

Original Research Article

Correlating X-Ray Fixer Induced Biochemical and Histological Renal Injuries in Wistar Rats

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Abstract: Renal problems are among the diseases that have fatal outcome if not diagnosed early and given proper treatment. Unfortunately renal problems are on the increase worldwide. Many intrinsic and extrinsic factors can cause renal problems. One of such factors is chemical substances. This study aimed at investigating and correlating x-ray film fixer induced biochemical and histological renal injuries in wistar rats. The study was carried out between 15th July and 14th August, 2014. Eighteen (18) apparently healthy wistar rats of 20-24 weeks and weighing between 208g and 210g were randomly divided into three groups (A-C) of six rats each. Groups A and B were the experimental groups exposed to different concentrations of the fixer solution for periods ranging from 15- 30 days while group C served as the control group. At the end of each desired period of time, two rats were randomly selected from each group, painlessly sacrificed, the kidneys harvested and blood sample collected and examine/analyzed at the Medical Laboratory Sciences department of Nnamdi Azikiwe University Nnewi Campus. Results showed raised serum urea and creatinine levels which were independent of the concentration of the developer solution in the first 15 days but concentration dependent at longer exposures. Varying histological changes were also observed, including distortion of tissue interstitial architecture, hypertrophy of the Bowmans capsule, infiltrate of inflammatory cells, loss of tubules and signs of necrosis. The biochemical and histological injuries correlated at short exposure times and low doses but showed divergence at higher doses.

Keywords: X-ray film fixer, Wistar rats, Biochemical renal injury, Histological renal injury, Serum urea, Serum creatinine.

INTRODUCTION

The kidneys are bean-shaped intra-peritoneal organs that perform many vital functions including homeostatic functions such as the regulation of electrolytes, maintenance of acid-base, regulation of blood pressure and elimination of waste products of metabolism such as urea from protein metabolism, uric acid from nucleic acid and creatinine from muscle metabolism. The kidneys accomplish these functions via filtration, reabsorption and secretion that take place in the nephrons which are the functional units of the kidneys. The kidneys also secrete a number of important hormones such as erythropoietin which stimulate erythropoiesis, and calcitriol (activated vitamin-D) that promotes the absorption of calcium and reabsorption of phosphates. The major parts of the kidney involved in the performance of these functions are the glomeruli, Bowman's capsule and the tubules. The glomerulus is composed of microscopic clusters of looping blood vessels contained in each nephron of the kidney through which blood is filtered. It

allows passage of water and small molecules but retains blood cells and larger molecules. Attached to each glomerulus is a tubule that collects the fluid and molecules that pass through the glomerulus and then reabsorb any that can be used by the body but pass out the rest as waste in urine. Injuries to these parts will result in kidney disease/damage and dysfunction.

The kidney is one of the primary sites of drug toxicity [1, 2]. It is the target for numerous xenobiotic toxicants including chemicals [3]. It's anatomical, physiological and biochemical features make it particularly sensitive to many environmental compounds [3]. Examples included the large blood flows within the kidneys [about 20% of the cardiac output], presence of variety of xenobiotic transporters and metabolic enzymes, and concentration of solutes during urine production [3]. Also, the conjugation of environmental chemicals to glutathione and /or cysteine make them show predilection to the kidneys with attendant inhibition of the kidney functions through

various mechanisms. The low solubility of substances such as ethylene glycol metabolites causes crystal formation within the lumen and nephrotoxicity [3]. X-ray film fixer is one of the processing chemicals that is essential for obtaining permanent images on the film emulsion base that can withstand abrasion but are known to contain substances/components that make it to have harmful effects on important organs such as the lungs, heart and the liver which have functional relationship with the kidneys [4-7].

The kidney can suffer a number of injuries/damages which could be inflammatory or non-inflammatory or acute or chronic. Often renal problems are fatal if not diagnosed and treated early. Early diagnosis often requires correlation of morphological evaluation with the clinical history and laboratory investigation results.

