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Acrylamide - Induced Acute Nephrotoxicity in Rats

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ABSTRACT

Acrylamide (ACR) has been shown to cause neurotoxic effects in humans and neurotoxic, genotoxic, reproductive, and carcinogenic effects in laboratory animals. To investigate the nephrotoxic effect of Acrylamide (ACR), 50 adult male rats (Wistar, approximately 250 g) housed in polycarbonate boxes as 5 per each, and randomly assigned in 5 groups including 4 exposure groups as A, B, C, and D groups of rats (10 rats per exposure group., total) and were exposed to 0.5, 5, 50, 100 mg/kg ACR per day×11days i.p. respectively. The remaining 10 rats were housed in group (E) as control group. Control rats received daily i.p. injections of 0.9% saline (3ml/kg). On day 12, four rats, were randomly selected, perfused, dissected and proper samples were collected from their kidneys. Results of histopathological studies based on H&E technique did show no morphologic changes in kidneys of rats belong to groups A, B and E, while moderate to severe morphologic changes including glomerular hypercellularity, global pattern of proliferative glomerulonephritis, occupation of capsular space, tubular cell swelling and hyaline cast formation, were observed in different stained sections obtained from the kidneys of rats belong to group, C, and D. This finding, beside neurotoxic, reproductive and carcinogenic effects, seems to indicate for the first time another important aspect of toxic effect of ACR, i.e., acute nephrotoxicity.

Keywords: Acrylamide, nephrotoxicity, glomerulonephritis, rats

I. INTRODUCTION

Acrylamide (ACR) is a water-soluble, vinyl monomer that is used primarily to produce polyacrylamides with different physical and chemical properties. These polymers are used extensively in chemical industries (e.g., water management, ore processing) and molecular laboratories (i.e., gel chromatography). ACR also appears to be a contaminant generated during the preparation of certain foods (LoPachin. et al. 2003).

Acrylamide (ACR) has been one of the most investigated toxic compounds among food carcinogens during recent years. In 2002 it was found in high concentrations in various food products, especially in potato chips. It has become evident that human exposure to AA ismainly via food, where it is formed when heating food rich in carbohydrates over 180 ◦C (Kirsi et al. 2008).

Although the polymer is nonneurotoxic, exposure of humans and laboratory animals to monomeric ACR produces ataxia, skeletal muscle weakness, and weight loss. Repeated daily exposure of laboratory animals (rodents, rabbits, dogs, cats and Guinea pigs) to ACR (0.5–50 mg/kg per day) is associated with neurological signs that, in many respects, resemble the neurotoxicity occurring in humans; i.e., ataxia and skeletal muscle weakness (LoPachin et al. 2003).

Early morphological studies indicated that this neurotoxic syndrome was associated with nerve damage characterized by multifocal paranodal swellings of preterminal distal myelinated axons. These swellings contained an abundance of tubulovesicular profiles, neurofilaments, and degenerating mitochondria and, as ACR intoxication continued, axonal regions below these swellings degenerated. Historically, this type of nerve damage has been classified as a central–peripheral distal axonopathy and has been presumed to be responsible for the associated neurological defects (LoPachin 2004).

Exposure to monomeric acrylamide results in a characteristic, well studied peripheral neuropathy, with

accompanying weakness of the limbs, especially hind limbs, in animals and humans. Neurologic signs are routinely observed in rats receiving acrylamide for 30 to 90 days at dosage levels of 5 to 20 mg/kg/day. These authors demonstrated a qualitatively progressive expression of the neurotoxicity, with morphologic indicators of neurotoxicity taking longer to appear than behavioral indicators. Light microscopic, histopathologic lesions in the peripheral nerve have been observed in rats given 5 mg/kg/day or more orally for 13 weeks, and minimal electron microscopic lesions were noted in rats given 1 mg/kg/day by the same regimen. Although the mechanism of this neurotoxicity is currently under investigation, it seems that acrylamide acts through inhibition of fast axonal transport. Acrylamide binds to neurofilaments in vitro and in vivo, and seems to inhibit the function of kinesin in catalyzing the transport process (Tyla et al. 2000).

