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Original Research Article

Hepatic cells and enzymes reactions in wistar rats exposed to x-ray film processing chemicals

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Abstract: Medical images play significant roles in the diagnosis and management of patients. In developing countries darkroom radiography is practiced and involves chemical processing of films using chemical substances known to be toxic when ingested or inhaled. This study aimed at investigating the histological changes and the changes in alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels in the liver of wistar rats exposed to different concentrations of x-ray film processing chemicals for varying periods of time. Thirty (30) apparently healthy wistar rats of 20-24 weeks, weighing between 190g and 210g were randomly put into five groups, A-E of 6 rats each. Rats in groups A-D were the experimental groups exposed to varying concentrations of the processing chemicals for periods ranging from 15-30 days while rats in the control group E were not exposed to any processing chemical. After each desired period of time, two rats from each group were randomly selected, painlessly sacrificed, the liver harvested and blood sample collected and examine/analyzed at the Nnamdi Azikiwe University Teaching Hospital, Nnewi Anambra State. Results showed that both x-ray film processing chemicals had adverse effects on the ALT and AST liver enzymes and liver tissues of wistar rats exposed to these chemicals. The effects were not significantly affected by the concentration of the solutions but were related to the period of exposure especially for the developer solution. While the major histological effect of the fixer was the dilation of central vein, the developer had varied histological effects.
Keywords: Hepatic cells, ALT and AST Liver enzymes, Wistar rats, X-ray film developer, x-ray film fixer

INTRODUCTION

Medical images play significant roles in the diagnostic and management of patient conditions. In developed countries of the world the production of radiographs is now by digital or filmless radiography. In developing countries including Nigeria, darkroom radiography is practiced in majority of radiology centres/facilities.

In darkroom radiography, the production of radiographic images involves x-ray exposure of the film emulsion and the processing of the films via the two processes of film development and fixing. Both of these later processes require the use of processing chemicals- the developer and the fixer respectively. Each of these processing chemical agents contains substances which are known to be toxic when ingested or inhaled.

The developer has its main agent as either monomethyl-p-amino-phenol hemisulphate (Methol) or 1-phenyl-3-pyrazolidione (Phenidone) mixed with hydroquinone to give metol hydroquinone or phenol hydroquinone respectively. Other additive components of the developer include sodium/potassium hydroxide, sodium sulphite, acetic acid, bromine/chlorine-containing compounds and a lot of others including gluteraldehydes.

Both the metol/phenol and the hydroquinone are known to have toxic effects and can cause severe respiratory problems [1-6]. Also exposure to the developer was associated with some cardiac symptoms [7-9]. Sodium hydroxide (NaOH), a component of developer was reported to be toxic to the heart [10]. After ingestion of several grams of pure hydroquinone, tinnitus, swelling of the tongue, exhaustion and tiredness, hypothermia, tachycardia with a fall in blood pressure, pallor, dyspnoea, cyanosis, convulsions and unconsciousness were observed in man. Doses of 1.3 and 12 g pure hydroquinone were reported not be fatal [11], but deaths occurred after ingestion of photographic developing agents containing 5 to 12 g

hydroquinone [12]. After medium-term and long-term application in animal experiments, hydroquinone led in rats and mice to increased liver weights and, after oral administration, to ulceration, inflammation and hyperplasia in the fore stomach[11]. In mammalian cells, gene mutation, micronuclei, chromosomal aberration and DNA single strand breaks were observed in vitro and in vivo, while sister chromatid exchange (SCE) was seen only in vitro [11]. In a study of the effects of hydroquinone on lympho haematopoietic system, a non-toxic concentration of hydroquinone showed a dose-dependent inhibition of differentiation to macrophages in a human promyelocytic leukemic cell line [13].

The fixer component of the processing chemical also contains substances that have some adverse effects. Its active agent is sodium thiosulphate ($Na_2S_2O_3$) or ammonium thiosulphate ($(NH4)_2S_2O_3$). Like the developer, the fixer also contains other additives. These substances are considered to be toxic, corrosive and potentially carcinogenic [14-15].

The liver is a very important and unique organ in the body that performs many vital and life-maintaining functions in the body some of which include detoxification of harmful substances, synthesis of blood-clotting proteins, production of biochemical substances necessary for digestion, filtering and processing blood as it circulates through the body, and many others. These functions are carried out by liver cells (hepatocytes) and are catalyzed buy liver enzymes, mainly alanine aminotransferase, ALT (formerly called serum glutamic pyruvic transaminase (SGPT) and aspartate aminotransferase, AST (formerly called serum glutamic oxaloacetic transaminase (SGOT). Ordinarily the liver enzymes (ALT and AST) do not pass into the blood. When however, there is injury to the liver cells, these enzymes leak into the blood. Liver enzyme tests (formerly liver function tests) are mainly to test for the enzymes ALT, AST, Alkaline phosphatase(ALP) 5' nucleotidase and Gamma-glutamyl transpeptidase (GGT), especially the ALT and AST. While the AST can also be found in cardiac and skeletal muscles and many other tissues, the ALT is almost found exclusively in the liver and simultaneous elevation of the blood ALT and AST invariably indicates liver damage [16].

