International Journal of Cell, Animal Biology and Genetics
Vol.8, No.1, pp., 25-35, 2023
Print ISSN: ISSN 2397-8147
Online ISSN: ISSN 2397-8155
Website: https://www.eajournals.org/
Publication of the European Centre for Research Training and Development -UK

Comparing the Effects of Formalin and Chloroform Inhalation on the Liver of Adult Male Wistar Rat

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doi: https://doi.org/10.37745/ijcabg.16/vol8n12535 Published November 10, 2023

Citation: Aguwa U.S, Ovie F.O, Olu S.I, Obinwa B.N, Eze C.E. (2023) Comparing the Effects of Formalin and Chloroform Inhalation on the Liver of Adult Male Wistar Rat, *International Journal of Cell, Animal Biology and Genetics*, Vol.8, No.1, pp., 25-35

ABSTRACT: *Staff and students working in medical laboratories especially in anatomy department are constantly exposed to chloroform and formalin inhalation. This work is designed to compare the effect of formalin and chloroform inhalation on the liver of adult male Wistar rats. Twenty-one male rats were randomly separated into 3 groups of 7 rats each. Group A had food and water only. Groups B and C were exposed to chloroform and formalin for 5 minutes daily for 2 weeks respectively. Animals were sacrificed on the 15th day and blood and liver tissue were collected for liver function enzymes and histological studies respectively. Our result showed that inhaling formalin and chloroform has negative health implications on the liver which could also adversely affect rat metabolism. We therefore recommend that those constantly exposed to these chemicals take proactive steps in regular detoxification and healthy diet.*

KEY WORDS: Inhalation, Anatomage, Liver, Formalin, Chloroform

INTRODUCTION

Chloroform is a dense colorless, volatile, non-flammable liquid compound, with a characteristic sweet odour. It is nearly insoluble in water but easily dissolves in alcohol, ether, acetone, gasoline and other organic solvents (National Institute for occupational safety, 2005). Chloroform can be produced with common household liquids including sodium hypochlorite solution (chlorine bleach) (Anderson and Washburn, 1946). Chloroform was used in the past as an extraction solvent for fats, oils, greases, and other products; as a dry cleaning spot remover; in fire extinguishers; as

a fumigant; and as an anesthetic (Atlanta, 1997). Chloroform is also used to extract and purify penicillin (Baeder and Hofman, 1991). In anatomy labs, chloroform is used as part of the embalming solution and mortuaries and for sedating rodents during sacrifice in experimental studies. However, there are continuous scientific evidences implicating chloroform as toxic to different organs and systems of the body, including the nervous system. These evidences notwithstanding, chloroform is still a major component of the fixatives used for cadavers across medical schools. Medical students are expected to spend hours in the dissecting room for gross anatomy practical. This study therefore intends to investigate the effect of chloroform inhalation on the liver of male Wistar rats. Chloroform is carcinogenic by oral administration to rats and mice, producing liver and kidney tumors in a sex- and strain- dependent manner (Page and Saffioti, 1976). Previous reports have shown liver damage in humans and animals exposed of chloroform vapor (Tongnit et al.,2004.

Formalin is a clear solution of formaldehyde in water. A 37% solution is used for fixing and preserving biological specimens for pathological and histological examination (Mosby's Medical Dictionary, 9th edition 2009). In air, it is readily broken down by sunlight, with a half-life of approximately 30-50 minutes (WHO 1999). Upon intraperitoneal, oral, or inhaler exposure, formalin quickly diffuses into many tissues, including the brain, testis, and liver [10].

Formalin is a pungent gas, with the chemical formula HCHO, used as an antiseptic, disinfectant and especially today as a fixative for histological studies. Formaldehyde is a naturally occurring organic compound; it is the simplest of the aldehydes and is also known by its systematic name methanal. The common name of this substance comes from its similarity and relation to formic acid. Formaldyhyde is a flammable gas at room temperature and has a strong odor. Exposure to formaldyhyde may cause adverse health effects. The primary way you can be exposed to formaldehyde is by breathing air containing off gassed formaldehyde. Formaldehyde can cause irritation of the skin, eyes, nose, and throat. High level of exposure may cause some types of cancers. Formaldehyde is produced industrially by the catalytic oxidation of methanol (Günther *et. al.*, 2002).

