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Nephrotoxicity of radiographic fixer effluent in wistar rats

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ABSTRACT

Background: Limited information exists concerning the cellular changes associated with toxicity of fixer effluents on kidney tissues. **Objectives:** The present study aimed at demonstrating the histopathological changes in the kidney tissues of wistar rats following exposure to fixer effluent. **Materials and Methods:** Eighteen young Wistar rats of weights 140-250g were divided into three groups. The control group was sub-divided into two groups of 3 rats each and orally administered with 1ml of distilled water daily for 14 days and 28 days respectively; each of the experimental groups II and III were further divided into two sub-groups of three rats each administered with 200mg/kg and 400mg/kg of fixer effluent daily for 14 days and 28 days respectively. **Results:** Histopathological findings indicated normal kidney tissues in the control group. In contrast, close adherence of the glomeruli to the Bowman's capsule was observed in groups IIA and IIIB. The glomeruli and renal interstitium appeared distorted in groups IIIA and IIIB. There were signs of glomerular necrosis in groups IIA and IIIB. In addition, inflammatory cell infiltrate within the tuft and stroma of renal interstitium, dense colloid materials on renal tubules and atrophy of tubular epithelium were observed in group IIIB. **Conclusions:** This study indicated adverse effects of acute/chronic and long-term/short-term exposures to sub-lethal doses of fixer effluent on Wistar rats' kidneys. Some of the histopathologic effects were marked in long-term and chronic exposures compared to short-term and acute exposures, thus indicating dose and duration-dependent effects of fixer effluent on kidney tissues of rats.

KEY WORDS: Environment; Workplace; Exposure; Radiography; Histopathology

INTRODUCTION

Radiographic images are indispensable and complementary diagnostic tools in health institutions [1, 2]. The techniques employed in obtaining these images involve the emission of x-rays into radiographic films, followed by the various steps of film processing, which include image developing, washing, fixing, final washing and drying [3]. A fixer is a chemical used in processing photographic or x-ray films, and applied after the developing phase in order to neutralize any developer remaining on the film and to remove undeveloped silver halides and harden the emulsion [4]. The silver halide solvent in ordinary fixers is sodium thiosulfate also known as 'hypo', while ammonium thiosulfate is a faster-acting silver halide solvent used in rapid fixers [5]. In actual reality, fixer itself is quite harmless, especially the 'plain fix'. However, the longer and/or more frequently a fixing bath is used, the closer it comes to its exhaustion point. The reaction products containing the silver (the silver complexes) become more and more 'complex', meaning they are larger and larger molecules, as the fixing bath proceeds through its working life and approaches exhaustion. At exhaustion, the silver complexes are at their most 'complex' point [6]. The exhausted fixer effluent that is generated is considered a hazardous chemical waste that can pose risks to public health or to the environment. This is because it contains organic and inorganic compounds that are toxic to the environment in cases where they are inappropriately disposed of [2].

In developed countries, there are clear legal rules that give guidance for the proper discharge of these effluents; however this situation is not common in developing countries [2]. What happens is that in most of the imaging diagnosis centers, including teaching and research institutions, the effluents are disposed into streams or into public sewer systems without previous treatment or recycling [2]. Such effluents are disposed with high levels of inorganic compounds such as silver above allowed limits. Additionally, such effluents are discarded with a high chemical oxygen demand (COD) and hydrogenic potential (pH), and color, total dissolved solids concentration, chlorides, sulfates and turbidity over allowed limits [2]. Exposure to harmful and toxic substances is known to likely occur through the diet, from medications, the environment and workplace [7] and even through the use of cosmetics [8]. Such exposures pose a great danger to the human body, particularly to organs associated with handling wastes such as the kidneys.

The impairment of organ function is a direct consequence of alterations in the histological structures of the organ, and this may be dependent on the degree or duration of exposure to toxic substances [9]. The kidneys of living organisms are vital organs that play essential roles such as metabolism, clearance of endogenous waste products and excretion of exogenously administered therapeutic and diagnostic agents as well as environmental exposures [10]. In its role as the primary eliminator of exogenous drugs and

toxins, the kidney is vulnerable to develop various forms of injury [10].

Limited information exists concerning the cellular changes associated with toxicity of radiographic fixer effluents on kidney tissues. It is believed that most radiographers and other health workers as well as workers in photo industries and the general public may be ignorant of the adverse effects of fixer effluents on body tissues especially, the kidneys. Clarification of fixer efflux toxicity is therefore important because of industrial and environmental exposure to this compound and its organic and inorganic harmful components. In the present study, we aimed to demonstrate the histopathological changes in the kidney tissues of wistar rats following acute and chronic exposures to a fixer effluent.