Unfortunately acute renal failure is currently on the increase with high morbidity and mortality rates [3, 8-9]. In Brazil for instance an increase of 115% was noted in the number of dialysis patients between 2000 and 2010, with a total of about 91,314 patients in 2011 with more than 77,000 patients undergoing haemodialysis as replacement therapy for kidney function in 2009[10]. Although there are many other waste products that can be used to evaluate kidney diseases, urea (Blood Urea Nitrogen, BUN/ Serum Urea Nitrogen, SUN) and creatinine are the most convenient to track [11]. Urea and creatinine are used to evaluate kidney functions in a wide range of circumstances to diagnose renal diseases and to monitor people with acute or chronic renal dysfunction or renal failure. Urea is a waste product formed in the liver from protein metabolism. It is carried to the kidneys from where it is filtered out of the blood into urine. Urea level in blood rises when there is kidney disease or damage which prevents the kidneys from filtering urea out of the blood. Increased level of serum urea is suggestive of impaired renal function, which could be due to acute or chronic kidney disease, damage or failure, or due to conditions that result in decreased blood flow to the kidney such as congestive cardiac failure, shock, stress, recent heart attack or severe burns or due to conditions that cause obstruction of urine flow or due to dehydration [12]. Elevation of urea level can also occur due to increased dietary protein or gastrointestinal bleeding [12]. Serum creatinine is a waste product from breakdown of dietary protein and muscle protein from muscle injury. Increase in serum creatinine level is also indicative of kidney injury.

This study was aimed at correlating x-ray fixer solution induced biochemical and histological renal injuries in wistar rats with a view to extrapolating to humans and hence create awareness to the possible health risks facing radiographers and darkroom staff of radiology facilities practicing darkroom radiography. Hence the need to quicken the transition to

filmless/digital radiography. Wistar rats are used because their genetic, biological, physiological and behavioural characteristics can be reasonably compared to those of human beings [13].

MATERIALS AND METHODS

The research was carried out between 15th July and 14th August, 2014. The experimental research design was adopted. Ethical approval was obtained from the Research and Ethical Approval Committee of the Faculty of Health Sciences and Technology, Nnamdi Azikiwe University, Nnewi Campus, Nnewi, Anambra State, Nigeria. Eighteen (18) apparently healthy wistar rats of 20-24 weeks and weighing between 208g and 210g obtained from the animal farm of Nnamdi Azikiwe University, Nnewi Campus were used. These rats were randomly divided into three groups (A-C) of six rats each. Groups A and B were the experimental groups while group C served as the control group. Each group of rats was put in a labeled metal cage and observed for one week to acclimatize in their new environment before the start of the study.

EXPERIMENTAL PROCEDURE

Using a standard procedure, a full strength fixer solution (0.05g per cm³) was prepared by dissolving the 500g packet of fixer powder in 10litres (10,000cm³) of water. Then five litres (5000cm³) of the prepared fixer solution was diluted with an equal volume of water to obtain a fixer solution with half the concentration of the original solution (0.025g per cm³).

Wistar rats in the experimental group A were exposed to the fumes from the full strength fixer solution contained in a plastic bowl placed close to the cage harbouring them. By the same method, the rats in the experimental group B were exposed to the fumes from the fixer solution of half strength concentration. The rats in the control group, C were not exposed to any fixer solution fumes. The cages harbouring the rats were kept in different rooms of a house. The rats were fed with water and Vital Feeds Growers palletized for feeding the rats as prescribed by the manager of the animal house from where they were obtained. The rats were made to experience 12 hours of light and 12 hours of light daily until sacrificed.

On the 15th day of the experiment, two rats were randomly selected from each group for dissection. Before dissecting the rats, each was weighed and their weights noted. Each of the rats was then anesthetized by placing it in a bell jar with a wire mesh floor over gauze moistened with chloroform and observed for signs of decreased motility and unsteady gait for about 20 seconds. Each rat was then brought out of the bell jar and painlessly sacrificed. With gloved hands, the proper incision was made on the midline of the ventral aspect from the thoracic region to the abdomen. The kidneys of each rat were then harvested, preserved in 10% formalin in a plain sample bottle and sent to the

Medical Laboratory Sciences department of Nnamdi Azikiwe University, Nnewi Campus for analysis.

