Original Article

Biochemical and Histopathological Effects of Acute Exposure to Vinyl Acetate Monomer Vapour in Wistar Rats

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ABSTRACT

Background: Vinyl acetate monomer is a commodity chemical widely used in the manufacturing of various products. The chemical is hazardous and exposure to it may occur in both occupational and non-occupational settings. The aim of this study was to characterize the effects of short-term exposure to Vinyl Acetate Monomer (VAM) vapour on the liver and lungs of Wistar rats.

Methods: Mice weighing 25-30g were used to determine the acute lethal dose, while Wistar rats weighing 120-140g were randomly assigned to a control group and two experimental groups, which were exposed daily to VAM vapour for 2 or 4 hours. On the 5th day, rats were sacrificed, the blood was collected for biochemical analysis while liver and lungs were examined for histological alterations.

Results: The acute lethal dose of VAM vapour was estimated to be 173.21 mg/kg body weight. A significant decline in total protein (6.725±0.10 g/dl; $p<0.05$) and increases in alanine aminotransferase (ALT; 33±1.47 u/l), aspartate aminotransferase (AST; 44±1.08 u/l), alkaline phosphatase (ALP u/l; 76.42±1.43), urea (22.89±0.93 mg/l), bilirubin (0.84±0.03 mg/dl) and creatinine (1.04±0.07 mg/dl) occurred in the experimental rats compared to the controls. Portal inflammation, fibrosis, and hepatitis were observed in the liver, while collapsed air spaces, thickened alveolar walls and haemorrhage were demonstrated in the lungs of the experimental rats. The extent of these lesions increased with rising exposure time to VAM vapour.

Conclusion: This study demonstrated that VAM liquid was moderately toxic, while short-term exposure to VAM vapour was injurious to the lungs and liver of Wistar rats.

Keywords: Hepatotoxicity, Histopathology, Liver Enzymes, Lungs, Vinyl Acetate Monomer.

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INTRODUCTION

Vinyl acetate monomer (VAM) is a key commodity chemical produced for the manufacturing of polymers, such as polyvinyl acetate, polyvinyl alcohol, polyvinyl acetals and the co-polymers, such as ethylene-vinyl acetate, and polyvinyl chloride-acetate [1]. The polymers are further used in the manufacturing of a wide range of commonly used products, such as adhesives for different materials including paper, glass and wood, water-based latex paints, inks, lacquers, heat sealing films, pesticides, food additives, and cosmetics [2].

Occupational exposure to VAM vapour may occur during the manufacturing of the vinyl acetate monomer, the synthesis of polymers and co-polymers, the application of the co-polymers to manufacture consumer products and during transportation or storage [3]. The presence of vinyl acetate in workplace occurs commonly due to its vapour [4]. Vinyl acetate concentrations of 0.25–2 mg/m^3 have been measured in air at manufacturing or processing facilities, while concentrations of 0.5$\mu g/m^3$ has been reported near a chemical disposal sites [5]. The environmental exposure may also occur from the industrial discharge of waste water. Vinyl acetate concentration of 50 mg/l was reported in waste water effluents from a polyvinyl acetate plant [2]. Non-occupational exposure can occur through the release of vinyl acetate monomer in polymerized forms, which may contain up to 1% monomer [4]. In the study by McNeal, and Hollifield [6], vinyl acetate concentrations of 0.002–0.14 $\mu g/cm^2$ was detected in food packaging heated in microwave ovens.

Vinyl acetate is metabolized into acetaldehyde and acetic acid by carboxyl esterase in animals, and the metabolites are responsible for the resultant toxicity [7]. Vinyl acetate has been shown to be genotoxic [8], cytotoxic [7], carcinogenic [8] and irritant to the respiratory system [9]. This study aimed to characterize the effects of short-term inhalation of vinyl acetate vapour on the lungs, kidney and liver of Wistar rats, about which there are very limited research knowledge available.

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MATERIALS AND METHODS

Test Chemical

Vinyl acetate monomer ($CH_3COOCH=CH_2$), a colourless liquid with % purity of 99.9, specific gravity of 0.932~0.936, density of 0.932 g/ml and vapour pressure of 92 mmHg at 20°C was sourced from an industrial plant which manufactures adhesives in Lagos, Nigeria.