In view of the numerous and vital functions of the liver and the fact that currently there is no means of compensating for the absence of liver functions in the long term[no artificial organ or device capable of performing or emulating all the functions of the liver][17], it becomes necessary to investigate the effects of the film processing chemicals on the liver.

Research showed that even at low level concentrations the processing chemicals have severe health problems such as asthma, headache, itchy eyes, cardiac arrhythmias and that the problems were more in radiographers and darkroom staff [18-20]. It was reported that these chemicals often cause subclinical injury to the liver which manifest as abnormal liver enzymes [21].

The literature findings points to the very high health risks radiology workers are exposed to but which they, and/or their .employers might not know. Establishing injuries to the liver caused by x-ray film processing chemicals will therefore be very useful to radiology workers and employers in developing countries as it will stimulate more efforts towards digital and/or filmless radiography. The aim of this research was therefore, to investigate the histological changes and the changes in the ALT and the AST levels in the liver of wistar rats following exposure to different concentrations of x-ray film processing chemicals-the developer and fixer for varying periods of time.

MATERIALS AND METHODS

This study adopted the experimental research design. Ethical approval was obtained from the Research and Ethical Approval Committee of the Faculty of Health Sciences and Technology, Nnamdi Azikiwe University, Nnewi Campu, Nnewi, Anambra State, Nigeria. The research was carried out between 1st and 30th June, 2014.

Thirty (30) apparently healthy wistar rats (male and female) of 20-24 weeks and weighing between 190g and 210g obtained from the animal house of Nnamdi Azikiwe University, Nnewi Campus were used. These rats were randomly put into five groups, A-E of 6 rats each and put into labeled metal cages and observed for 7 days to acclimatize in the new environment before the start of the experiment.

Rats in groups A-D were the experimental groups (A and B, for the developer and C and D, for the fixer) while rats in group E served as the control. Rats in group A were exposed to the full strength developer solution while rats in group B were exposed to half strength developer solution. Similarly, rats in group C were exposed to full strength fixer solution and rats in group D were exposed to half strength fixer solution. Rats in the control group E were not exposed to any chemical solution but were allowed in their normal environment.

Materials used for the study include 5metal cages, bell jar, weighing balance, dissecting set, an electron microscope, 5ml syringes, palletized vital feed growers produced by Grand Cereals Limited, water, feeding and water troughs, 10% formalin, cotton gauze, surgical gloves, chloroform, plain sample bottles,

EDTA bottles, 500g weight packet of developer powder and 500g weight packet of fixer powder (products of Be good company, each with production date as 26/10/12 and expiry date of 26/10/16) , two-10litre water tanks , 50ml measuring jugs , 4 small (3 liter) plastic bowels, Reitman-Frankel ALT Kit (Span diagnostics, code MB913).

Experimental Procedure

Using the standard procedure, a full concentration of the developer solution (0.05g per cm^3) was prepared by dissolving the 500g packet of developer powder in 10litres (10,000cm^3) of water. Five liters (5000cm^3) of the prepared developer solution was then diluted by equal volume of water to obtain a developer solution with half the concentration of the original solution (0.025g per cm^3). The full strength and half strength fixer solutions were similarly prepared from the 500g weight packet of fixer powder.

Wistar rats in the experimental group A were exposed to the fumes from the full concentration developer solution contained in a plastic bowel 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 developer solution of half concentration. Rats in the experimental groups C and D were similarly exposed to the respective strength fixer solutions. The rats in the control group, E were exposed to neither developer nor fixer solution fumes. The cages harbouring the rats were kept in different rooms of a building. 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 environment of the rats was maintained at a temperature of 26-30 ^{0}C as provided in the guide for the care and use of laboratory animals. Each of the rooms housing the rats was lit by a 15watt energy bulb during the night.

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 the weight 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 20seconds. 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. Blood samples were collected from each sacrificed rat by cardiac puncture and discharged into plain sample bottles and centrifuged to obtain the serum. The samples were then biochemically analyzed at the Medical Laboratory Sciences Department of the Nnamdi Azikiwe University Teaching Hospital Nnewi. The liver of each rat was then harvested, preserved in 10% formalin in a plain sample bottle and also sent to the Medical Laboratory Sciences Department of Nnamdi Azikiwe University, Nnewi Campus for examination and histological analysis.

On that same 15th day, two rats were randomly selected from each of the experimental groups (A-D) for pharyngeal aspiration with the respective developer and fixer solutions. Before aspirating the rats with the developer solutions, the rats were labeled A1, A2, B1, B2, C1, C2, D1, and D2 and weighed.

To aspirate the rats with the respective developer or fixer solutions, Rao et al technique was adopted: 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 and placed on a board in a near vertical position (supine 45^o head up), the mouth gently opened and the rat's tongue extended with small forceps. Using a 5ml syringe each rat was aspirated with 0.5 ml of the respective solution instilled at the back of the tongue: rats A1 and A2, with the full strength developer solution, rats B1and B2, with the half strength developer solution, rats C1and C2 with full strength fixer solution and rats D1 and D2 with half strength fixer solution. The tongue was maintained in the extended position and the rat monitored to take a few breathes after the aspiration [22]. 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. Feeding with the vital feeds and water was continued.