In laboratory animals ACR exposure is also associated with carcinogenicity and reproductive toxicity, whereas in humans, neurotoxicity is the only demonstrated effect of this toxicant (Shan-xia Li et al. 2006).

Acrylamide is probably carcinogenic to humans by the International Agency on research of Cancer. Health risks of the general population are based on an average exposure to 4mg/kg bw/day. It is reported that exposure to acrylamide at 200 mg/kg bw/day places humans at risk for neurotoxicity, while 300 mg/kg bw/day puts humans at carcinogenic risk (Hao Wanga et al. 2010)

Effects of acrylamide on various reproductive parameters of mice have been extensively tested including decreased sperm count and increased abnormal sperm morphology, severe testicular damages such as vacuolation and swelling of the round spermatid, and DNA breakage during specific germ cell stages. Male rats administered acrylamide exhibited significant reductions in mating, fertility, and pregnancy as well as transport of sperm in uterus, suggesting that acrylamide exerts reproductive toxicity in male rodents, whereas has no effect on the females (Hye-Jin et al. 2005).

In spite of numerous studies have been taking place so far on neurotoxicity, carcinogenicity, and reproductive toxicity of ACR in rats, no study has yet been done on the effect of this substance on other visceral organs. In author's personal experiences on ACR acute neurotoxicity (Jamshidi et al. 2009, Jamshidi and Panahi 2011), certain changes in visceral organs of rats including kidney and liver were observed. Thus the present study was performed to confirm the potential of oral (gavage) exposure of male Wistar rats to acrylamide monomer for 11 days, to evaluate the possible nephrotic changes detected by light microscopy.

II. METHODS AND MATERIAL

Chemicals

The chemical substance, acrylamide monomer, used in this study, was of the best analytical grade available and obtained from Merck (99.7%) and kept at room temperature throughout the study period. The solvent used was normal saline solution (0.9%). The treatment doses used were as follow: 0.5, 5, 50, 100 and 500 mg/kg bw per day i.p. with dose volume of 3 ml/kg.

Animals and trial

In this study 50 adult male rats (Wistar, ± 250 grams, from Pasteure Institute) were used. Animals, after being weighted, were randomly divided into 5 groups including 4 treatment groups (A, B, C, and D), containing 10 rats each, and 1 control group (F), containing 10 rats. Rats were housed in polycarbonate boxes, and drinking water and laboratory rodent chewing pellets were available ad libitum. The animal room was maintained at approximately 22^{oC} and 50% humidity with a 12 h light/dark cycle.

The study period was 11 days, during which the randomly assigned groups of rats (5 treatment groups) were exposed exposed to 0.5, 5, 50, and 100 mg/kg bw per day×11days i.p. respectively. The remaining 10 rats in group F, received daily i.p. injections of 0.9% saline (3ml/kg). On day 12 of experiment, two rats were randomly selected from each group to be dissected and sample collection for light microscopy.

Necropsy and Sample Collection

To dissect animals, each randomly selected rat was first anasthetized with ether and then transcardially perfused by a blood-washing solution consisting of 0.8% sucrose,

0.8% NaCL and 0.4% glucose which was followed by fixation. Fixative solution containing 4% paraformaldehyde in 0.1 M phosphate buffer was perfused through the same way.

Histopathology

The removed kidneys were fixed in same fixative used for perfusion, and processed using standard laboratory procedures for histology. The tissues were embedded in paraffin blocks, sectioned perpendicular to the longest axis of the kidneys at 5µm thickness, and stained with Hematoxylin and Eosin technique (Putchler et al 1962). Sections were mounted with dextran- plasticizer xylene and examined by light microscopy (×400).

III. RESULT AND DISCUSSION

Group A (0.5 mg/kg bw)

Body Weight: In this group the mean (±S.E.M.) starting body weight was 237.95 ± 32.52, after 11 days of exposure, mean weight increased by 5.91% to 260.21 ± 37.46(Graph – 1).

Kidney**:** in histopathological evaluation of kidneys of rats belong to this group no morphologic changes were observed throughout the cortex and medulla (Figs: 1&2).