On that same 15th day, two rats were randomly selected from each of the experimental groups (A and B) for pulmonary aspiration with the respective fixer solutions. Before aspirating the rats with the fixer solutions, the rats were labeled A1, A2, B1, and B2 and weighed. To aspirate the rats with the fixer solutions, each rat was anesthetized using the same procedure described earlier for the rats sacrificed above. Each rat was then brought out of the bell jar. Using a 5ml syringe each rat was aspirated with 0.5ml of the solution: rats A1 and A2, with the full strength fixer solution and rats B1 and B2, with the half strength fixer solution. To instill the solution, the mouth of the rat was opened. The tongue was pulled to a side using a swap stick and the solution was introduced to pass down through the pharynx. The aspirated rats were then kept on a slab and observed to recover from the effects of the anesthesia and were put back into their respective cages where they stayed and continued with the non-aspirated rats in the group.

On the 30th day (end of the experiment) all the rats in each group were painlessly sacrificed after anesthetizing with chloroform as described earlier for the rats sacrificed on the 15th day.

Tissue collection

With a surgical blade a ventral midline incision was made from the thoracic region to the abdomen of each rat. The kidneys were harvested and each preserved in a vial of 10% formalin. The organs were labeled according to their groups and mode of exposure to the fixer solution (i. e. inhalation only or inhalation plus aspiration). The specimens were then sent to the

Medical Laboratory Sciences department for examination and analysis of the biochemical and histological changes that might have occurred in the kidneys.

Tissue Preparation and Processing

The kidney tissues were processed using standard operative procedures and embedded in molten paraffin wax. The embedded tissues were then mounted on wooden blocks, sectioned with microtome knife and stained with Ehrlich's haematoxylin and Eosin staining method [14].

The stained slides were then cleared in xylene and mounted in dibutyl phthalatepolystyrene xylene (DPX).

Microscopy and photomicrography

Microscopic examination of the cut sections was carried out using Swift binocular microscope with in-built lighting system. Sections with striking features were selected for photomicrography using Olympus photomicroscope with coloured films. Results were expressed in terms of observed biochemical and histological changes in kidney tissues.

BIOCHEMICAL ANALYSIS

Serum Urea

Serum urea is estimated using the Berthelot Reaction [15, 16]

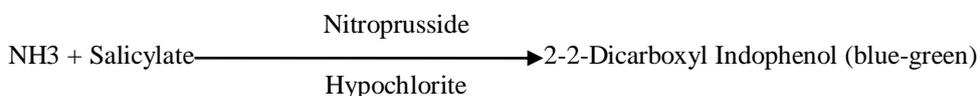
Principle:

Serum urea, in the presence of urease enzyme is hydrolyzed to ammonia and carbamic acid with the carbamic acid spontaneously decomposing to ammonia and carbon dioxide.



The ammonia then reacts with Salicylate (or phenol) nitroprusside and alkaline solution of hypochlorite to yield a blue-green

chromophore(indophenols) which absorbance can be measured photometrically and is directly proportional to the concentration of urea in the serum sample.



In the procedure, three test tubes labeled: TEST, STANDARD and BLANK were set up with 10µl of serum sample added to each. Distilled water was then pipette to each test tube. Reagent 1(phenol) (50µl) was pipette into each test tube, mixed and incubated for 10minutes at 37°C. Then reagent 2(1.5ml) urease enzyme was pipette into each test tube, followed

by 1.5ml of reagent 3(sodium hydroxide/sodium hypochlorite solution). They were then properly mixed and incubated for 15minutes at 37°C. The absorbance was then read at 540nm against the blank. The concentration of the TEST was calculated using the formula:

$$\text{TEST conc.} = \frac{\text{TEST absorb.} \times \text{STD conc.}}{\text{STD absorb}}$$

Where, TEST conc. = Concentration of the Test

TEST absorb. =Absorbance of the Test
 STD conc. = Concentration of the Standard
 STD absorb. = Absorbance of the Standard.

Serum Creatinine

Serum creatinine is estimated using Jaffe Slot’s Akaline Pictrate method[17]

Principle

In an alkaline medium (and the creatininase enzyme) serum creatinine reacts with picric acid to produce creatinine pictrate with orange/red colour which can be measured spectrophotometrically and the intensity of the colour is directly proportional to the concentration of creatinine in the serum sample.