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. Blood samples were collected and the liver harvested for examination and analysis.

Tissue collection/Blood collection

With a surgical blade a ventral midline incision was made from the thoracic region to the abdomen of each rat. Blood samples were collected from each sacrificed rat by cardiac puncture and discharged into plain sample bottles and centrifuged to obtain the serum. The liver of each rat was then harvested, preserved in 10% formalin in a plain sample bottle The blood and liver specimen were labeled according to their groups and mode of exposure to the developer and fixer solutions (i.e. inhalation only or inhalation plus aspiration) and then sent to the Medical Laboratory Sciences Department of Nnamdi Azikiwe Teaching Hospital for examination and analysis of the biochemical changes in the blood and the histological and morphological changes that might have occurred in the liver of the rats.

Tissue Preparation and Processing

The liver tissues were cut into thin slices of 3-5mm thick and 1cm long and studied under an electron microscope. The microscopic appearance was first observed for irregularities in shape, consistency, colour, firmness and presence of lesions and ulcers. The tissues were also measured (length x breadth x height), weighed. After microscopic examination, the small pieces of tissues (3-5mm thick and 1cm long) were cut and put into labeled tissue cassettes and preserved in 10% formalin before processing.

The tissues were processed manually by immersing them into a 10% formalin bath for 1hour after which they were passed through 70%, 90% and 95% absolute alcohol I, II and III for 2hours each and absolute alcohol IV overnight to dehydrate the tissues completely. On removing the tissues from the absolute alcohol, the tissues were passed through three changes of xylene (I, II, III) for 1hour 30minutes each to remove the alcohol. The tissues were then impregnated with paraffin wax by passing them through two wax baths (I and II) for 2hours and 1hour respectively. After this, the tissues were embedded in molten paraffin wax in a mould. This was achieved by using a heated forceps to orient the tissue in the mould until it lay in the desired plane. The corresponding labels (I and II) were transferred from the paraffin wax bath and placed against the side of the mould adjacent to the tissue. The mould was then transferred to ice block to solidify. On solidification, the tissue block was removed from the mould and attached to a wooden block using a heated knife.

Sectioning the tissue block

The wooden block to which the tissue block was attached was placed in the block holder in the microtome parallel to the microtome knife. Before sectioning, the tissue block was trimmed to expose the surface of the tissue by adjusting the microtome knife to 10µm. Sections were then cut with the rotary microtome and the ribbons were placed onto 20% alcohol on a large (5cm x 7.5cm) slices to remove minor folds and creases from the sections. The ribbon was gently placed on a water bath preheated to about 45^{oC} so as to float out the tissue. Using a clean slide, the tissue was collected, allowed to dry and then labeled using diamond pencil. The slide was then placed on a hot plate at 5^{oC} for the tissue to adhere to the slide.

Staining the tissue sections

The sectioned tissue was stained using Erhlich's hematoxylin and Eosin staining technique [23] for demonstration of the general tissue structure. The slides were taken to water and stained for 20minutes in Erhlich's hematoxylin. The stained slides were then washed in distilled water with agitation for 10minutes after which they were differentiated in 1% acid alcohol for few seconds and then blued in Scott's tap water for 2minutes. This was followed immediately by counter staining in 1% aqueous eosin for 2minutes. The stained slides were rinsed in water for 30seconds, then dehydrated in ascending grades of alcohol (70%, 90% and 95% absolute I and II) for 2minutes each. The stained slides were then cleared in xylene and mounted in dibutylphthalate polystene 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 colored films. Results were expressed in terms of observed physical/behavioral changes in the rats and histological/morphological changes in the liver tissues.

BIOCHEMICAL ANALYSIS

Serum ALT and AST activities were estimated using Reitman-Frankel method [24]. The principle of ALT and AST estimation using this method is the same except that their respective substrates (alanine/α-ketoglutarate and aspartate/α-glutarate) are used. In the presence of glutamic acid, the ALT enzyme form pyruvate when treated with its substrate while the AST enzymes treated with its substrates forms oxalaoacetates with glutamic acid. The ketoacids (pyruvates and oxaloacetates) formed are then treated with 2, 4-dinitrophenylhydrazine (2, 4-DNPH) to produce the brown colored dinitrophenylhydrazone whose absorbance is measured spectrophotometrically at 540nm.