Group B (5.0 mg/kg bw)

Body Weight: In this group the mean (± S.E.M.) starting body weight was 224.38 ± 34.45, after 11 days of exposure, mean weight increased by 2.07% to 249.82 ± 32.23(Graph – 1).

Kidney**: I**n histopathologic evaluation of kidneys of rats belong to this group no morphologic changes were observed throughout the cortex and medulla (Figs:3&4).

Group C (50 mg/kg bw)

Body Weight: In this group the mean (± S.E.M.) starting body weight was 241.7 ± 20.06, after 11 days of exposure, mean weight decreased by 5.46% to 228.51 ± 21.53(Graph – 1).

Kidney: In histopathologic evaluation of kidneys of rats belong to this group certain morphologic changes indicating hypercellularity of the glomerular tufts, global pattern of proliferative glomerulonephritis, occupation of capsular space, and tubular cell swelling were observed throughout the cortex and medulla (Figs:5,6&7).

Group D (100 mg/kg bw)

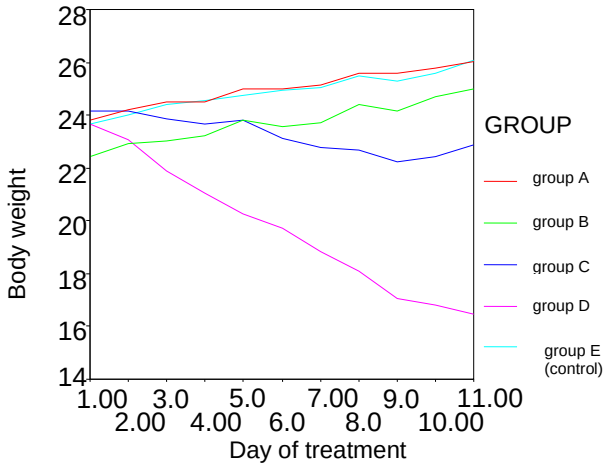
Body Weight: In this group the mean (± S.E.M.) starting body weight was 236.7 ± 33.9 g, after 11 days of exposure, mean weight decreased by 30.52% to 164.46 ± 30.11 (Graph – 1).

Kidney**:** In histopathologic evaluation of kidneys of rats belong to this group in addition to certain morphologic changes indicating hypercellularity of the glomerular tufts, global pattern of proliferative glomerulonephritis, occupation of capsular space, tubular cell swelling as well as hyaline cast formation, were also observed throughout the cortex and medulla (Figs: 8, 9 & 10).

Group E (Control group)

Body Weight: In this group the mean (S.E.M.) starting body weight was 236.49 ± 26.1g, after 11 days of exposure, mean weight increased by 10.17% to 260.55 ± 30 (Graph – 1).

Kidney: In histopathologic evaluation of kidneys of rats belong to this group no morphologic changes were observed throughout the cortex and medulla (Figs:11&12).



Graph 1: Comparison of body weight among different group. As indicated in this graph, the highest percentage of body weight increase (10.17%) and the lowest percentage of body weight decrease (30.52%) belong to group E and Group D respectively.

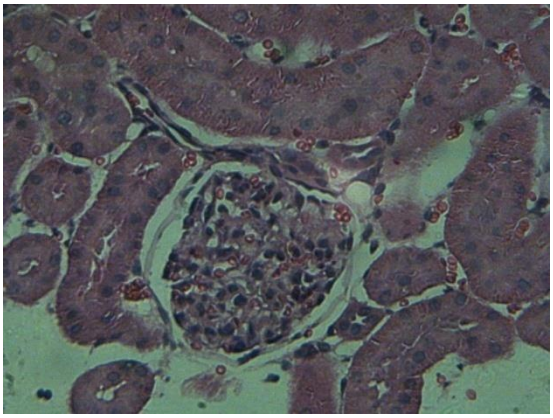


Figure 1 Rats, kidney. Group A. No morphologic changes were observed throughout the cortex and medulla. 100X