The liver is the largest of the abdominal viscera, occupying a substantial portion of the upper abdominal cavity. It performs a wide range of metabolic activities necessary for homeostasis, nutrition and immune defense. It is composed largely of epithelial cells (hepatocytes), which are bathed in blood derived from the hepatic portal veins and hepatic arteries. There is continuous chemical exchange between these cells and the blood. Hepatocytes are also associated with an extensive system of minute canals, which form the biliary system into which products are secreted

(Moore and Dalley, 2006). The liver is important in the removal and breakdown of toxic or potentially toxic materials from the blood. It regulates blood glucose and lipids, and plays a role in the storage of certain vitamins, iron, and other micronutrients as well as breaking down or modifying amino acids. It is involved in a plethora of other biochemical reactions. Since the liver is the primary site of detoxification and is generally the major site of intense metabolism, it is therefore prone to various disorders as a consequence of exposure to the toxins of extrinsic as well as intrinsic forms (Guyton and Hall, 2006).

Naturally we associate problems with inhalants to the lungs. But there could be another dimension to it. The liver may also be affected when toxic chemical products are inhaled regularly. This study intends to elucidate this possibility in rats. The liver is the site of biotransformation in which a toxic compound is transformed to a less harmful form to reduce systemic toxicity (Hodgson, 2004). However, these results in progressive damage to the liver cells and produce hepatotoxicity. Alanine transaminase (ALT) is an enzyme that helps metabolize protein. When the liver is damaged, ALT is increased in liver and is subsequently released in the bloodstream. Aspartate transaminase (AST) is an enzyme that plays a role in the metabolism of the amino acid alanine. An increase in AST levels may indicate liver damage or disease. Aspartate transaminase is the mitochondrial enzyme, predominantly found in the liver, skeletal muscles and kidneys. Alanine transaminase is a cytosolic enzyme, which is more specific for the liver than aspartate transaminase (Hodgson, 2004). Alanine aminotranferase (ALT) and aspartate aminotransferase (AST) are located in liver cells and leak out into the general circulation when liver cells are injured. These two enzymes were previously known as the SGPT (serum glutamic-pyruvic transaminase) and SGOT (serum glutaicoxaloacetic transaminase) respectively. ALT and AST are present in highest concentrations in cells from the liver, heart, skeletal muscles, and red blood cells (Infante, 2008).

MATERIALS AND METHODS

Twenty-one (21) male Wistar rats weighing between 150-200g were obtained from Omacilia farms, Ika North, Delta state, Nigeria. The rats were housed in Wire gauze cages and allowed to acclimatize for one week before exposure. The rats were fed with rat chow and tap water throughout the duration of the experiment ad libitum. Rats were handled according to global best practices. Analytic grade chloroform and formalin manufactured by May and Baker Ltd.
were used for this study. A gas meter was used to determine the extent of gas released at various durations of exposure as follow: for 2min : 409.2, 3ppm : 455.9, 4ppm : 495.6, 5ppm : 495.6ppm.

After acclimatization, the rats in Group A rats (control) continued to feed normally and were not exposed to any chemical. Group B and C were exposed to 50ml of chloroform and formalin poured into a beaker and placed in a glass inhalation chamber for 5minutes daily for 14 days respectively. For the purpose of exposure, the rats were put in a well-ventilated plastic cage which was in turn placed within the inhalation chamber. Each day after exposure, the animals were transferred back to their cages. On day 15 the animals were sacrificed by cervical dislocation. Blood was collected via cardiac puncture for serum chemistry to assess liver function enzymes and the liver harvested for histopathological studies.

The mean body weight for each group was determined for each group, analyzed and compared using the student's T-test. Data were expressed as Mean ± SD. Difference were considered significant at $P \leq 0.05$.

RESULTS AND DISCUSSION

Results of weight changes in the rats as presented in tables 1 and 2 indicated that all the rats had increased weight in the course of the research. In Table 1, all the rats showed weight increase between the time of commencement of exposure and the time prior to sacrifice. Differences in weight were statistically significant at 95% confidence level ($P \leq 0.05$). On further investigation into the percentage increase in rat weight as presented in Table 2, we observed that the percentage increase in weight in the experimental groups B and C were lower than that of the control. However, we saw that the percentage weight increase was higher in group C (23.22%) exposed to formalin compared to group B (20.16%) exposed to chloroform. This could imply that the weight of the rats was less affected by formalin as compared to chloroform. Simply put, chloroform inhalation had more negative effect on rats weight compared to formalin inhalation

Table 1: Changes in Weight of Rats

Group	Initial weight	Final weight
A	162.86± 4.90	227.14 ± 28.70*
B	177.14± 16.03	212.86 ±19.76*
C	146.43±17.01	180.43± 8.14*

Results were presented as Mean ± Standard deviation of 7 animals.