Measurement of the ALT and AST levels

Two clean test tubes labeled "Test" and "Blank" were set up for each of the ALT and AST. Serum (50µl) was pipetted into each of the test tubes labeled 'Test'. Then 250µl of the respective ALT and AST substrates were added into their respective 'Test' tubes and into their 'Blank'. The tubes were then incubated at 37^0C for 30mins after which 250µl of 2, 4-DNPH was added into each of the test tubes and incubated for 20mins at room temperature. At the end of the 20mins, 2.5ml of 0.4NaOH was pipetted into each test tube and mixed properly. The absorbance was then read at 540nm. The concentration of the test (CONC test.) was obtained as the ratio of the product of the absorbance of the test (ABS test) and the concentration of the standard (CONC std) to the absorbance of the standard (ABS std).

$$\text{CONC test} = \frac{\text{ABS test x CONC std}}{\text{ABS std}}$$

The enzymatic activities were read off the standard calibration curve.

Data Analysis

Statistical analysis was done using a computer package – statistical package for social sciences version 20 (SPSS version20). T-test statistics was used to compare two groups of variables. P-values of < 0.05 were considered statistically significant.

RESULTS

Physical/Biochemical results

All the rats in the experimental groups showed insignificant weight loss, decreased social activities and poor response to feeds and water were observed in the experimental rats towards the end of the experiment. After the first 15days, the ALT levels in the rats exposed to either full or half concentration of developer solution were significantly lower than the ALT levels of the rats in the control group ($p < 0.05$). On the other hand, the AST levels were significantly higher than those of the control group (Table 1). After 30days inhalation of the developer fumes (without aspiration), only the rats in the experimental group A (exposed to full concentration of developer solution fumes) showed statistically significant higher ALT levels. The ALT level of the rats exposed to half concentration of developer solution was significantly lower than that of the control. Also the AST levels were significantly lower than that of the control group. In the aspirated rats, both the ALT and AST levels were significantly lower than that of the control group (Table 2).

For the fixer solution, after 15days the ALT levels in the rats exposed to full concentration of the fixer solution showed significant decrease. For rats exposed to full concentration of fixer solution AST level was significantly higher than that of the control group but significantly lower for rats exposed to half concentration of the fixer solution (Table 2). Table 2 also showed that for the aspirated rats, both the ALT and AST levels were statistically lower than those of the control group.

Table 1: Comparing Enzyme values for full and half concentration of developer solution and the control values.

Duration of exposure/condition	Enzymes	Control: Mean±SD	Concentration	
			Full: Mean±SD	Half: Mean±SD
15 days inhalation	ALT	132.50 ± 3.53	21.00±0.00 P = 0.001*	18.50±0.71 P =0.000*
	AST	26.00 ± 1.41	50.00 ± 0.00 P = 0.027*	49.00±0.71 P =0.009*
30days inhalation only	ALT	132.50 ± 3.53	156.00 ± 0.00 P= 0.011*	71.00±0.00 P = 0.002*
	AST	26.00 ± 1.41	13.00 ± 0.00 P = 0.006*	9.00±0.00 P = 0.003*
30 days inhalation & 15 days post aspiration	ALT	132.50 ± 3.53	56.80±0.00 P = 0. 001*	107.00±0.00 P = 0. 009*
	AST	26.00 ± 1.41	14.00±0.00 P = 0. 007	13.00±0.00 P = 0. 006*

*P < 0 .05 Significant.

Table 2: Comparing Enzyme values for full and half concentration of fixer solutions and the control values.

Duration of exposure/condition	Enzymes	Control: Mean±SD	Concentration	
			Full: Mean±SD	Half: Mean±SD
15 days inhalation	ALT	132.50 ± 3.53	20.50±0.71 P =0.001*	47.00 ± 0.00 P =0.001*
	AST	26.00 ± 1.41	60.00±1.41 P =0.002*	17.00 ± 0.00 P =0.012*
30days inhalation only	ALT	132.50 ± 3.53	7.10 ± 0.00 P = 0.000*	213.00 ± 0.00 P = 0.001*
	AST	26.00 ± 1.41	35.00 ± 0.00 P = 0.012*	13. 00 ± 0.00 P = 0.006*
30 days inhalation & 15 days post aspiration	ALT	132.50 ± 3.53	85.20 ± 0.00 P = 0.003*	85.20 ± 0.00 P = 0.003*
	AST	26.00 ± 1.41	10.00 ± 0.00 P = 0. 004*	10.00 ± 0.00 P = 0. 004*

*P < 0. 05 Significant.

No significant difference existed between the AST levels of the rats exposed to full or half concentration of the developer solution under any condition. Similarly, no statistically significant difference existed in the ALT levels of rats that inhaled the developer fumes for 15days. Significant difference existed only in the ALT levels of rats that inhaled the developer fumes for 30days and those aspirated with the developer solution (Table 3).

Table 3: Comparing effects of concentration of developer solution on the ALT and AST liver enzymes

Duration of exposure	Enzymes	Concentration		Calculated t-value	T-test p-value	Inference
		Full: Mean±SD	Half: Mean±SD			
15 days inhalation	ALT	21.00±0.00	18.50±0.71	3.16	9.925	NS
	AST	50.00±0.00	49.00±0.71	0.50	9.925	NS
30days inh. only	ALT	156.00±0.00	71.00±0.00	41.06	9.925	S
	AST	13.00±0.00	9.00±0.00	2.46	9.925	NS
30days Inh. and 15days post asp.	ALT	56.80±0.00	107.00±0.00	-10	-9.925	S
	AST	14.00±0.00	13.00±0.00	0.74	9.925	NS

S= significance, NS = Non-significance

Table 4 below showed the effects of concentration of the fixer solutions on the ALT and AST liver enzyme levels. As can be seen from the table, significant difference occurred in the ALT levels of rats the inhaled the fixer solution fumes for 15 days and in the AST levels of rats that inhaled the fumes from fixer solution for 30days without aspiration.