Experimental Animals

The mice (25-30g) and Wistar rats (120-140g) were procured from the College of Veterinary Medicine, Michael Okpara University of Agriculture, Umudike, Nigeria. They were acclimated for one week in the laboratory prior to the start of the experiment. The experimental animals were fed with rat chow and water ad libitum before and throughout the experiments.

Experimental Group and Administration of Vinyl Acetate Monomer

Twenty five mice were used to evaluate the oral and acute toxicity of vinyl acetate monomer. Using a completely randomised design, five mice were assigned to five groups each (Groups 1-5). Each mouse in groups 1, 2, 3, 4, and 5 were administered one dose of liquid VAM in this order: 10, 100, 300, 600 or 1000 mg/kg body weight, respectively. The mortality was observed in all mice for 24 hours.

In a separate set of experiments, 15 rats were randomly assigned to a control group and two experimental groups (5 rats/group) in a randomised design. The 10 rats in the experimental groups were exposed daily to an air saturated with VAM vapour at a concentration of 259 ppm for 2 or 4 hours, by placing a plastic box containing cotton wool soaked with 5ml VAM inside the cage ($0.018m^3$) with limited ventilation to ensure that the rats inhaled the vapour. After 2-4 hours of daily exposure, the rats were moved to a location free of VAM vapour. The experiments were conducted at room temperature (27^oC) and lasted for 5 days after, which the animal blood samples were collected for biochemical analysis, while liver and lungs were extracted for histological analysis. The biochemical analysis was only performed for the control and experimental rats that were exposed to VAM for 4 hours daily. The animals were handled in accordance with the international principles on the care and use of experimental animals [10] and the guidelines of the Department of Physiology, Pharmacology, Biochemistry, and the Animal Health Ethics Committee, Michael Okpara University of Agriculture, Umudike, Nigeria.

Determination of Acute Oral Toxicity (LD_{50}) of Vinyl Acetate Monomer

Lethal dose was determined using Lorke [11] method by the following formula:

$LD_{50} = \sqrt{a \times b}$, where; "*a*" was the maximum dose that did not kill any mice and "*b*" was the minimum dose that killed all of them. In other words, LD_{50} was the dosage that killed 50% of the animals exposed to the toxin.

Biochemical Analysis

The collected rat blood samples (3 ml each) were allowed to clot at room temperature for 15 min and then centrifuged at 300g for 5 min to prepare the sera. The clear supernatants from the blood samples were used to estimate the serum enzymes. The serum sample from each rat was used for the quantitative determination of ALT, AST, ALP, blood urea and creatinine. The ALT, ALP and AST levels were measured against the available standards for the respective enzymes using appropriate kits from Randox Labs (London, UK). The urea level was measured against the available standards, using the kit from Randox Labs (London, UK). The plasma creatinine level was measured against the standards kit also from Randox Labs (London, UK) by the photometric determination protocol, based on Jaffe kinetic method [12].

Histopathological Investigations

Samples for histological investigations were cut into thin slices of about 0.3–0.5 cm with a scalpel blade to enhance penetration of the processing reagents and fixed in 10% formal-saline for 6 hours. The specimens were then dehydrated sequentially in graded alcohol (70% & 90% I, II, 1 hr.; III, 2 hr.; absolute I, 2 & 3 hr) and then transferred into xylene I for 1hr, and xylene II for 2 hrs followed by wax impregnation and embedding in paraffin. The resultant sections were mounted on glass microscope slides and air dried prior to staining with hematoxylin and eosin [13]. The stained sections were viewed and analyzed, using light microscopy.

Statistical Analysis

The mean value of the biochemical parameters of the exposed rats to VAM were compared with that of the controls, using student T-test. The statistical analysis was performed with SPSS software, version 19, and the results were presented using boxplots. Liver and lung biopsy results were categorized as: - = none; + = mild; ++ = moderate, and, +++ = severe pathological damages.

RESULTS

Acute Oral Toxicity (LD_{50}) of Vinyl Acetate Monomer

The mortality was recorded in rats, based on a dose of 300mg/kg body weight (Table 1), with the LD_{50} established at 173.21mg/kg body weight.

Table 1. Mortality of mice exposed to vinyl acetate.

