soil and absorbs in ground water(22-24). As a result butachlor may have adverse effect on aquatics and humans(25). In this study we examined, histopathological

changes and adverse effect of butachlor on liver and kidney tissue of rats in chronic exposure. Our results show that chronic 12 weeks exposure to 72.2 mg/kg butachlor, ¼ $LD_{50,}$ in adult male Wistar rats make significant pathological changes in liver including single cell necrosis, lobular hepatitis, interface hepatitis and bile duct proliferation. Liver is one of the largest organ in the body, which is responsible for detoxification. In hepatic damage, if the cause is poison, because it enters through the blood stream, involves the areas around the portal tracts and zone 1 at first. But, if the cause of injury is hypoxemia, it involves the areas farthest from the portal tract, because there is still some oxygen in portal tracts. Damaged hepatocytes can exhibit potentially inflammatory changes, such as lipid accumulation and bilirubin (cholestasis), but when the damage is irreversible, hepatocytes are destroyed by necrosis and apoptosis. Necrosis is commonly seen after hypoxic or ischemic liver damage. On the other hand apoptosis is commonly seen in viral and autoimmune hepatitis and toxic liver injuries(26). Butachlor metabolism occurred in the liver. On the other hand, studies showed that butachlor induces apoptosis in hepatic cells by increasing the amount of genes and proteins related to apoptosis through mitochondrial pathway. Butachlor also induces oxidative stress in hepatic cells(10, 27). So through this mechanism butachlor has toxic effect on liver. Our results demonstrated that butachlor has toxic effect on kidney. Tubular necrosis and degeneration, colloidization and cast formation were the renal pathological alterations. This changes may be associated to the fact, that kidney is the most important organ in the body responsible for exertion. Also the toxins such herbicides may be accumulated in renal tissue and cause injury. Tubular damage is a clinical-pathological category that is morphologically related to the damage of epithelial cells of the tubules and clinically associated with acute decreased in renal function in form of decreased in GFR and oliguria (urinary output less than 400 mL/day) along with presence of tubular cells and granular cylinders in urine. Renal damage has different causes like severe glomerular diseases which manifest as RPGN, diffuse vascular diseases such as microscopic polyangiitis and thrombotic microangiopathies, drugs and toxins. There are generally two types of underlying causes for tubular damage: first, Ischemic damage as a result of insufficient blood flow to all or some peripheral organs such as the kidney and in the context of hypotension and shock. This condition occurs in various situations such as severe traumas, blood loss, acute pancreatitis sepsis, malignant hypertension, inappropriate blood transfusion and hemolytic crisis. The second is nephrotoxic damaged caused by various toxins and drugs. The epithelial cells of proximal tubules have especial sensitivity to anoxia and are vulnerable to toxins. Several factors play a role in toxic damage of tubular cells including increasing intracellular concentration of different molecules that are absorbed or secreted by the proximal tubule, exposure to high concentration of soluble substance in urinary tract and high oxygen consumption to produce ATP. Ischemia causes several structural changes in epithelial cells. Loss of cell polarity is an early reversible event that changes the distribution of membranes proteins such as change in position of Na-K-ATPase pump from the basal-lateral surface to ductal surface of tubules and as a result reduce Na reabsorption from proximal tubules and increase Na delivery to distal tubules. Increasing the amount of sodium delivered to the distal tubules, activates tubule-glomerular feedback system and causes pre-glomerular arterioles contraction and decreases GFR and worsens blood supply. Tubular cell injury cause them to separate from basement membrane and spill into urine. Enough tubular derbies accumulations obstruct urine outflow and as result intra-tubular pressure increase and cause decrease in GFR. The fluid from damaged tubules may leak into interstitial tissues (backward leakage) and causes an increase in the pressure of the interstitial tissue and as a result the tubules lie on top of each other. Injured tubular cells express chemokines, cytokines, and adhesion molecules such as P-selectin, which attract leukocytes and play a role in tissue damage and interstitial tissue inflammation. Necrotic tubular cells activate an inflammatory response which is associated with tubular damage(26). In one study, the toxic effect of 500 mg/kg butachlor for 4 weeks on rat Rattus rattus frugivorouswere observed(28). Dilation in the central veins, portal veins and sinusoids, focal mononuclear leucocytes inflammatory cells infiltration in portal area, kupffer cells proliferation after 3 weeks and degeneration of hepatic cells after 4 weeks was the pathological changes in liver. In other study. 262 mg/kg butachlor was administered orally in Wistar rats for 28 days(29). Hepatocytes degeneration and fatty change were the pathological alterations in liver. Tubular degeneration and mildly fatty changes were the histopathological changes in renal tissue. Similar to these studies, we showed that butachlor cause cell necrosis and inflammatory changes in liver structure. Although the time of exposure was longer in our study, but the dose of butachlor was higher in both studies than ours. So that might lead to less remarkable changes in our studies. In other study on fish, the toxic effect of two dose (3.2 and 0.64 μmol/L) butachlor on four main organs (brani, gill, liver and kidney) of gold fish Carassius*auratus*in ten days, was studied.The pathologicalchanges in liver tissue was increase of cell size, obscure cell boundary, bright cytoplasm, and severe edema and in kidney increased size of renal tubular epithelial cells, fadedstaincytoplasm, increased transparency of cell baseand moderate degeneration(17). In Ahmadivand study on Oncorhynchusmykiss fish, fishes were exposed to 0.39 mg/l butachlor for ten days. Vacuolar degeneration of tubular epitheliumespecially in proximal

tubules, desquamation of epithelium and necrosis of tubular epithelium were the pathological changes in kidney (6). The pathological changes in kidney and liver of fishes in these studies were similar to our study and both of them show degrees of necrosis of epithelial cellsbecause the function of two organ is somehow similar in both animals. To summarize, butachlor has toxic effect on kidney and liver and makes pathological changes in organs tissues. We suggest other suggest that other studies with higher sample size and different doses of butachlor will be performed.

Conclusion

We still have not enough information about interaction between butachlor and mammalian cells. This study demonstrate that butachlor has significant deleterious effects on liver and kidneys of rats.Single cell necrosis, lobular hepatitis, interface hepatitis and bile duct proliferation were the histopathological changes in liver chronic exposure of butachlor in rats and tubular necrosis and cast formation were the changes in kidney. Considering that herbicides are important part of agricultural industry and food chain, it is mandatory to find out toxicological consequences of herbicides.

Conflict of Interest

The authors declare that there is no conflict of interest.

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