* Indicated statistical significance at 95% confidence level ($P \leq 0.05$).

Table 2: Percentage changes in rat Weights

GROUP	INITIAL WEIGHT	FINAL WEIGHT	% WEIGHT GAIN
A	162.86	227.14	39.63%
B	177.14	212.86	20.16%
C	146.43	180.43	23.22%

The result of the Liver Function Test (LFT) as presented in table 3 below indicates that chloroform and formalin inhalation led to significant increase in serum levels of Aspartate aminotransferase (AST), Alanine aminotransferase (ALT) and Alkaline phosphatase (ALP) compared to the control. In comparing the extent of increase between chloroform and formalin groups, we saw that AST was significantly higher in the chloroform group compared to the formalin group. However, ALT and ALP were higher in the formalin group (C) compared to the chloroform group (B). Reports from the works of Hodgson (2004) indicates that ALT is a more specific determinant of liver function impairment than the other two. Also, the report of Aguwa et al., (2016) indicates that rise in serum levels of AST, ALP but especially ALT is a valid indicator of liver damage. Liver enzymes ALT and AST are normally present in normal hepatocytes. These enzymes however, leak out into the circulation when hepatocytes or their cell membranes are damaged as reported by Charles (2012). These results therefore imply that formalin adversely affected liver function enzymes more than chloroform. However, the two showed deleterious adverse effect on the function of the liver.

Table 3: Result of Liver Function Test (LFT)

LIVER ENZYME	A	B	C
AST	128.00±2.31	216.25±4.79**	194.00±11.43**
ALT	51.50±3.32	61.75±3.86**	66.75±4.50**
ALP	39.00±8.08	55.25±9.78*	66.50±7.77**

Where * represents statistical significance at $P<0.05$ and ** indicates high statistical significance.

Result of Histological Studies

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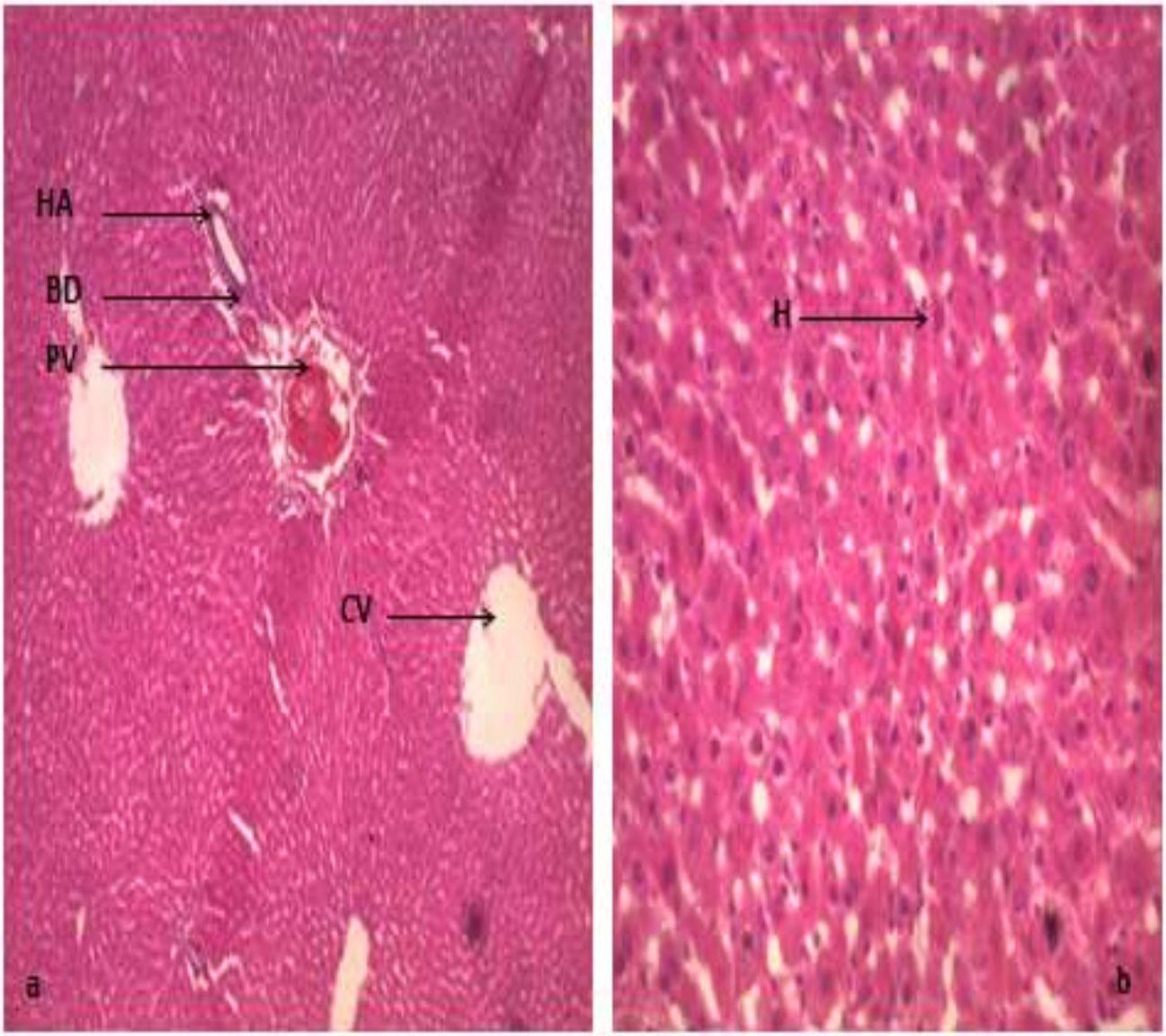