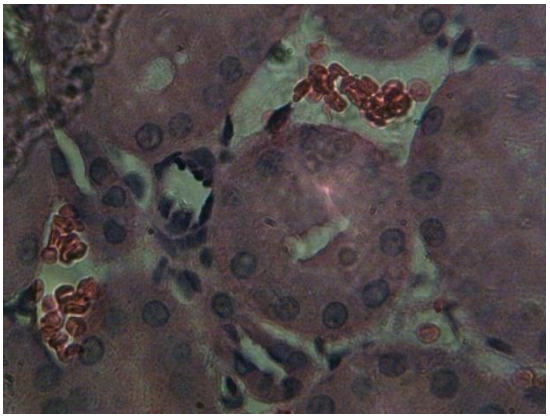


Figure 2 Rats, kidney. Group A. No morphologic changes were observed throughout the cortex and medulla. 400X

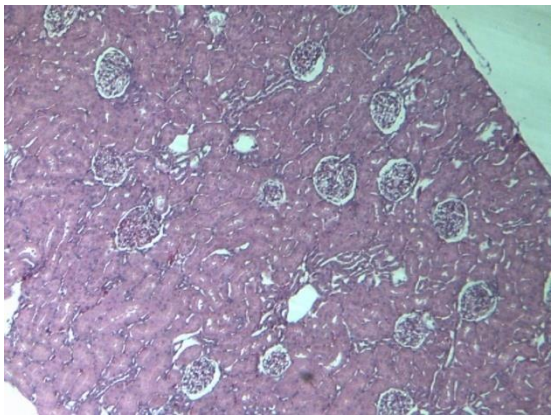


Figure 3 Rats, kidney. Group B. No morphologic changes were observed throughout the cortex and medulla. 40X

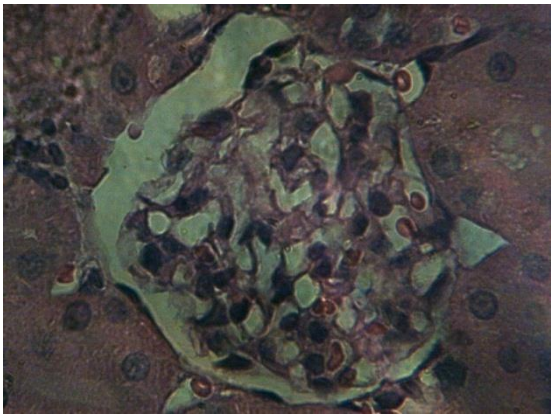


Figure 4 Rats, kidney. Group B. No morphologic changes were observed throughout the cortex and medulla. 400X

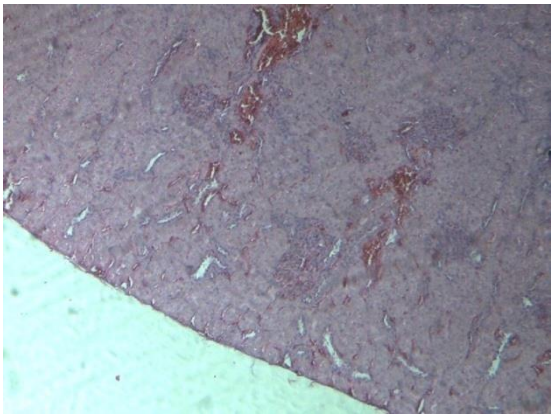


Figure 5 Rats, kidney Group C. Certain morphologic changes indicating hypercellularity of the glomerular tufts, global pattern of proliferative glomerulonephritis, occupation of capsular space, and tubular cell swelling were observed throughout the cortex and medulla. 40X

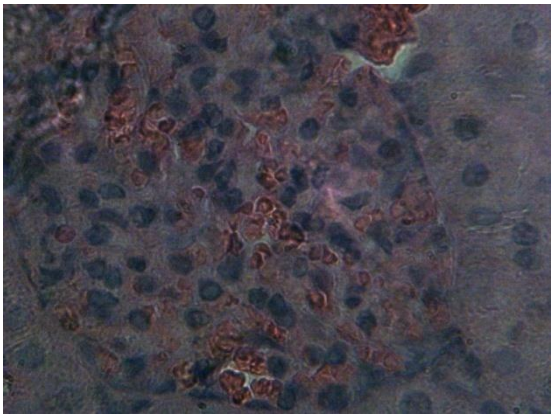


Figure 6 Rats, kidney Group. C. Certain morphologic changes indicating hypercellularity of the glomerular tufts, global pattern of proliferative glomerulonephritis, occupation of capsular space, and tubular cell swelling were observed throughout the cortex and medulla. 400X