MATERIALS AND METHODS

Animals

Eighteen apparently healthy Wistar albino rats weighing 140-220 g were used. They were housed in the animal house of the Department of Human Anatomy, Nnamdi Azikiwe University, Nnewi Campus, under standard conditions (29 ± 2°C temperature, 40-55% humidity, good ventilation) and have free access to water and diet (normal rat chow). They were acclimatized for two weeks before the start of the experiment.

Test Chemical

The original product, a commercially prepared fixer (a chemical used in processing photographic or x-ray films) was purchased from Begood Manufacturing Company Ltd, China. The main components of the fixer are sodium thiosulfate and ammonium thiosulfate. The fixer effluent, the liquid waste material generated from radiographic processing comprises of high silver-concentrated solutions, sodium thiosulfate, sodium sulfite, acetic acid, boric acid and low pH. The lethal dose (LD50) concentration of the fixer effluent was calculated as 2450 mg/kg body weight using the formula: $LD_{50} = \sqrt{a \times b}$ (where: a = the lowest dose that brought death i.e. 3000 and b = the highest dose that did not kill i.e. 2000). The lethal dose test of the fixer effluent was carried out at the Faculty of Pharmacy and Pharmaceutical Sciences, Nnamdi Azikiwe University, Agulu Campus according to the method employed by Lorke [11]. The concentrations of the fixer effluent used for the experiment were sub-lethal doses of 200mg/kg (lower dose) and 400mg/kg (higher dose) of body weight.

Experimental Design

The animals were divided randomly into three groups of 6 rats each - group I (control) and experimental groups II and III respectively. Group I was further divided into two groups of 3 rats each (i.e. groups IA and IB) and administered with 1ml of distilled water for 14 and 28 days respectively. The other groups II, III which served as the experimental groups were orally administered with 200mg/kg and 400mg/kg of fixer effluent respectively. The experimental groups II and III were further sub-divided into two groups of three rats each (i.e groups IIA, IIB, and groups IIIA, IIIB), which were orally administered with 200mg/kg and 400mg/kg of fixer effluents daily for 14 days and 28 days respectively. Thus group IIA rats were administered with low dose (200mg/kg) of fixer effluent for a short term period of 14 days; the group IIB rats were administered with the higher dose (400mg/kg) of fixer effluent for a short term period of 14 days; group IIIA rats were administered with low dose (200mg/kg) for long term period of 28 days; and group IIIB rats were administered with higher dose (400mg/kg) for long term period of 28 days. The average fixer effluent consumption was 0.2 ml/day for the low dose (200mg/kg) group and 0.42ml for the high dose (400mg/kg) group. The present experiment was designed to be time and dose-dependent with the same low dose (200 mg/kg or 400mg/kg) being administered daily to Group II or III for 14 days and 28 days respectively. After 14 days, three rats from groups I, II and III were sacrificed (using the chloroform inhalation method), while the remaining three rats in each of the groups were sacrificed after 28 days and their kidneys harvested.

Tissue preparation

As soon as the animals were sacrificed, they were quickly dissected and their kidneys removed and immediately fixed in a fixative (Bouni's fluid) and transferred into specimen bottles and kept frozen for 48 hours before undergoing routine processing (dehydration, clearing and infiltration with melted paraffin). The kidney tissues were embedded in paraffin wax, sectioned at 5 µm placed on a hot water bath, after which they were dried and stained by hematoxylin and eosin [12]. The photomicrographs were observed using Nikkon research microscope (Novex, Holland). The micrograph pictures were taken with digital camera (DCM 510.5M Pixels, CMOS chip) connected to the microscope.

Ethical Consideration

All procedures used in this study conformed to the criteria and guiding principles for research involving animals as outlined in the "Guide for the Care and Use of Laboratory Animals " prepared by the National Academy of Sciences and published by the National Institutes of Health (NIH publication 86-23 revised 1985) [13]. The experiments were carried out following the ethical approval of the Ethical Board of Faculty of Health Science and Technology, College of Health Sciences, Nnamdi Azikiwe University.

RESULTS

Fig 1 showing the kidney of the Control rats with normal renal histology (H&Ex400). There were no changes observed.