Plate 1: Representative photomicrograph of rat liver in group A (a. X100, b. X400) showing HA-hepatic artery, BD-bile duct, PV-portal vein, CV- central vein, H-hepatocyte.

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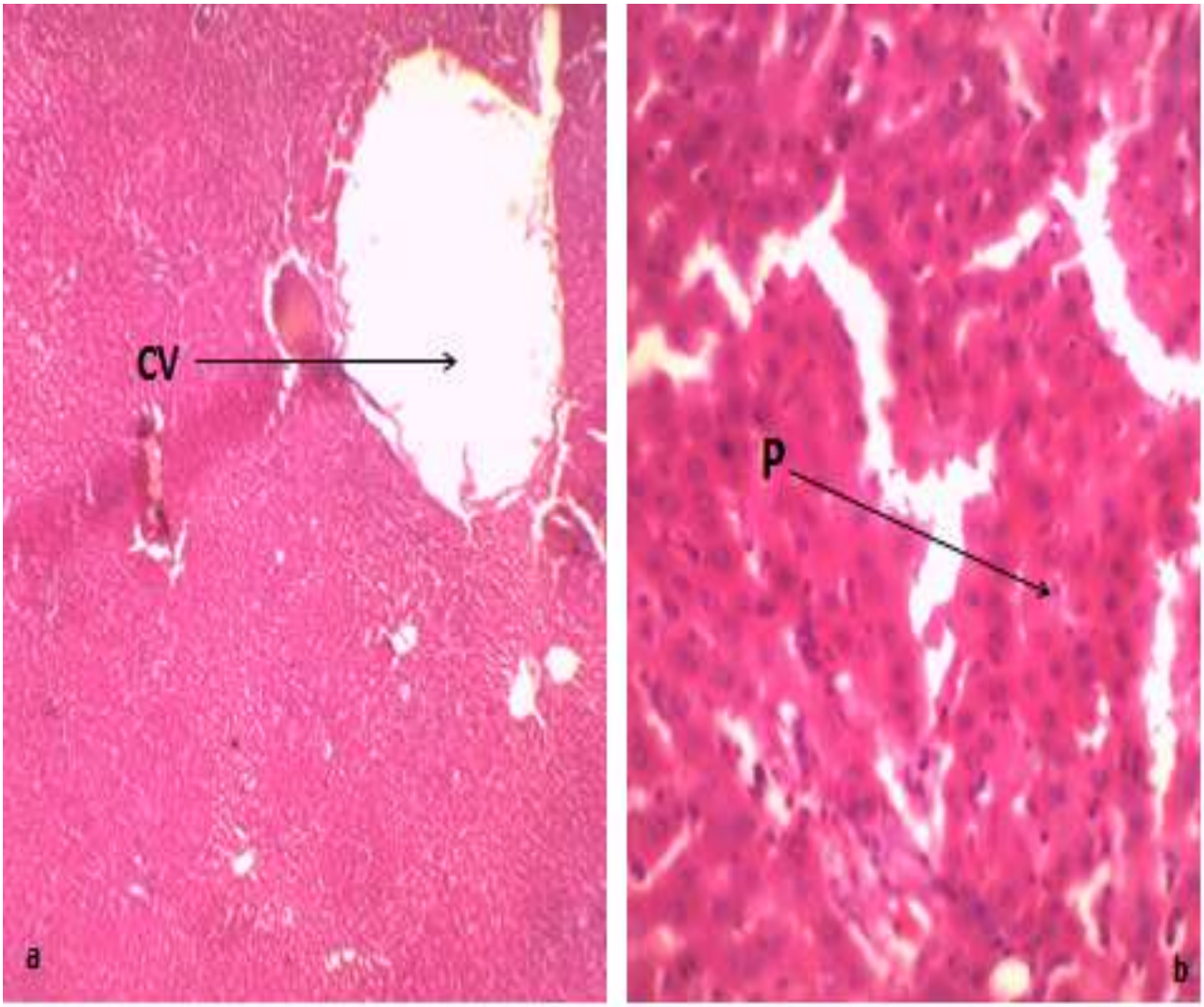