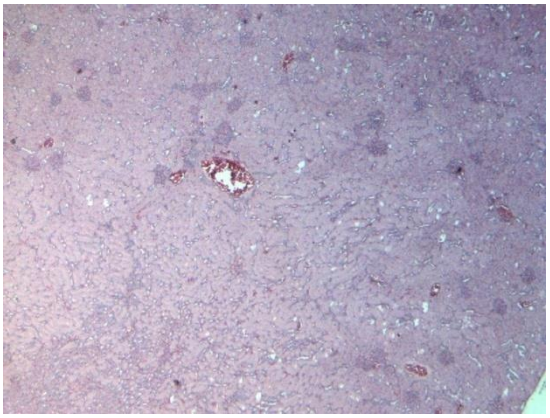


Figure 7 Rats, kidney Group D. Certain morphologic changes indicating hypercellularity of the glomerular tufts, global pattern of proliferative glomerulonephritis, occupation of capsular space, and tubular cell swelling were observed throughout the cortex and medulla. 4X

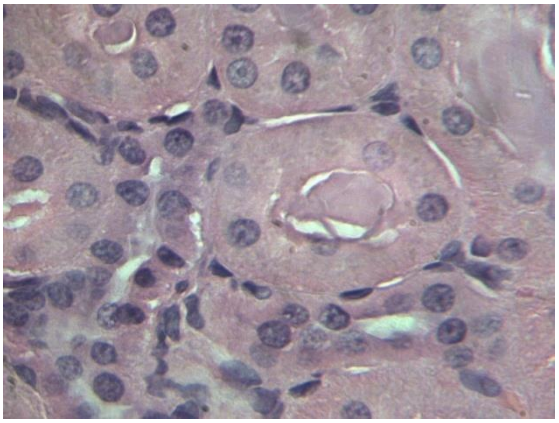


Figure 8 Fig- 8: Rats, kidney. Group D. Hyaline cast formation.100X

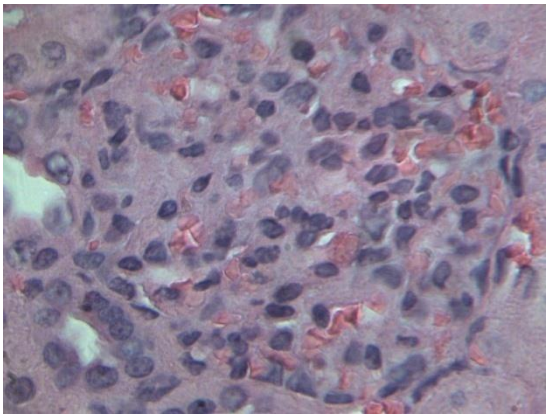


Figure 9 Rats, kidney. Group D. Certain morphologic changes indicating hypercellularity of the glomerular tufts, global pattern of proliferative glomerulonephritis, occupation of capsular space, tubular cell swelling were also observed throughout the cortex and medulla. 40X

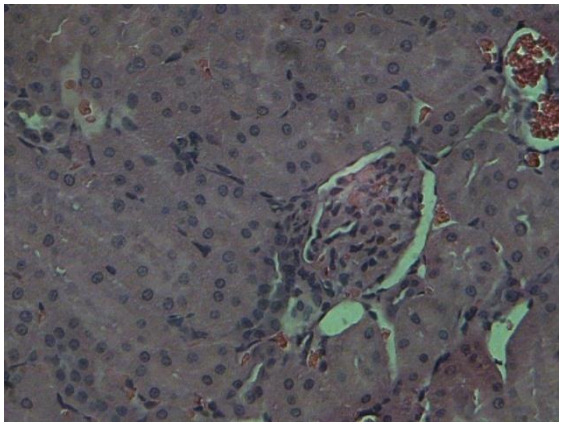


Figure 10 Rats, kidney. Group D. Certain morphologic changes indicating hypercellularity of the glomerular tufts, global pattern of proliferative glomerulonephritis, occupation of capsular space, tubular cell swelling were also observed throughout the cortex and medulla. 10X