Group of Animal	Dose (mg/kg body weight)	Number Exposed	Mortality
Group 1	10	5	0
Group 2	100	5	0
Group 3	300	5	5
Group 4	600	5	5
Group 5	1000	5	5

Biochemical Indices of Wistar Rats Exposure to Vinyl Acetate Monomer

The effect of inhalation of VAM vapour on the liver enyzmes, AST, ALT, ALP and serum total protein levels are presented in Figures 1a, 1b, 1c and 1d, respectively. The AST, ALT and ALP were significantly increased by 83%, 132%, 13%, respectively, in the rats exposed to vinyl acetate for 4 hours daily compared to the control group ($p<0.05$). The serum total protein decreased significantly by 8% in these rats compared to the controls ($p<0.05$).

Figures 2a, 2b, and 2c represent the effect of VAM vapour on the levels of serum urea, creatinine and bilirubin, respectively. Urea, creatinine and bilirubin levels were significantly increased in the experimental rats exposed to VAM vapour for four hours daily by 28%, 87% and 8%, respectively, compared to that in the control group ($p<0.05$).

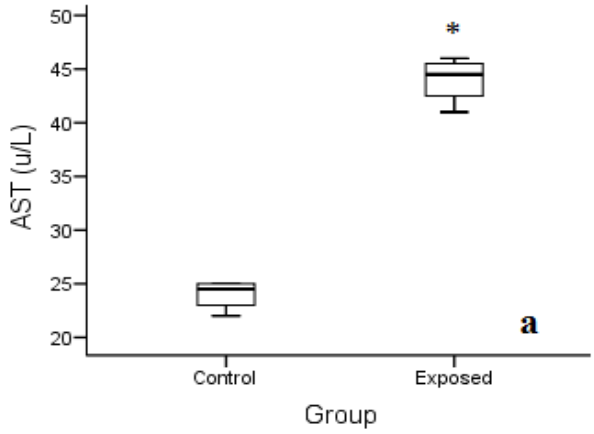

Figure 1a. Box plot of liver enzymes aspartate aminotransferase (AST) of rats exposed to VAM vapour 4 hours daily compared to controls. * indicates statistically significant difference ($p<0.05$).

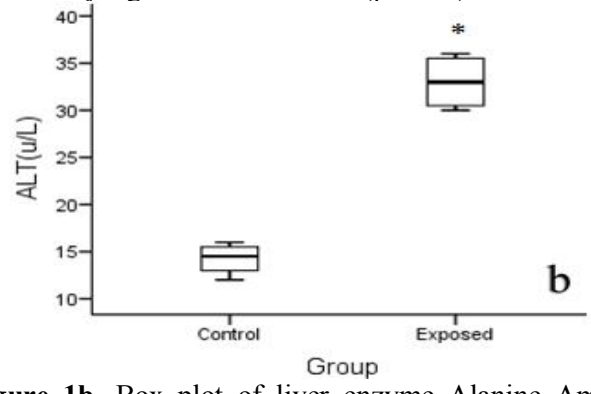

Figure 1b. Box plot of liver enzyme Alanine Amino Transferase (ALT) of rats exposed to VAM vapour 4 hours daily compared to controls. * indicates statistically significant difference ($p<0.05$).

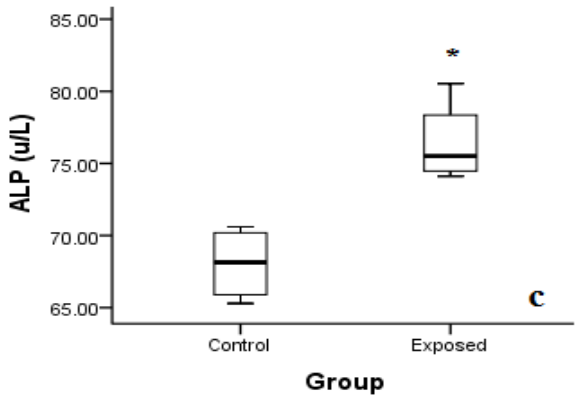

Figure 1c. Box plot of liver enzyme alkaline phosphatase (ALP) of rats exposed to VAM vapour 4 hours daily compared to controls. * indicates statistically significant difference. ($p<0.05$).