Table 4: Comparing effects of concentration of Fixer solution on the ALT and AST liver enzymes

Duration of exposure	Enzymes	Concentration		Calculated t-value	T-test p-value	Inference
		Full : Mean±SD	Half: Mean±SD			
15 days inhalation	ALT	20.50±0.71	47.00±0.00	21.25	9.925	S
	AST	60.00±1.41	17.00±0.00	7.38	9.925	NS
30days inhalation only	ALT	7.10±0.00	213.00±0.00	-8.40	-9.925	NS
	AST	35.00±0.00	13.00±0.00	13.92	9.925	S
30days Inhalation & 15days post aspiration	ALT	85.20±0.00	85.20 ±0.00	0	9.925	NS
	AST	10.00±0.00	10.00±0.00	0	9.925	NS

S=Significance, NS = Non-significance

Changes in the ALT and AST levels of the liver of the rats exposed to the developer and fixer solutions was compared in table 5. Significant differences in ALT and AST levels were observed in the rats that inhaled half concentration of the solutions for 15days. No such difference existed for the rats that inhaled the full concentration of the solutions for 15 days. For the rats that inhaled the solutions for 30days, significant differenced existed in the ALT and AST revels of rats that inhaled the half concentration of the solutions. For the aspirated rats, significant difference in ALT level was observed in the rats that inhaled and were also aspirated with the full concentration of the solutions.

Table 5: Comparing effects of developer and fixer solutions on the ALT and AST liver enzymes

Duration of exposure/Concentration	Enzymes	Solutions		Calculated t-value	T-test p-value	Inference
		Developer: Mean±SD	Fixer: Mean±SD			
15 days inh. Full	ALT	21.00±0.00	20.50±0.71	0.45	9.925	NS
	AST	50.00±0.00	60.00±1.41	-1.715	-9.925	NS
Half	ALT	18.50±0.71	47.00±0.00	-25.49	-9.925	S
	AST	49.00±0.71	17.00±0.00	16.00	9.925	S
30days inh. Only. Full	ALT	156.00±0.00	7.10±0.00	80.18	9.925	S
	AST	13.00±0.00	35.00±0.00	-15.09	-9.925	S
Half	ALT	71.00±0.00	213.00±0.00	-71.00	-9.925	S
	AST	9.00±0.00	13.00±0.00	-1.79	-9.925	NS
30days Inh. & 15days post asp. Full	ALT	56.80±0.00	85.20 ±0.00	-16.40	-9.925	S
	AST	14.00±0.00	10.00±0.00	2.48	9.925	NS

Half	ALT	107.00±0.00	85.20±0.00	8.98	9.925	NS
	AST	13.00±0.00	10.00±0.00	1.70	9.925	NS

S =Significance, NS = N0n-significance.

Histological results

Photomicrographs of the liver tissues of the rats in the control group E and rats in the experimental group A (that inhaled fumes from the full concentration of developer solution for 15days) showed normal tissue structures and hepatocytes (Plates 1A and B). The liver of the rats in the experimental group B that inhaled fumes from the half concentration of developer solution for 15 days showed distorted interstitial tissue architecture and mild infiltrates of macrophages with pleomorphic nuclei (Plate 2). Infiltration of macrophages and signs of inflammatory oedema were noted in the liver of the rats that inhaled fumes from the half concentration of developer solution for 30days(Plate 3) while the aspirated rats which also inhaled the fumes from the same half concentration of developer solution for 30days showed normal tissue structures and hepatocytes.

Photomicrographs of liver of rats that inhaled fumes from the full concentration of the developer solution for 30days without aspiration showed mild vaculations of cytoplasm (Plate 4) while the aspirated rats that inhaled fumes from the same full concentration of developer solution for 30days showed marked vaculations of the cytoplasm and distorted interstitial tissue architecture (Plate 5).