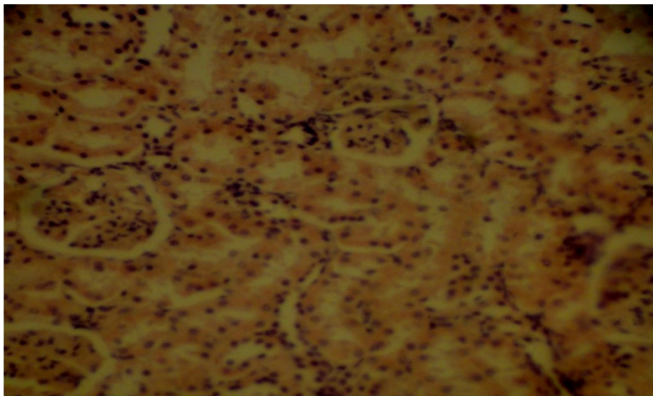

Figure 1. Photomicrograph showing normal architecture of the kidney of group I (control) rat.

Fig 2. Photomicrograph of kidneys of the group IIA rats after low dose (200mg/kg) and short term (14 days) daily oral administration of fixer effluent showing a section of the renal interstitial tissue with observable distorted glomeruli with necrosis alongside severe loss of tubules (H&E x 400)

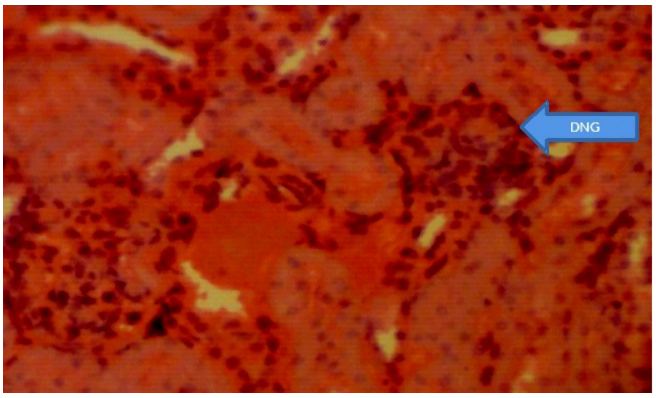

Figure 2. DNG = Distorted necrotic glomerulus

Fig 3. Photomicrograph of kidneys of the group IIB rats after high dose (400mg/kg) and short term (14 days) daily oral administration of fixer effluent showing normal interstitial tissue with glomerular tuft closely adherent to the Bowman's capsule. The tubules show normal histology (H&E x 400).

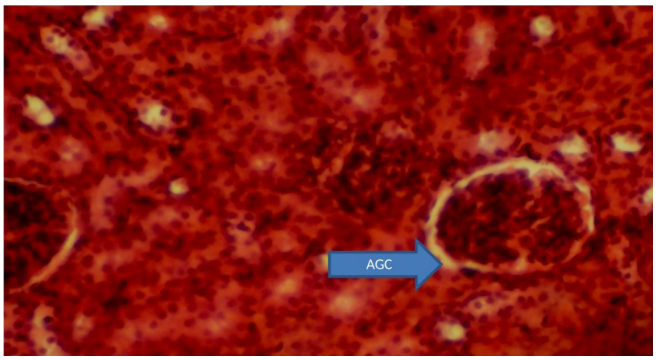

Figure 3. Abbreviation: AGC = Adherence of glomerular tuft to capsule

Fig 4. Photomicrograph of kidneys of the group IIIA rats after low dose (200mg/kg) and long term (28 days) daily oral administration of fixer effluent showing distorted interstitial tissue with glomerular tuft closely adherent to the Bowman's capsule. There is inflammatory cell infiltrate

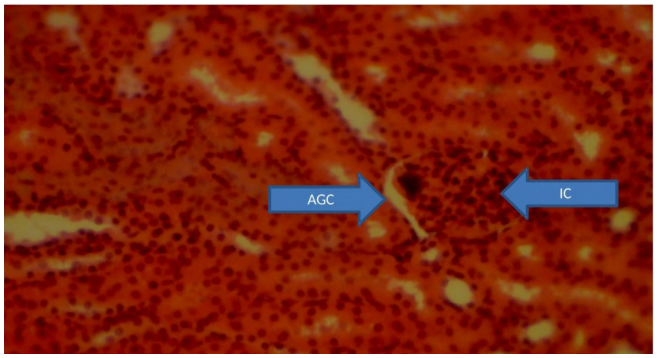

Figure 4. Abbreviation: AAGC = Adhesion of the glomerular tuft and capsule; 1C = Inflammatory cells infiltration

within the glomerular tuft (H&Ex400).