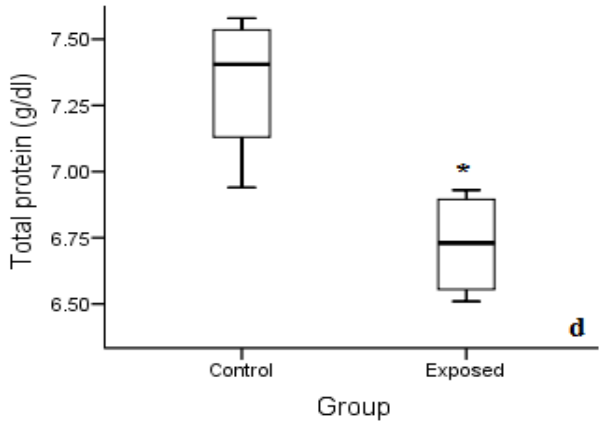

Figure 1d. Box plot of total protein of rats exposed to VAM vapour 4 hours daily compared to controls. * indicates statistically significant difference ($p<0.05$).

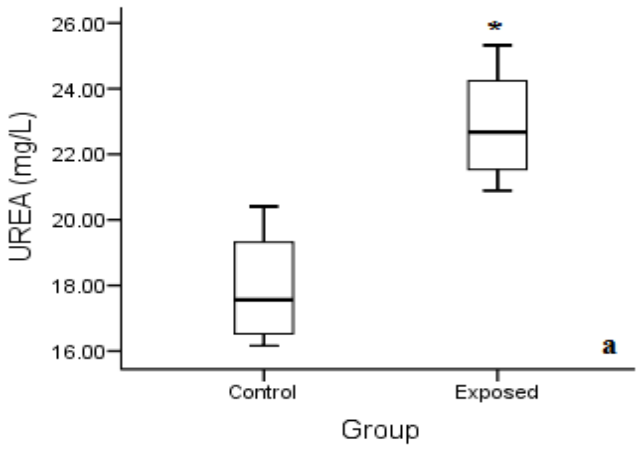

Figure 2a. Box plot of serum urea of rats exposed to VAM vapour 4 hours daily compared to controls. * indicates statistically significant difference ($p<0.05$).

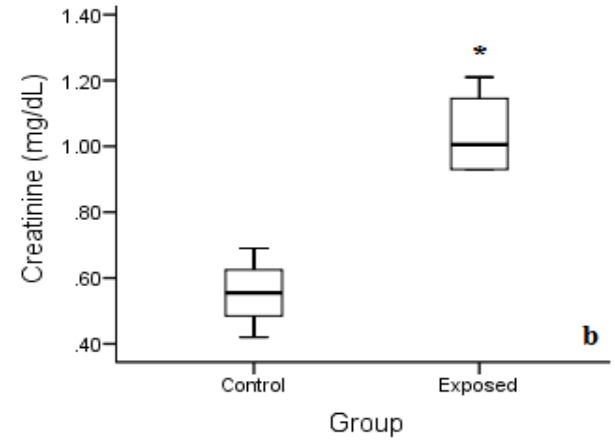

Figure 2b. Box plot of rat creatinine exposed to VAM vapour 4 hours daily compared to controls. * indicates statistically significant difference ($p<0.05$).

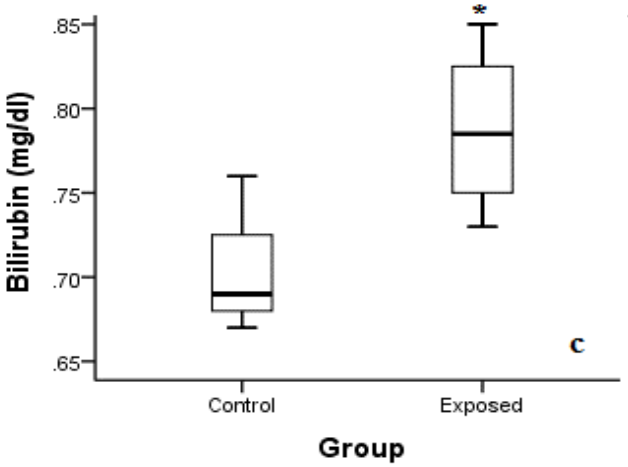

Figure 2c. Box plot of bilirubin level of rats exposed to VAM vapour 4 hours daily compared to controls. * indicates statistically significant difference ($p<0.05$).