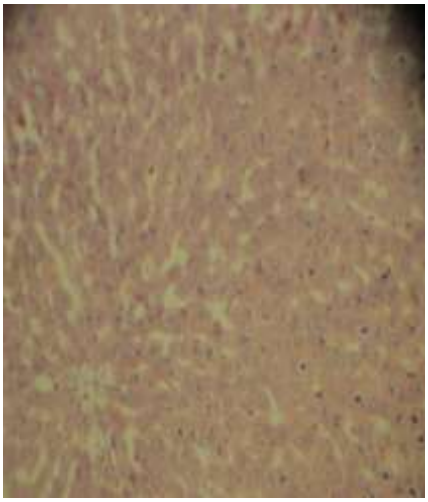

Plate 1A: Photomicrograph of liver of rats in the control group showing normal tissue structures and hepatocytes (Mag: X100)

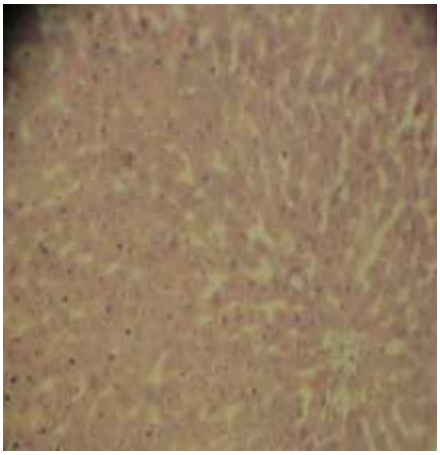

Plate 1B: Photomicrograph of liver of rats in the experimental group that inhale fumes from the full concentration developer solution for 15days showing normal tissue structures and hepatocytes(Mag: X100).

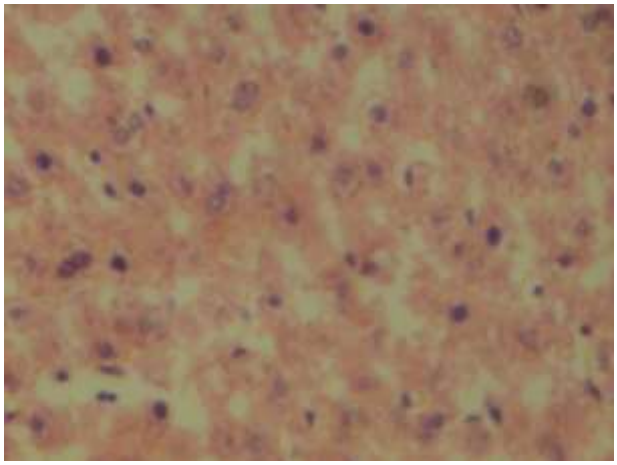

Plate 2:Photomicrograph of liver of rats in experimental group that inhaled fumes from half concentration of developer solution for 15days showing distorted interstitial tissue architecture and mild infiltrates of macrophages with pleomorphic nuclei(Mag: X100).

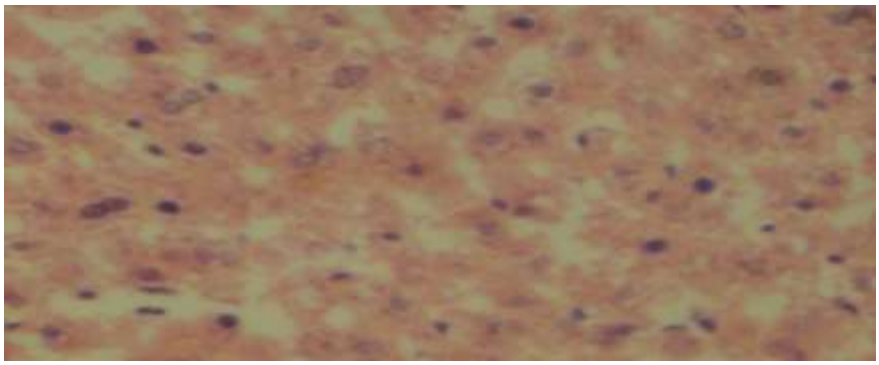

Plate 3: Photomicrograph showing infiltration of macrophages and signs of inflammatory oedema in the liver of the rats that inhaled fumes from the half concentration of developer solution for 30days.(Mag: X100)

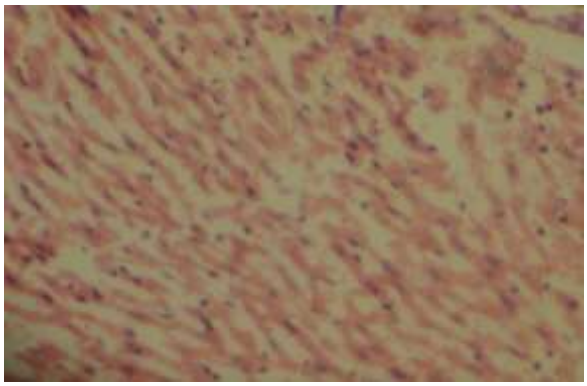

Plate 4 Photomicrographs of liver of rats that inhaled fumes from the full concentration of the developer solution for 30days without aspiration showing mild vaculations of cytoplasm (Mag: X100).

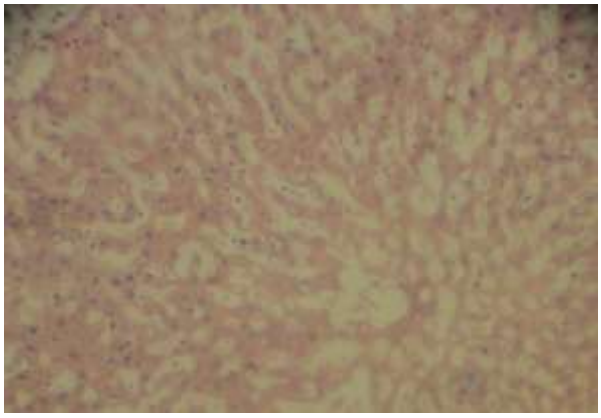

Plate 5: Photomicrograph of liver of aspirated rats that inhaled fumes from the full concentration of developer solution for 30days showing marked vaculations of the cytoplasm and distorted interstitial tissue architecture.