The procedure is in two stages. In the first stage, two test tubes labeled ‘TEST’ and ‘BLANK’ were set up. About 0.5ml serum sample was pipetted into the TEST test tube. Then 1.5ml distilled water was

added to the TEST test tube and 2.0ml distilled water to the BLANK test tube. This was followed by 0.5ml of 2/3NH₂SO₄ and 0.5ml of 10% sodium tungstate added to each of the test tubes. The tubes were then centrifuged for 5minutes and the supernatant solution in each tube was collected.

In the second stage, 0.5ml of 10% sodium tungstate was pipette into each test tube followed by 0.5ml picric acid. Then 0.5ml of 0.7N NaOH was added and the set up was incubated for 20 minutes at room temperature after which the result was read at 520nm.

The concentration of the TEST was then calculated using the formula:

$$\text{TEST conc.} = \frac{\text{TEST absorb.} \times \text{STD conc.}}{\text{STD absorb}}$$

Where, TEST conc. = Concentration of the Test
 TEST absorb. =Absorbance of the Test
 STD conc. = Concentration of the Standard
 STD absorb = Absorbance of the Standard.

Data Analysis

Statistical analysis was done using a computer package – statistical package for social sciences version 20 (SPSS version 20). Analysis of variance (ANOVA) test was used to test for differences in the urea and creatinine levels among the groups of rats. Scheffe’s

test was used to find the source(s) of any difference among the groups. A P-value of < 0.05 was considered statistically significant.

RESULTS
Biochemical Analysis

Table 1: Serum urea and creatinine levels of the wistar rats at varying concentrations of the fixer solution and different exposure conditions

Period/condition of exposure	Concentration	Rat no	Serum urea (mmol/L)		Serum creatinine (mmol/L)	
			SURK	SULK	SCr RK	SCr LK
15 days inhalation	Full	1	11.8	11.8	159	159
		2	11.7	11.8	159	159
	Half	1	9.9	9.8	114	114.2
		2	9.8	9.8	114	114
	Control	1	4	4	78	78
		2	4	4	78	78
30 days inhalation without aspiration	Full	1	3	3	93.3	93
		2	3	3.1	93	93
	Half	1	4.5	4.5	101	101
		2	4.5	4.5	101	101
	Control	1	3.9	4	78	78
		2	4	4	78	78
30 days inhalation plus aspiration	Full	1	3.8	3.9	78	78
		2	3.8	3.8	78	78
	Half	1	3	3.1	78	78
		2	3	3	78	78
	Control	1	3.9	4	78	78
		2	4	4	78	78

SURK=Serum urea right kidney; SULK=Serum urea left kidney
 SCr RK =Serum creatinine right kidney; SCr LK =Serum creatinine left kidney

Table 2: Mean serum urea and creatinine levels and their Scheffe's p-values

Period/condition of exposure	Concentration	Mean serum urea (mmol/L)		Scheffe p-value	Mean serum creatinine (mmol/L)		Scheffe p-value
		RK	LK		RK	LK	
15 days inhalation	Full (group A)	11.75	11.8	0.000 (0.000)+	159	159	0.000 (0.000)+
	Half (group B)	9.85	9.8		114	114.1	
	Control (group C)	4	4		78	78	
30 days inhalation without aspiration	Full	3	3.05	0.000 (0.002)+	93.15	93	.000 (0.000)+
	Half	4.5	4.5		101	101	
	Control	3.95	4		78	78	
30 days inhalation plus aspiration	Full	3.8	3.85	0.001 0.078* (0.003)+	78	78	0.171
	Half	3	3.05		78	78	
	Control	3.95	4		78	78	

*Statistically non-significant at the 0.05 level

(+) + Scheffe's p-value between rats exposed to half concentration of fixer solution and the control.

Table 2 above showed that significant difference ($P < 0.05$) exists in the mean urea and mean creatinine levels of rats exposed to the full strength fixer solution and the rats exposed to the half strength solution and those in the control group for the 15 days and 30 days inhalation of the fumes from the fixer solutions.