Histopathology of the Liver

The photomicrographs of rats in the control group demonstrated a well preserved liver tissue and cellular architecture, evenly spaced portal triads around a central vein with no portal inflammation or steatosis (Plates 1a & 1b). However, mild portal inflammation without interface hepatitis was observed in the liver of rats exposed to VAM vapour for 2 hours. The portal triads were also evenly spaced around a central vein without steatosis or alterations to the liver architecture (Plates 1c & 1d). Examination of liver samples from the rats exposed for to VAM vapour for four hours revealed the presence of moderate to severe portal inflammation with interface hepatitis and moderate portal fibrosis and ductular formation. The portal triads were evenly spaced around a central vein and no steatosis found (Plate 2). The extent of liver tissue alterations increased with an increase in the exposure duration. The extent of liver tissue damages detected in rats exposed to VAM vapour for four hours daily was significantly higher compared to those exposed for two hours only (Table 2).

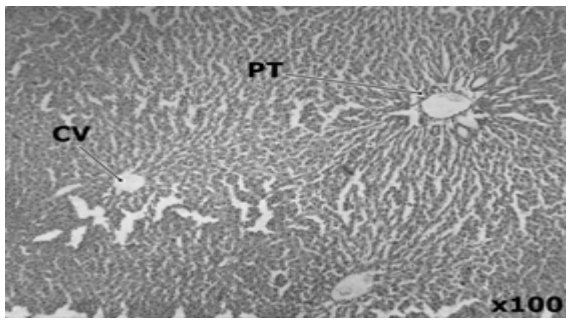

PLATE 1a. Photomicrograph of the liver section from rats in the control group. CV = Central vein, D = Portal triad.

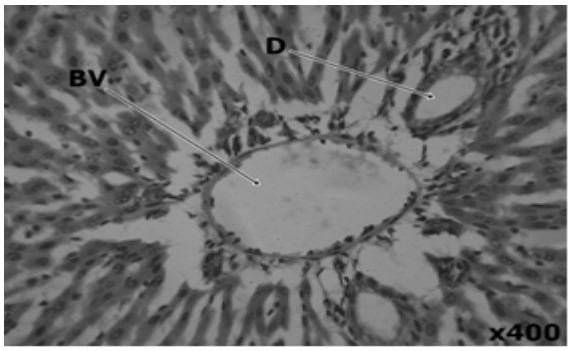

PLATE 1b. Photomicrograph of the liver section from rats in the control group. BV = Blood vessel, D = Ductule.

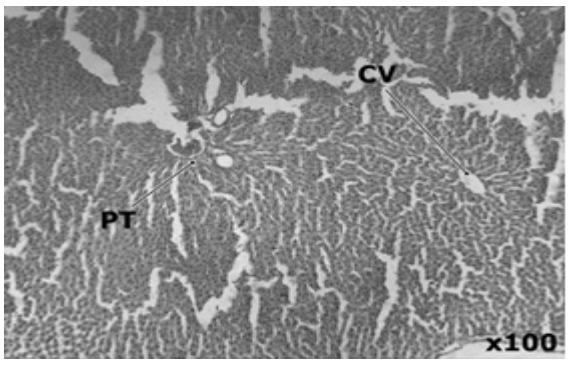

PLATE 1c. Photomicrograph of the liver section in rats exposed to vinyl acetate x 2hr daily. PT = Portal triad,CV = Central vein.

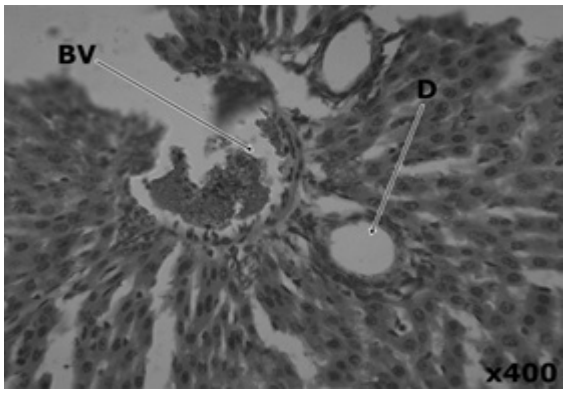

PLATE 1d. Photomicrograph of the liver section in rats exposed to VAM for 2 hours**.** BV = Blood vessel, D = Ductule.