For the rats exposed to the fixer solutions, normal tissue structures were observed except for the rats that inhaled fumes from full concentration of fixer solution for 15days and the aspirated rats that also inhaled the same fixer solution for 30days. Photomicrograph of the rats that inhaled fumes from the full concentration of fixer solution for 15days showed marked enlargement of the central vein with normal hepatocytes and slight inflammatory oedema(Plate 6) while the aspirated rats that also inhaled the same fixer solution fumes for 30days showed mild enlargement of the central vein and normal hepatocytes (Plate 7).

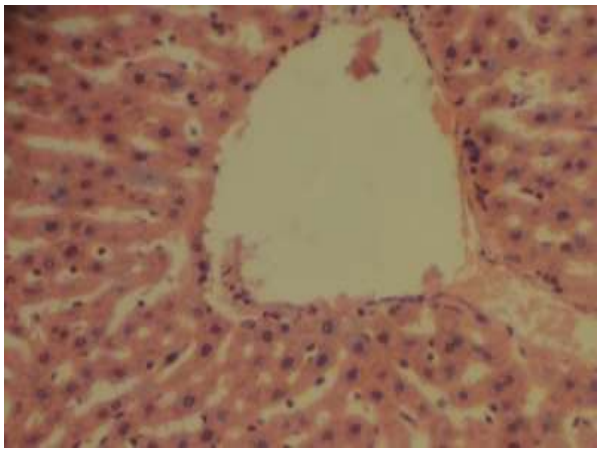

Plate 6: Photomicrograph of the liver of rats that inhaled the fumes from full concentration of fixer solution for 15days showing marked enlargement of the central vein with normal hepatocytes and slight inflammatory oedema(Mag: X 200).

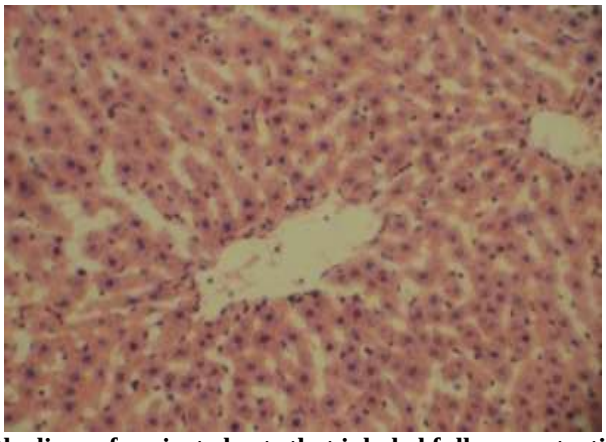

Plate 7: Photomicrograph of the liver of aspirated rats that inhaled full concentration of fixer solution fumes for 30days showing mild enlargement of the central vein and normal hepatocytes(Mag: X 200).

DISCUSSION

The liver is the organ responsible for metabolism and plays major roles in the detoxification of harmful substances. Injuries to the liver often end up with a fatal outcome if not given prompt and adequate attention. Prompt attention can only be given when early diagnosis is made. Unfortunately, many hepatic diseases appear asymptomatic for reasonable period of time. Early diagnosis is therefore necessary and is achieved by performing liver function tests (liver enzyme tests).

Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) are liver enzymes whose detection in elevated levels in blood indicates hepatic injuries. While the AST is found in many other tissues apart from the liver, the ALT is found mainly in the liver and the elevation of its level in blood gives strong indication of hepatic injury.

In our experimental study investigating the effects of x-ray film processing chemicals on the liver of wistar rats, it was observed that towards the end of the study, the rats in the experimental group showed weight loss, reduced social activities and poor response to feeding.

Biochemical analysis of the ALT and AST enzymes after 15days of the experiment revealed significant increase ($p<0.05$) in level of AST enzyme in the rats exposed to the developer solution than those of the control group while the reverse was the case with the ALT enzyme. The AST Level was higher than the ALT level. This finding indicates probable acute toxic effects of the developer. This agrees with the finding of Kim *et al.;* [25].