Fig 5. Photomicrograph of kidneys of the group IIIB rats after high dose (400mg/kg) and long term (28 days) daily oral administration of fixer effluent showing interstitial tissue (stroma) infiltrated by lymphocytes. Some tubules have survived but are small and atrophic. Several tubules contain dense oeosinophic(colloid) material. The glomeruli appear distorted with signs of necrosis. The tubules are

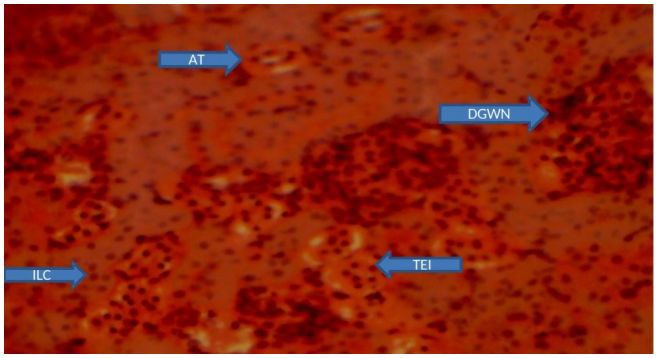

Figure 5. Abbreviation: AT = Atrophic tubules; DGWN = Distorted glomerulus with signs of necrosis; ILC = Inflammatory lymphocyte cells; TEI = Tubular esinophilic infiltration

lined with atrophic epithelium.

The present study indicated that the control group I (IA, IB), administered with distilled water, maintained their normal histology at the end of the study (Figure 1). The low-dose, short-term, daily oral administration of fixer effluent (group IIA) indicated observable glomeruli necrosis as well as severe loss of tubules in the renal interstitial tissue. Rats administered with low-dose fixer effluent over long-term period (group IIB) presented with normal interstitial tissue with glomeruli closely adherent to the Bowman's capsule (space). The renal tubules also showed normal histology. There was a distorted interstitial tissue with glomeruli closely adherent to the Bowman's capsule (space) in high-dose, short-term group IIIA. There was inflammatory cell

infiltrate within the tuft of group IIIB administered with high-dose, long-term daily fixer effluent. A section of the kidney showed interstitial tissue (stroma) infiltrated by lymphocytes, while some of the tubules which survived appeared small and atrophic. Several tubules also contained dense oeosinophic (colloid) material. In addition, the glomeruli appeared distorted with signs of necrosis and the tubules lined with atrophic epithelium.

DISCUSSION

The present findings indicated normal kidney tissues in the control group I, administered with distilled water. In contrast, close adherence of the glomerular tuft to the Bowman's capsule was observed in groups IIB and IIIA. The glomeruli and renal interstitium appeared distorted in groups IIA, IIIA and IIIB. There were signs of glomerular necrosis in groups IIA and IIIB. In addition, inflammatory cell infiltrate within the tuft and stroma of the renal interstitium, dense colloid materials on renal tubules and atrophy of tubular epithelium were observed in group IIIB.

In the process of filtering waste products from the blood the kidneys are potentially exposed to high concentrations of endogenous and exogenous toxic substances. Thus, some kidney cells are exposed to concentrations a thousand times higher than in blood [14]. Chemicals can affect the renal function or structures through a direct toxic action or through various systemic effects, such as intravascular hemolysis, rhabdomyolysis, or cardiac failure [15]. Toxic injury can affect the cells, glomerulus, interstitium or tubules with release of corresponding biomarkers. Xenobiotics may affect more than one compartment or may cause biomarker changes because of the interdependence of cells within the compartment. Inflammatory changes, autoimmune processes and immunological processes further promote the release of biomarkers [14]. About 10% of renal failure cases have been attributed to environmental exposures to toxic compounds or iatrogenic induction by various compounds, such as antibiotics, or procedures such as administration of kidney x-ray contrast to a diabetic [14].

Most of the transport systems are localized in the proximal tubule, thus that part of the nephron is the most frequent target of nephrotoxic chemicals. According to some authors, up to 20% of the cases of acute renal failure might be ascribed to toxic injury [15]. Most chronic kidney diseases which present with chronic interstitial nephritis have been associated with exposure to toxic agents such as lead or cadmium [16]. The tubulo-interstitial distortion and tubular atrophy observed in the short and long term high dose groups in the present study may be due to the nephrotoxic effects of the toxic agents in the fixer effluent. It is noteworthy that acute interstitial or tubular damage can produce acute renal failure, and chronic changes are good prognosis factors in glomerular and/or vascular diseases (www.kidneypathology.com).