For the aspirated rats that also inhaled fumes from the solutions for 30 days significant difference ($P < 0.05$) occurred in the mean urea levels of rats

exposed to the full strength fixer solution and those exposed to the half strength solution and also between the rats exposed to the half strength fixer solution and the rats in the control group (Table 2). No difference ($P = 0.078$) exists in the mean serum urea level between the rats exposed to full concentration of the fixer and rats in the control group (Table 2). Also, no difference ($P = 0.171$) exists in mean creatinine levels in any of the aspirated rats groups nor between them and the rats in the control group (Table 2).

Histological

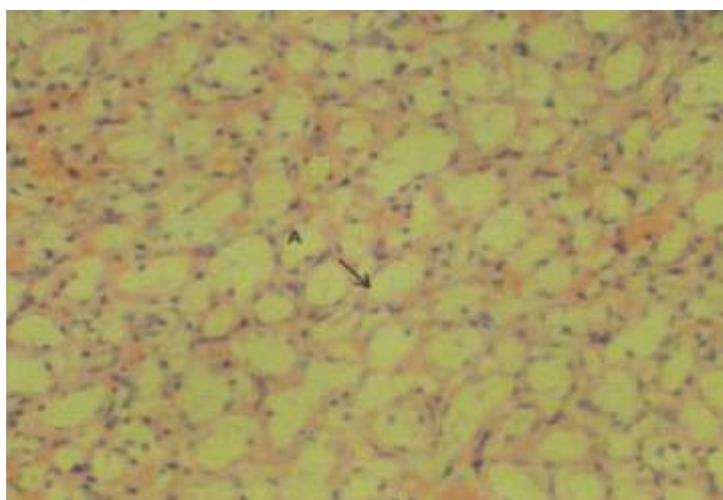


Fig. 1: Photomicrographs of the kidney of rats from the control group showing normal tissue architecture (Mag. X 100). A → normal intercellular boundary

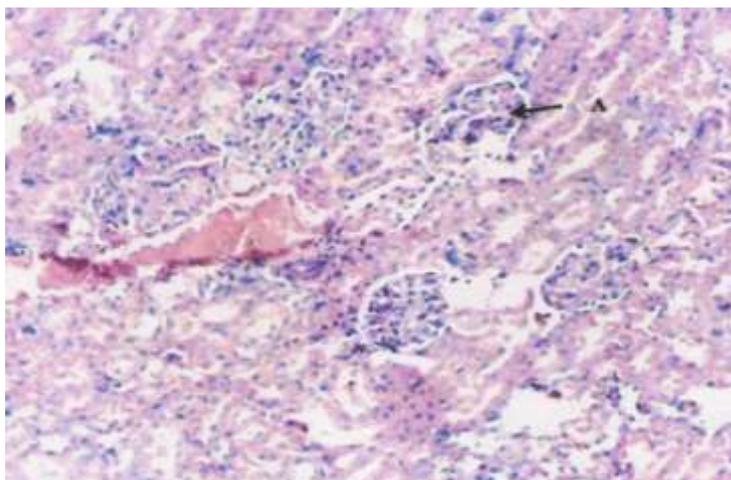


Fig. 2: Photomicrograph of the kidney of wistar rats that inhaled fumes from full concentration of x-ray film fixer solution for 15days showing mild distortion of interstitial tissue architecture, hypertrophy of Bowman's capsule, and sign of necrosis (Mag. X 100) A→Bowman's capsule