Plate 2: Representative photomicrograph of rat liver from group B which were exposed to chloroform inhalation. (a) shows wide central vein (CV). The hepatocytes in the higher magnification (b) show numerous necrotic cells; several pyknotic (P) cells. Also, we see unusual cellular gap, indicative if tissue hypotrophy.

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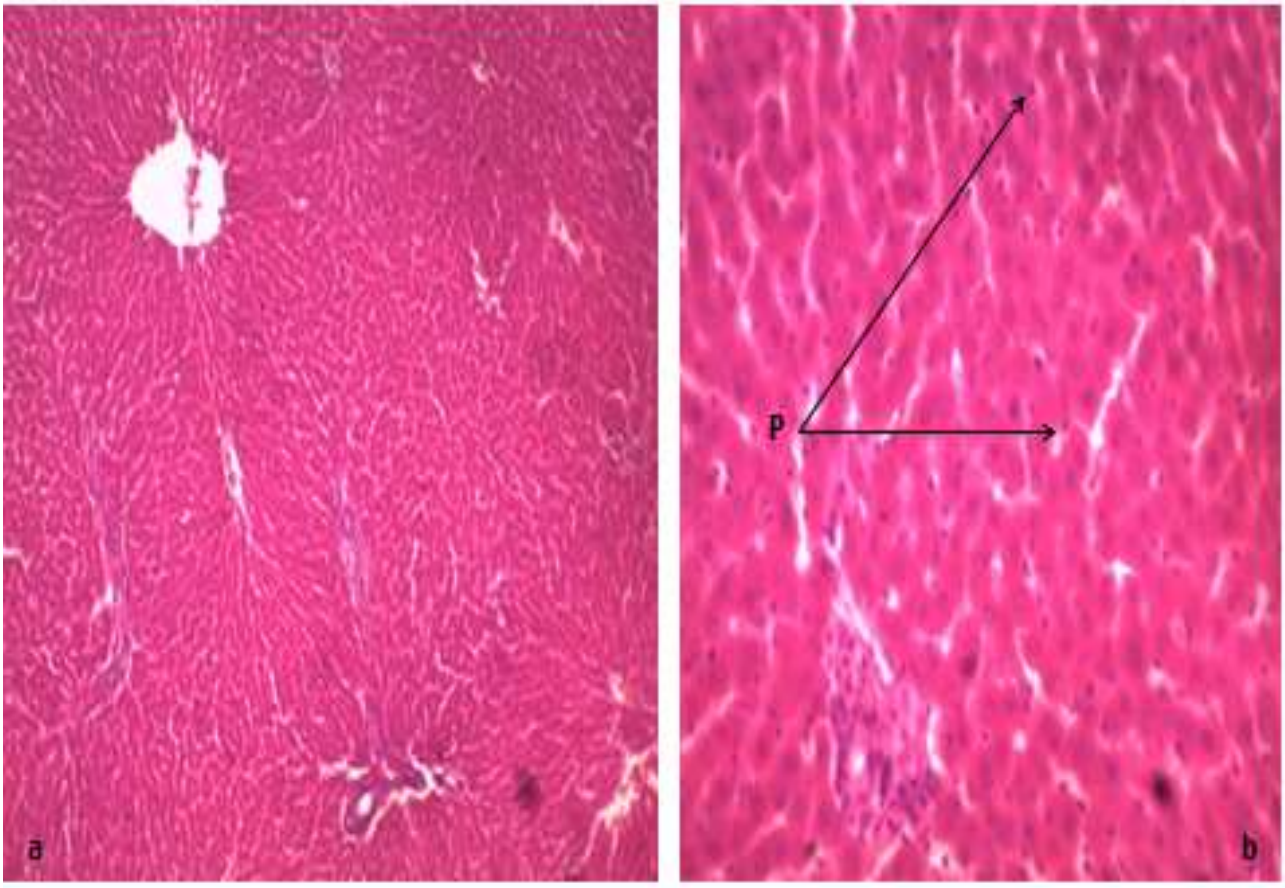