In general, glomerular lesions after occupational or environmental exposures are very uncommon. However, acute and chronic forms of the glomerulonephritis are caused by a variety of infectious, autoimmune or inflammatory conditions or by direct exposure to toxic agents [14]. The present findings revealed signs of glomerular necrosis and different degree of shrinkage of the glomerular tufts after long-term, high-dose exposure to fixer effluent. There was also infiltration of inflammatory cells within the glomerular tuft and stroma, and the presence of eosinophilic colloid substances. The inflammatory infiltration may be due to deposition of immune complexes probably generated by the fixer efflux [17]. The presence of the numerous chronic inflammatory cells in the renal interstitium may explain the destruction of the renal interstitium observed in this study, which may cause loss of kidney function [18]. Furthermore, the above effects may destroy the basic functions of the nephrons which are ultra-filtration, tubular reabsorption and tubular secretion.

The mechanisms behind the nephrotoxicity effects of fixer efflux in the present study are not clear and were beyond the scope of this study. However, it has been reported that renal toxicity can be a result of hemodynamic changes, direct actions of toxins on cells and tissue, immunological reactions due to inflammatory tissue injury, and/or obstruction of renal excretion [19]. It is possible that the nephrotoxicity effects observed in renal tissues in the present study may be a direct toxic effect of the constituents of the fixer effluent. One of the major constituents of fixer effluent, the silver metal, has been implicated as one of the toxic metals involved in acute renal failure [20, 21]. Ingestion is the primary route of entry for silver compounds and colloidal silver proteins [22]. The body's uptake of silver is often much higher when taken orally as medication, as opposed to occupational exposure, which is predominantly through inhalation. In other studies, silver could not be definitively linked to an adverse health outcome due to the presence of confounders. The study by Rosenman et al. [23] attempted to assess the effects of silver on kidney function. Creatinine clearance was significantly depressed and urinary NAG was significantly higher in the exposed group. However, it was difficult to determine if silver caused any adverse effect on kidney function because the workers had also been exposed to other agents that were known nephrotoxins [24]. Similarly, sub-acute boric acid (a component of fixer effluent) exposure has been reported to cause dose-dependent histopathological degenerative changes in kidney tissue [25]. Furthermore, Koschier et al., acetic acid (bis(p-chlorophenyl)) produced renal failure in vivo which was associated with a reduction in renal perfusion pressure [26]. However, perfused kidney experiments indicated that acetic acid (bis(p-chlorophenyl)) could have caused a direct effect on nephron function [26]. The US National Library of Medicine has also reported that the toxic effects of acetic acid observed in human body are due to its irritant properties as well as its effect on the central nervous system and kidneys [27].

Limitations of study

We could not carry out further investigations to elucidate the exact mechanisms behind the nephrotoxic effects of fixer effluent on the wistar rats. Our major focus was to ascertain if there were morphological alterations or histopathological changes in rat kidney tissues due to acute or chronic fixer effluent exposures. We therefore recommend further studies in this regard.

CONCLUSIONS AND RECOMMENDATIONS

The present study indicated that both acute/chronic and long-term/short-term exposures to sub-lethal doses of fixer effluent caused alterations in the histology of the kidney of Wistar rats. These alterations may account for the various nephrotoxic effects associated with exposure to fixer effluent. In addition, some of the histopathologic effects were marked in long-term and chronic compared to short-term and acute exposure to fixer effluent, thus indicating dose-duration-dependent effect of fixer effluent on the kidney of wistar rats.

In view of the nephrotoxicity effects of fixer effluent observed in the present study and the need to minimize other environmental hazards and risks to public health, we recommend that, appropriate treatment and disposal of fixer effluent should be done before disposal and by companies duly licensed by the environmental agency. There should be avoidance of the silver complexes arising from the exhausted fixer effluent entirely by using a fixer only part way to exhaustion. This will help deal only with the silver complexes that are relatively easy to wash out of paper fibers and minimize the need to dump the harmful silver compounds into the environment. There is a strong need to provide increased awareness and guidance and improve the knowledge on specific regulations among professionals involved in health services which generate radiographic processing effluents. Furthermore, training courses on health services waste management for different institutions should be encouraged and provided by health and environmental agencies.

CONFLICT OF INTEREST

"The authors declare that they have no competing interests."

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