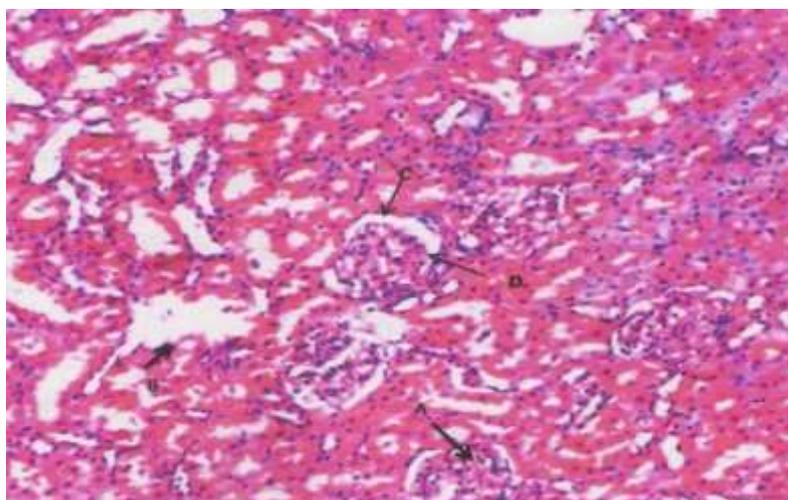
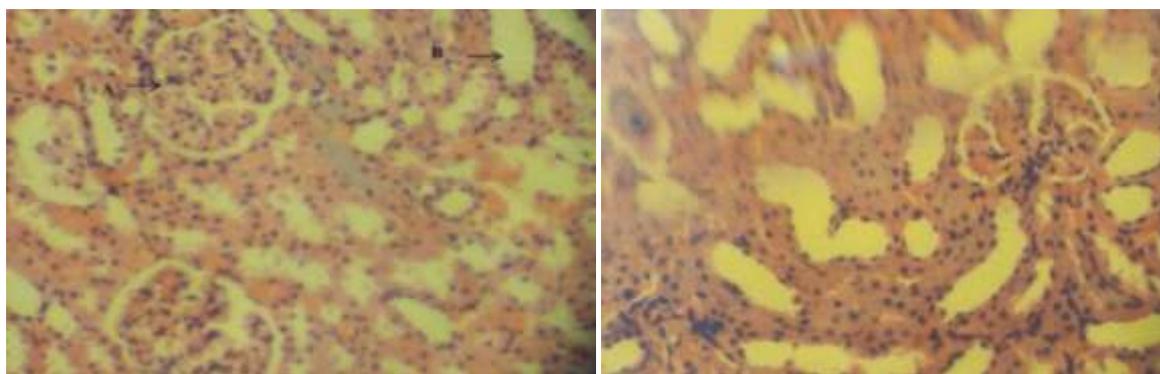


Fig. 3: Photomicrograph of the kidney of wistar rats that inhaled fumes from half concentration of x-ray film fixer solution for 15 days showing mild distortion of interstitial tissue architecture, hypertrophy of Bowman's capsule and signs of necrosis. (Mag. X100), A→Bowman's capsule, B →Collecting tubule, C→Visceral layer, D→Parietal layer



A. Full concentration

B. Half concentration

Fig. 4A and B: Photomicrograph of the kidney of wistar rats that inhaled fumes from x-ray film fixer solution for 30 days showing distortion of interstitial tissue architecture, hypertrophy of Bowman's capsule, severe loss of tubules, infiltrate of inflammatory cells and atrophied surviving tubules. (Mag. X2 00)

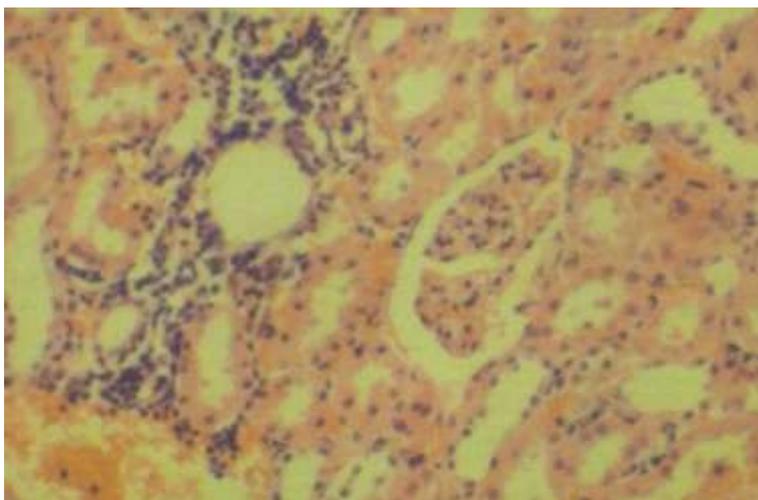


Fig. 5: Photomicrograph of the kidney of aspirated wistar rats that inhaled fumes from full concentration of x-ray film fixer solution for 30 days showing distortion of interstitial tissue architecture, hypertrophy of Bowman's capsule, loss of tubules, signs of necrosis and infiltrate of lymphocytes (Mag. X200)