Plate 3: Representative photomicrograph of rat liver in group C which were exposed to formalin inhalation. The higher magnification shows some shrinkage in the nucleus of many hepatocytes (H) many of which are pyknotic, indicative of ongoing cell death.

The result of these histological slides shows that unlike the control group where the liver show normal histological features, the experimental groups are not so. The chloroform group shows wide sinusoidal gaps and vacuolations of the liver tissue. This corresponds with the tissue hypertrophy observed after tissues were removed after sacrifice. Plate 3 shows the liver tissue from rats exposed to formalin. We see tissue shrinkage, with different areas of cellular congestion and necrosis. Also prominent are the distortions seen on the tissue cytoarchitecture. Reports from the works of Mamun et al., (2014) shows that oral exposure to formalin produced some severe

histological alterations. Congestion in the central vain, (this results in dilatation of central veins and pooling of blood in the sinusoids towards the center of the liver lobule), deleterious degenerations in tissues, enlargement in the sinusoids, mild hemorrhages inside the tissue and reduced compactness of the cells by the formation of intercellular spaces.

This also corresponds to the physical observations made at the point of sacrifice which shows tissue shrinkage in the liver as well as the other organs removed from the rats exposed to formalin.

Our results therefore presents a trend in which chloroform leads to tissue hypertrophy while formalin leads to tissue shrinkage. We also see that exposure to these chemicals for a period as short as 5 minutes a day for 14 days produced adverse effects on the rats. This gives a total of 70 minutes (one hour and 10minutes). If 70 minutes can lead to this extent of health consequence, then we might as well revisit the state of the dissecting labouratories across our medical schools and device further ways to shorten the time spent in the cadaver room. Students of medical sciences, including Anatomy, physiology and medicine are supposed to dissect for a minimum of 3 hours every week for 15 weeks in a semester, giving us about 90 hours per semester. Students of Medicine and physiology as well as other allied medical courses may go through this for about 4 semesters in 2 years, giving an exposure time of about 180 hours per annum and 360 hours for the two years of their preclinical studies. For students studying anatomy, it lasts through out their 3 years post basic (year one) training (540 hours). Today, several technologies abound which could be used as supplements to dissection to reduce the time spent in the dissecting room. One of the most recent is the Anatomage machine which offers very flexible methods of demonstration in anatomy that can efficiently improve students' understanding during dissection and also reduce the time they spend there.

CONCLUSION

The results of our study show that inhaling formalin for as little as five minutes daily every day for 2 weeks can bring about adverse health consequences on the liver. Problems with the liver affect normal metabolic activities which may be fatal over time. I therefore recommend that those who by profession or training are under obligation to work around environments prone to chloroform and formalin inhalation must be proactive in detoxifying their system and paying attention to proper medical care from time to time to avert the destruction of their liver through the inhalation of these chemicals.

Acknowledgement:

We hereby acknowledge the input of every member of this team for their tireless efforts during this work.

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