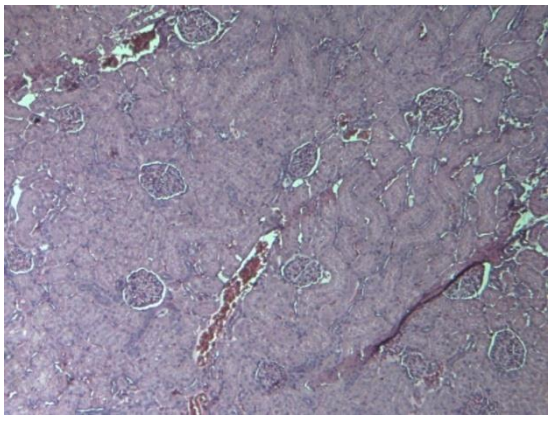


Figure 11 Rats, kidney. Group F. In histopathologic evaluation of kidneys of rats belong to this group no morphologic changes were observed throughout the cortex and medulla. 4X

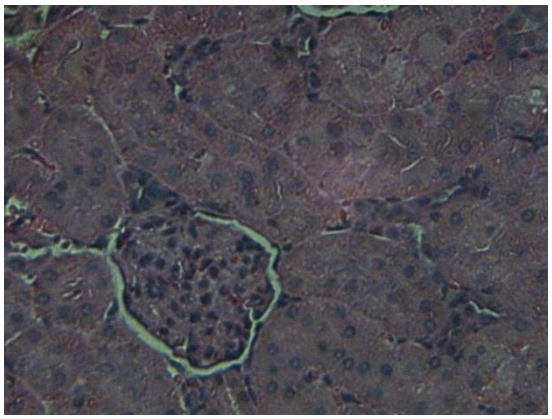


Figure 12 Rats, kidney. Group F. In histopathologic evaluation of kidneys of rats belong to this group no morphologic changes were observed throughout the cortex and medulla. 10X

IV. CONCLUSION

In animals ACR is neurotoxic, mutagenic and carcinogenic, and disturbs fetal development and reproductive functions (Tyl and Friedman, 2003).

ACR is a well-documented neurotoxicant in both humans and laboratory animals. Subchronic, low- level occupational exposure of humans to ACR produces neurotoxicity characterized by ataxia, skeletal muscle weakness and numbness of the hands and feet (Spencer and Schaumburg, 1974a).

Furthermore, studies of ACR-exposed laboratory animals (primarily rodents) have revealed an increased incidence of tumors in certain tissues e.g., mammary gland fibroadenomas in female rats, tunica vaginalis mesotheliomas in male rats (LoPachin, 2004).

Epidemiological studies of occupationally exposed human populations have, however, failed to establish a relationship between ACR exposure and an increased risk for cancer (Collins et al., 1989; Marsh et al., 1999).
In addition, reproductive toxicity is another important aspect of the toxic effect of AA. When mice were exposed to AA at higher doses, sperrm concentration and morphology were affected (Sakamoto and Hashimoto, 1986), and in the similar condition for rats, morphology, motility and transport of sperm in uterus were affected too (Sublet et al., 1989; Tyl et al., 2000; Yang et al., 2005), indicating that AA exposure decreased the mating frequency.

The results of our present study clearly demonstrated that i.p administration of different doses of acrylamide to male rats led to macroscopic and microscopic changes in the kidneys.