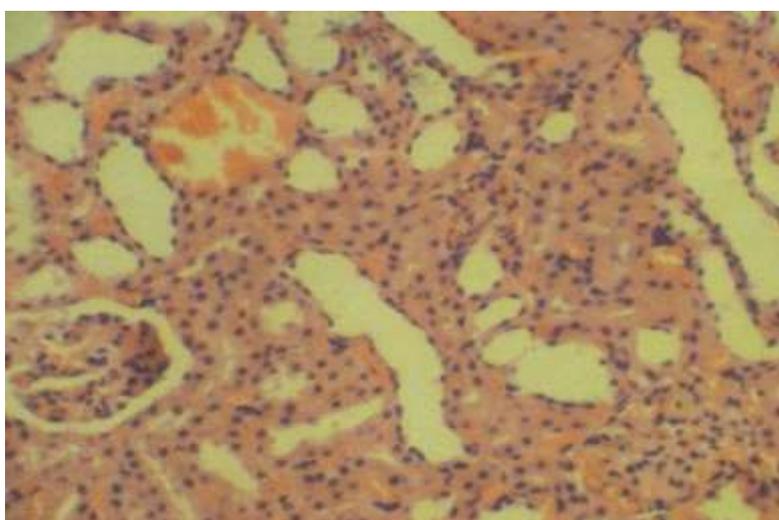


Fig. 6: Photomicrograph of the kidney of aspirated wistar rats that inhaled fumes from half concentration of x-ray film fixer solution for 30 days showing distortion of interstitial tissue architecture, hypertrophy of Bowman's capsule, severe loss of tubules and infiltrate of inflammatory cells. (Mag. X 200)

DISCUSSION

Marked increase in the serum urea and creatinine levels were noted in the kidneys of group A and B rats (experimental groups) inhaling fumes from the respective fixer solutions for 15 days. The rise in the urea and creatinine levels was in accordance with what obtains in acute renal injuries/damage[12] possibly resulting from vasoconstriction [18]. Both the biochemical and histological results indicated significant difference in the findings between the rats in the control group and the rats in each of the experimental groups that inhaled fumes from the respective fixer solutions for 15 days and 30 days. Biochemically, there was a significant difference between kidneys of rats that inhaled the fixer solution fumes for 15 days and those that inhaled fumes from the same solution for 30 days but the histological results were similar. This could be due to acquired immune response by the tissue defense mechanism. The fall to

the normal levels of urea and creatinine in the aspirated rat which also inhaled the fumes from the fixer solution might represent a situation of reversed vasoconstriction. A similar result was noted by Robert *et al* [18]. This kind of condition could be deceiving as was shown by the abnormal histology result (Plates 5 and 6). This implies that with long exposure to the fixer fumes can mask the true situation. That is to say that on long term exposure, even though the kidneys are being injured, biochemical/laboratory investigations of the urea and creatinine levels will give a false negative result. With respect to the radiology staff (radiographers and darkroom technicians) that are exposed to the fixer for long periods, their kidneys will be dying without their knowledge of it. Since many renal patients often present late, both biochemical investigations are necessary to arrive at proper diagnosis. In other words, biochemical investigation of urea and creatinine levels give better results in acute cases while histological investigations

serve better in chronic conditions. The histology results also indicate the affected portions of the kidney. From the result it was shown that the fixer solutions cause acute injuries via attacks on the main functional apparatus of the kidneys-the tubules and the Bowman's capsules/glomerulus.

CONCLUSION

X-ray film fixer solutions can cause both acute and chronic renal injuries in wistar rats. Acute short exposure injuries can be diagnosed by investigating the serum urea and creatinine levels while long term chronic injuries are better diagnosed by histological tests. At short exposures and low doses(indicated by exposure by inhalation without aspiration), the biochemical and histological result match but at high doses(indicated by inhalation and aspiration) the biochemical and histological results differed and the histological results gave better information.