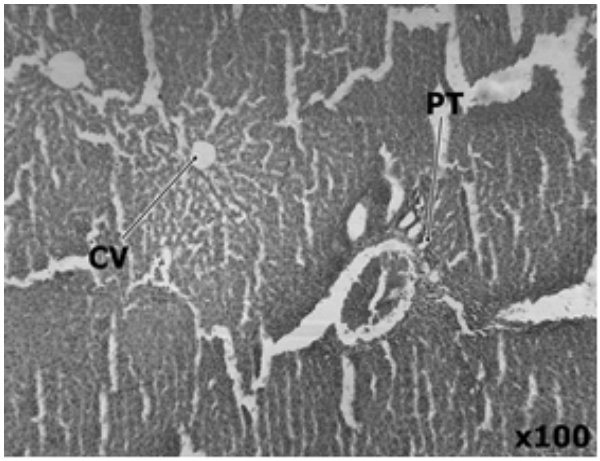

PLATE 2a. Photomicrograph of the liver section from rats exposed to VAM for 4 hours. PT = Portal triad, CV = Central vein.

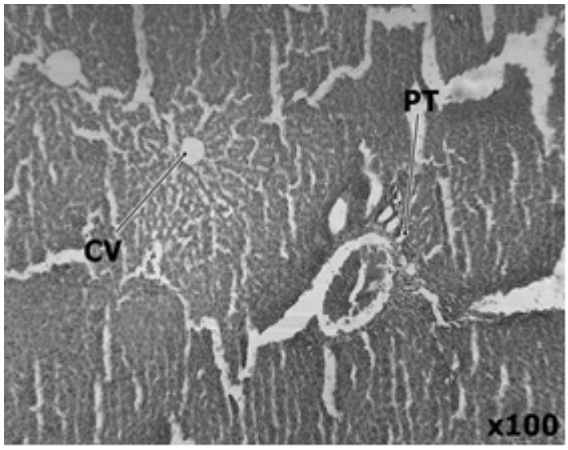

PLATE 2b. Photomicrograph of the liver section in rats exposed to VAM for 4 hours daily.BV = Blood vessel, D = Ductule, IC = Inflammatory cells.

Table 2. Histopathological effects of VAM vapour inhalation on the liver.

Experimental Group	Liver Architecture	Portal inflammation	Steatosis	Hepatitis	Portal Fibrosis	Ductular formation	Portal triads
Control	P	-	-	-	-	-	ES
2 hours daily	P	+	-	-	-	-	ES
4 hours daily	P	+++	-	++	++	++	ES

Key: None (-), mild (+), moderate (++), severe (+++), P (preserved), ES (Evenly spaced)

Histopathology of the Lungs

The photomicrographs of rats in the control group showed a lung tissue with numerous open alveolar air spaces bordered by thin walls, and lined by simple epithelium and few alveolar macrophages, with the bronchioles surrounded by lymphoid cells. However, up to 5% of the alveolar air spaces in the lungs of rats exposed to vinyl acetate for 2 hours were collapsed with the surrounding walls mildly thickened. Also, there was mild haemorrhage into the alveolar walls (Plate 3). Up to 10% of the alveolar air spaces are collapsed with the surrounding walls moderately thickened. Also, there was moderate haemorrhage seen in the alveolar walls of the lung tissue exposed to VAM vapour for four hours (Plate 4). The extent of lung tissue alterations increased with an increase in the duration of VAM vapour exposure (Table 3).

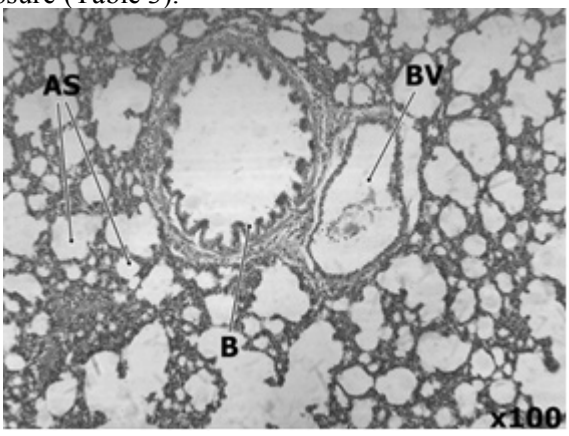

PLATE 3a. Photomicrograph of the lungs section in rats from the control group. B = Bronchiole, AS = Alveolar spaces, BV = Blood vessels.