The fall in the AST level (lower than the ALT level) after 30 days of inhalation of fumes from the developer solution could be attributed to the shorter half life of the AST enzyme (17 ± 5 hours) compared to that of the ALT enzyme(47 ± 10 hours). The higher level of the ALT enzyme in the rats that inhaled fumes from the full concentration of the developer (than in the control) for 30 days but lower level (than the control) in rats that inhaled fumes from the half strength developer solution could be that while the full concentration of developer solution was strong enough to cause injury to the liver, the hepatic defense mechanism was able to combat the effect of the half strength solution. This was supported by the observed infiltration of hepatocytes and signs of inflammatory oedema seen the histological analysis (Plate 3). The significant fall ($p<0.05$) in ALT and AST level in the aspirated rats are suggestive of more injuries to the liver caused by the aspiration of the developer solutions and could mean that the hepatic cells were yielding to the insult as could occur in chronic situations. The fall in level of the liver enzymes in the aspirated rats agrees with the work of Obi et al [26] which attributed such a fall to prolonged intoxication due to damage to the liver. The marked vaculations of cytoplasm and distortion of interstitial tissue architecture (Plate 5) tends to confirm this.

The higher levels of AST and lower levels of ALT in the rats exposed to the full concentration of fixer solution compared to the control in the first 15days is suggestive of acute insults to the liver and showed that both the developer and fixer solutions can cause acute hepatic injuries. The rise in the ALT and fall in the AST levels in the rats that inhaled fumes from the half concentration of fixer solution for 30days compared to their levels on the 15^{th} day showed the effect of length of exposure to the to the liver tissue [25] . In the aspirated rats the levels of ALT and AST was respectively equal under the full and half concentration, possibly indicating a case of chronic exposure where variation in concentration has no effects. Significant difference ($p<0.05$) observed in the effects caused by the developer and fixer on the enzymes was observed in the rats exposed to the half concentrations of the chemicals, and occurred in the rats that inhaled the fumes from the fixer in the first 15 days and those rats that inhaled fumes from the full concentration for 30 days. The lack of difference ($p> 0.05$) in the ALT and AST between the aspirated rats and control group showed that the changes in the ALT and AST were caused by exposure to the processing chemicals. This was confirmed by the histological results.

Histological findings in the experimental rats exposed to the developer solutions correlated the biochemical findings. The observation of macrophage infiltration and signs of inflammatory oedema in the rats that inhaled fumes from the half concentration of developer solution for 30days (without aspiration), the presence of mild vaculations in the rats that inhaled fumes from full concentration of developer solution for 30 days and the marked vacuolation in the aspirated rats indicated that as the quantity of the developer received by the rats increases, the more the injury. This agreed with the findings by Monique [27] and Greenhough and Hay [28] who reported various symptoms in workers exposed to processing chemicals.

The contrasting biochemical and histological results obtained with the fixer solutions could be misleading and very dangerous, and points to the advantages of biochemical tests over histological tests. As seen from the biochemical results there was always significant difference ($p< 0.05$) between the ALT and AST levels in the experimental rats and the rats in the control group (indicating a problem) but the histological results were normal except for the rats that inhaled fumes from concentrated fixer solution for 15 days and the aspirated rats that inhaled the same fumes from full

concentration of fixer solution for 30days (Plates 6 and 7). The finding of normal histological findings disagrees with the report from the Health Safety Executives; HSE that most of the problems associated with processing chemicals could eventually be traced to the fixer [29]. The dilation of the central vein agreed with the findings by Adjene and Enaibe[30] who observed dilated central vein that contained lyses blood cells, cyto architectural distortion by histiocytes, atrophic and degenerative changes attributed to the presence of monosodium glutamate. The marked dilation of the central vein and inflammatory oedema in the rats exposed to the fixer solution for 15 days, and the increase in the AST level within the same period were indicative of acute toxicity similar to the findings by Kim *et al.;* [25].

From the finding from this study, it became obvious that inhalation or ingestion of the x-ray film processing chemicals can be injurious to liver of radiology personnel exposed to them. This becomes of concern when we note that the effects on the rats occurred following short exposure periods of 15-30 days. It can then be imagined what will happen to radiology workers who work almost daily with the chemicals over the 35 years period of service allowed in developing countries like Nigeria. It is also important to note that while the rats used for this experiment were separately exposed to the developer or fixer solutions, the radiology darkroom worker is exposed simultaneously to these processing chemicals. This implies experiencing the summation of the effects of these chemicals and over a long period of time and with probable greater damage to the exposed individual. Again, the decrease in the dilation of the central vein in the aspirated rats which also inhaled the fumes from the fixer solution for 30 days is suggestive of development of immune response system or adaptive features by the liver cells. The development of such adaptive features to toxic substances will have serious consequences for the radiology darkroom staff because it means dying without knowing it.

CONLCUSION

Findings from this study showed that x-ray film processing chemicals had negative effects on the ALT and AST liver enzymes and liver tissues of wistar rats exposed to these chemicals. The effects produced by these chemicals was not significantly affected by the concentration of the solutions but was related to the period of exposure especially for the developer solution. While the major histological effect of the fixer was the dilation of central vein, the developer solution had varied histological effects. For the radiology darkroom staff, simultaneous exposure to these chemicals in work place means summation of the effects of these chemicals. This means greater damage to the liver. Based on this and the possible respiratory problems likely to develop within 25 years of exposure at work in the darkroom [31], it becomes necessary that measures are taken to hasten the transition from darkroom radiography to digital/filmless radiography in developing countries as is the practice in the advanced countries of the world.

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