All functions of the glomerulus including plasma ultrafiltration, blood pressure regulation, peritubular blood flow regulation, tubular metabolism regulation, and circulating macromolecule removal are affected by processes that target this structure in disease. Damage to the glomerular filtration barrier can result from several causes and produce a variety of clinical signs. The major clinical finding of glomerular disease is the leakage of various low molecular weight (small molecule size) proteins, such as albumin, into the glomerular filtrate. As a result, large quantity of albumin overload the protein reabsorption capabilities of the proximal convulated tubular epithelium to such an extant that protein-rich glomerular filtrate accumulate in the variably dilated tubular lumina and protein subsequently appears in the urine. In such diseases, the proximal tubular cells often have microscopic eosinophilic intracytoplasmic bodies referred to as hyaline droplets, which represent accumulation of intracytoplasmic protein absorbed from the filtrate. Renal diseases that result in proteinuria are called protein-losing nephropathies.

Different forms of glomerulonephritis including bacterial, viral, chemical and immune-mediated glomerulonephritis have been identified so far. Chemically induced glomerular disease, although much less common than the immune mediated forms of glomerulonephritis, occurs in variety of different ways (McGavin and Zachary 2007).

Generally, a combination of physiologic and biochemical events contributes to the susceptibility of the kidney to several distinct classes of nephrotoxicity. Compared with other organs, the kidney is uniquely susceptible to chemical toxicity, partially because of its disproportionately high blood flow (25% of cardiac output), and due to its complexity both anatomically and functionally. Kidneys also play an instrumental role in regulating overall blood pressure. Urine is the principal route by which most toxicants are excreted. As a result, the kidney concentrates toxicants in the filtrate, transports toxicants across the tubular cells, and bioactivates certain toxicants. All these attributes make kidneys extremely vulnerable to a variety of adverse effects (Hickey et al. 2001). Toxic doses of DCLF can cause nephrotoxicity in humans and experimental animals (Hickey et al. 2001).

Cyclophosporine (CyA) is a potent immunosuppressant. But it has a nephrotoxic side effect (Ishikawa et al.1999). It alters renal perfusion and ultimately the glomerular filtration rate by damaging glomerular endothelial cells (McGavin and Zachary 2007). Cisplatin is an antineoplastic drug highly effective against several human cancers, such as testis and ovarian cancer,head and neck cancer, and lung cancer. However, nephrotoxicity is one of the most important side-effects

of cisplatin therapy, affecting primarily the S3 segment of the proximal tubule (Blachley and Hill 1981). The severity of cisplatin nephrotoxicity is related to platinum concentrations in the kidneys. There is an increasing amount of evidence that cisplatin-induced nephrotoxicity is ascribed by oxidative damage resulting from free radical generation and that the administration of antioxidants is efficient in inhibiting these side effects (Weijl et al, 1997).

Besides the histomorphological changes, several other observations were very striking. At the time of animal sacrifice, ACR-exposed kidneys in groups C and D, were clearly distinguishable from those in groups A, B and E, vehicle-treated control group, by their colors and sizes. ACR-exposed kidneys in groups C and D appeared dark red brown and abnormally enlarged, compared to groups A, B and E.

Kidney sections from the animals in groups A, B and E, showed normal kidney cellular architecture with no evidence of injury. In contrast, ACR induced chaotic changes in groups C and D were manifested by numerous histomorphological alterations including glomerular hypercellularity, global pattern of proliferative glomerulonephritis, occupation of capsular space, tubular cell swelling, hyaline cast formation and hyaline droplet formation.

Although researchers have made much progresses in understanding ACR-induced mechanisms of neurotoxicity, reproductive toxicity, and carcinogenicity, the precise pathways by which ACR produces nephrotoxicity and glomerulonephritis still remain obscure. Moreover, its nephrotoxic potential may involve multiple pathways, which are not known.

In conclusion, the present findings demonstrated for the first time that the administration of acute nephrotoxic doses of ACR (50 and 100 mg/kg ACR per day×11days i.p) to male wistar rats could result in severe kidney injury leading to glomerular hypercellularity, global pattern of proliferative glomerulonephritis, occupation of capsular space, and tubular cell swelling and hyaline cast formation.

Future studies of renal tissues in acrylamide-treated rat kidneys will contribute to the understanding of the mechanisms of acrylamide toxicity in this organ and potentially in overall renal function.

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