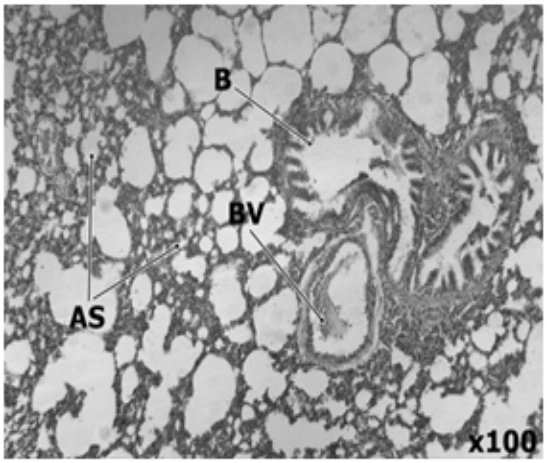

PLATE 3b. Photomicrograph of the lungs section in rats exposed to VAM for 2 hours.B = Bronchiole, AS = Alveolar spaces, BV = Blood vessels.

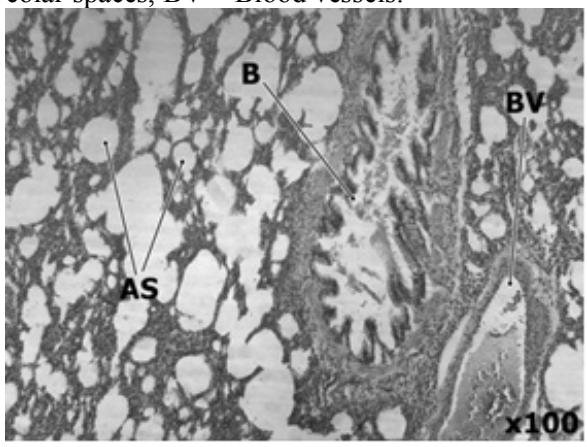

PLATE 4. Photomicrograph of the lungs section in rats exposed to VAM for 4 hours. B = Bronchiole, AS = Alveolar spaces, BV = Blood vessels.

Table 3. Histopathological effects of VAM vapour inhalation on the lungs of Wistar rats.

Experimental Group	Alveolar air space collapse	Alveolar wall thickening	Haemorrhage
Control	-	-	-
2 hours exposure daily	+	+	+
4 hours exposure daily	++	++	++

None (-), mild (+), moderate (++), severe (+++), P (preserved), ES (Evenly spaced)

DISCUSSION

Vinyl acetate is generally considered to be of mild but acute toxicity based on the previously reported LD_{50} values [4,14,15]. However, the oral LD_{50} of vinyl acetate, as documented in the current study (173.21mg/kg body weight) was lower than that (1,600 mg/kg body weight) reported by a previous study on mice [14]. Thus, based on the LD_{50} values determined in this study, VAM can be classified as a moderately toxic chemical.

The hepatotoxicity found for vinyl acetate in this study was based on the documented injury to the hepatocytes as evidenced by high AST, ALT, and ALP, a decline in total protein and a rise in bilirubin synthesis levels. The total protein synthesis may decline due to decreases in the synthesis of globulin and albumin due to impaired liver functions, while bilirubin may increase due to liver cell damage, leading to lower liver capacity to conjugate and excrete it through the bile [16]. We suggest that the hepatotoxicity observed for vinyl acetate may be linked to the induced acetaldehyde level. Blood, liver, kidney and lung contains carboxyl esterease [17], which hydrolyzes vinyl acetate to acetic acid and acetaldehyde [18]. The liver further metabolizes the acetaldehyde into acetate [4]. Acetaldehyde can damage liver tissue and its functions by binding to tubulin which can impair protein secretion and promote its retention, with the concomitant inflammatory response in the hepatocytes [19]. Hepatocytes are the primary functional cells in the liver, making up 70-85% of the liver mass and perform various vital functions, including synthesis, utilization and secretion of proteins [20].