ACKNOWLEDGEMENTS

Author acknowledge with thanks the help rendered by the Medical Laboratory Sciences department of Nnandi Azikiwe Uninersity, Nnewi Campus, Nnewi, Anambra State, Nigeria.

REFERENCES

1. Vaidya VS, Ramirez V, Ichimura T, Bobadilla NA, Bonventre JV; Urinary kidney injury molecule-1: a sensitive quantitative biomarker for early detection of kidney tubular injury. *American Journal of Physiology - Renal Physiology*, 2006; 290(2): 517-529.
2. Mark PM;Renal Vulnerability to Drug Toxicity.Clinical Journal of the American Society of Nephrology,Department of Medicine, Yale University, New Haven, Connecticut.
3. Van-Vleet TR,Schnellmann RG; Toxic nephropathy: environmental chemicals. *Seminars in Nephrology*; September 2003, 23(5):500-508.
4. National Institute of HealthNIH; A Service of the U.S National Library of Medicine, Sodium Hydroxide Poisoning. Available from www.nlm.gov/. Accessed 12th June 2014.
5. Sherwood BP; Occupational risks of gluteraldehyde may cause respiratory, nasal and skin problems at low concentrations. *British Medical Journal*, 1989; 299-342.
6. Neelam B, Uppal V, Pathak D; Toxic effect of formaldehyde on the respiratory organs of rabbits: A light and electron microscopic study. *ToxicolIndust Health*, 2011; 27(6): 563–569.
7. Chiegwu HU, Ugwuanyi DC, Ogenyi SI, Chuks HC, Mbakwe KL; Hepatic cells and enzymes reactions in wistar rats exposed to x-ray film processing chemicals.Scholars Journal of Applied Medical Sciences, 2016; 4(1B):112-123.
8. Bonventre V Joseph, Weinberg M Joel; Recent Advances in the Pathophysiology of Ischemic Acute Renal Failure. *Journal of the American Society of Nephrology*, 2003;14: 2199–2210.
9. Tolkoff-Rubin NE, Rubin RH, Bonventre JV; Noninvasive renal diagnostic studies. *Clin Lab Med.*, 1988; 8: 507–526.
10. Abreu de Castro BB, Colugnati FAB, Cenedeze MA, Giovanni de Albuquerque Suassuna P, Pinheiro HS; Standardization of renal function evaluation in Wistar rats (*Rattusnorvegicus*) from the Federal University of Juiz de Fora's colony. *Official Organ of the Brazilian Society of Nephrology-* Apr/May/June 2014; 36 (2):
11. Hines R; Chronic Kidney Disease In Your Pet Rat - What It Is, And What You Can Do About It. <http://www.2ndchance.info/> in
12. American Association for Clinical Chemistry(AACC); Blood Urea Nitrogen. *LabTests Online*, 2001-2016, reviewed 31st Aug. and modified 29 Oct. 2015. Accessed 6th Dec. 2015.
13. Taksshi KG, Nakanishi S, Ochiai M, Nakagama H, Voigt B, Serikawa T; Origins of albino and hooded rats: Implications from molecular genetic analysis across modern laboratory rat strains. *PLoS ONE*. 2012; 7:e43059.
14. Godwin A; Histological uses of Haematoxylin: a Review. *Journal of Pharmacy and Clinical Sciences*, 2011; 1: 24-34.
15. Urea Nitrogen (BUN); (Berthelot/Colorimetric). Pointe Scientific, Inc, 5449 Research Drive. Canton MI 48188. USA. www.pointescientific.com
16. Shakya PR; Digital Home 2013.Biochemistry Class notes. Urea estimation: Principle, Methodology and clinical significance. Posted Saturday, November 10, 2012. Reposteted Dec 21, 2015. Accessed 15th Jan. 2016.
17. Peake M, Whiting M; Measurement of Serum Creatinine – Current Status and Future Goals. *Clin Biochem Rev*. 2006; 27(4): 173–184.
18. Schrier RW, Wang W, Poole B, Mitra A; Acute renal failure: definitions, diagnosis, pathogenesis, and therapy. *Journal of clinical investigation*, 2004; 114(4): 598.