Injured hepatocytes may induce inflammatory response, leading to the appearance of inflammatory cells, edema and congestion around the hepatocytes, and liver fibrosis [21, 22]. The fibrosis can also occur as a result of inflammation associated with the proliferation of bile ducts or ductular reactions [23]. In this study, the portal inflammation, hepatitis, portal fibrosis and ductular scar formation observed in the experimental rats are likely to be the direct consequences of the injured hepatocytes due to VAM metabolites. The ductular scar formations suggest impairment in the regenerative capacity of the hepatocytes. The extent of these alterations increased with an increase in the exposure time to VAM vapor. Thus, VAM vapour is likely to be hepatotoxic, since the interaction of its metabolites (acetaldehyde) with the liver cells resulted in the leakage of liver enzymes into the blood and the pathological alterations such as proliferation of the bile ducts. Nevertheless, histopathological changes or signs of liver dysfunction were noted in rats exposed to vinyl acetate (200 & 600 ppm) over 104 weeks [24].

The kidney is responsible for the filtration and excretion of waste materials including urea and creatinine. Renal biomarkers in the blood, such as creatinine and urea have typically been used to diagnose renal injury [25]. The significant increase in blood urea and creatinine observed in this study suggests a possible impairment of renal function in rats. However, a decrease in blood urea has been reported in rats exposed to 1,000 ppm of vinyl acetate via inhalation for 3 months [26]. The duration of exposure may account for the decrease in urea compared to its increase, as recorded in our study. Organisms can exhibit different responses to acute or chronic exposure to toxicants. For instance, acetylcholine esterase activity was reported to increase in rats after acute exposure to chlorpyrifos [27] but it deceased after chronic exposure to the same pesticide[28].

In our study, acute exposure to VAM vapour through inhalation resulted in the collapse of alveolar air spaces, thickening of the walls and haemorrhage into the alveolar spaces and walls. The lung is a complex organ composed of thousands of alveoli that facilitate the exchange of O_2 and CO_2 via diffusion [29]. Vinyl acetate can be hydrolysed to form acetic acid and acetaldehyde in the lung tissue, which can be rapidly absorbed through the lungs [30], forming adducts with surfactant proteins [31]. These proteins which make up 10% of surfactant composition include the hydrophilic proteins A (SP-A) and D (SP-D), and the hydrophobic proteins B (SP-B) and C (SP-C) [32]. Surfactants, produced by type 2 alveolar cells, help maintain the shape and surface tension of the alveoli and reduce the likelihood of alveolar collapse [33]. The collapse of the alveolar air spaces observed in this study suggests inactivation of surfactants possibly through the formation of adducts by the interaction of acetaldehyde with surfactant proteins. Generally, adduct formation by acetaldehyde leads to functional impairments of key proteins [34]. Haemorrhage into the alveolar walls suggests injury to the type 1squamous alveolar epithelial cells and damage to the capillary membrane. Type 1 alveolar epithelial cells make up to 95% of the alveolar surface area and their main function is to maintain a barrier to prevent leakage of fluid, protein and blood from entering the alveolar space [35]. Haemorrhage was also observed in experimental animals following inhalation of VAM

vapour for four hours [1]. Consequently, collapses in the alveolar air spaces and the thickened walls may have impaired the gas exchange capacity in the lungs, which may have accounted for the gasping and laboured breathing pattern observed in the experimental rats [1].

CONCLUSION

Our results demonstrated that vinyl acetate monomer is hepatotoxic. VAM was associated with injury to the hepatocytes and cholangiocytes shown by release of liver enzymes and the concomitant lesions including ductular scar formation. This compromised the functioning of the liver, resulting in both decreased protein synthesis and bilirubin excretion. VAM is also deleterious to the lungs. Surfactant proteins in type 2 alveolar cells and type 1 alveolar epithelial cells appear to be the target site of VAM and/or its metabolites in the lungs. Changes in kidney function biomarkers also suggest that VAM may be nephrotoxic. While considerable research efforts are made to investigate the carcinogenic and genomic effects of vinyl acetate, there is still a need for assessing the effects of chronic inhalation of vinyl acetate on vital organs, including the human liver and kidneys.

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CONFLICT OF INTEREST

The authors declare no conflict of interests in conducting this study.

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