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Effects of Formalin Inhalation on Physical Characteristics and Renal Profile of Albino Wistar Rats

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Authors' contributions

This work was carried out in collaboration between all authors. Author JNE designed the study, wrote the protocol, performed the statistical analysis and wrote the first draft of the manuscript. Authors MCO, CNE, FUO, BCA, IGE and EEN managed the analysis of the study. Authors PCU, DII, PN and MUE managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Background: Formalin exposure is common in our environment. This study assessed the effects of formalin inhalation on renal function and physical features of albino wistar rats.
Materials and Methods: Thirty apparently healthy male albino wistar rats, weighing between 100-160 g, divided into five groups of six rats each were used for this study that lasted for four weeks.

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Group A, the control group, exposed to ambient air, received normal rat chow and portable water *ad libitum*, while the test groups B, C, D and E had 2, 4, 6, and 8 hourly daily exposure to formalin respectively via inhalation in the cadaver dissecting hall of the Anatomy laboratory for medical students, in addition to normal rat feed and water *ad libitum* when not exposed to formalin. Fortnightly, four rats were randomly selected from each group, anaesthetised with chloroform and blood samples collected through cardiac puncture for the analysis of serum electrolytes, urea and creatinine.

Results: Results showed yellow discoloration of the skin, drowsiness, reduced movements and feeding habits in groups C-E when compared with those in groups A and B. The results also showed statistically significant duration of exposure dependent increase in serum concentrations of sodium, potassium, urea and creatinine in test groups C to E after 2 & 4 weeks exposure when compared with control ($p<0.05$). After 2 weeks exposure, only group E had a significant increase in serum concentrations of chloride and bicarbonate while all test groups except group B had significant elevation after 4 weeks exposure.

Conclusion: Persistent inhalation of formalin may have deleterious effects on the kidney, skin, brain and appetite. We, therefore, recommend that medical students and lecturers, as well as those using Formalin, should have time-limited exposure, well-ventilated rooms, use of less toxic chemicals for embalming as well as a restriction to the utilisation of this chemical as a food preservative, and use of personal protective equipment during procedures.

Keywords: Formalin inhalation; renal functions; albino wistar rats; time-limited exposure; medical education.

1. INTRODUCTION

Formalin, a commercial preparation of formaldehyde, has a vapour density of 1.04, a boiling point of 101®C, a specific gravity of 1.08, and a pH of 2.8 to 4.0 [1]. Formaldehyde (FA) is a noxious, colourless, flammable, highly water soluble gas discovered in 1867 by the British chemist, August Wilheld Von Hofman [2-3].

FA is a common contaminant in our environment as a result of its wide use in industries such as the production of building materials, textiles, sterilisation of products, plastics, paper, cosmetics, plywood and for the preservation of tissues, organs and viscera in various universities/schools, biological, forensic and pathological laboratories [4-6]. FA has also been found in cigarette smoke, in car emissions, fuel oil and natural gas, in smoke due to combustion of wood or liquid-based fuels, in the exhaust of vehicles with burning fossil fuels and in the fumes of paints used for surfaces and furniture, particleboard, plywood and medium-density fiberboard [7-13]. Glycine and serine are the most important endogenous sources of FA. Furthermore, N-methyl amino acids and sarcosine can be converted into FA via oxidative demethylation by specific enzymes. In the body, endogenous tissue levels range from 3-12 ng/g and about 40% of this occurs in the free form [14].

When FA is taken into the body, irrespective of the route of entry, it forms a water addition product, FA acetal (methylene glycol, $CH_2(OH)_2$) which reacts with glutathion (GSH), forming S-hydroxymethylglutathione (FA glutathione thioacetal; HO-CH_2-SG). This intermediate is oxidised by the glutathione-dependent FA dehydrogenase FDH [15] to S-formylglutathione and later hydrolysed to GSH and formate [16]. FA with its very short half-life of about 1.5min is metabolised into formic acid in the liver and erythrocytes. Formaladehyde dehydrogenase requires glutathione as a co-factor during this metabolic reaction. Therefore, as the formaldehyde level increases, the blood glutathione concentration decreases. This reduction of glutathione, an antioxidant, exacerbates the toxicity of FA [17-20].

Individuals are constantly being exposed to FA both in their jobs and in non-occupational settings [21-23], FA is metabolised in the liver and erythrocytes into formic acid and later excreted in the faeces or urine or via the respiratory tract as carbon dioxide. It is usually completely eliminated within a few days [24-25] and its toxicity is most often ignored [26] probably due to adaptation and duration of exposure effects.

The health effects of FA exposure affect people differently; while some people are very sensitive, others may exhibit no noticeable reactions to a

particular level of the compound. Formalin affects almost all systems and organs in the body. It can be toxic, allergenic and carcinogenic [27-28]. Formalin exposure associated health effects include: squamous cell carcinoma in rats and nasopharyngeal cancer and sinonasal cancer in humans [29-31]; sick-building syndrome [32]; sensory irritation from the eyes and upper airways [10,32-34]; genotoxicity [10, 33,35-36], cytogenetic [37], anaemia and leukemia [28,3], spontaneous abortions, menstrual irregularities and congenital malformations [38,39], ocular irritations and corneal clouding [38], testicular dysfunction, reduced sperm viability and motility, reduced testosterone concentration [40], bladder cancer [41] and nephrotoxic effects such as glomerular and tubular degeneration, tubular dilatation and congestion, acute tubular necrosis and renal failure [42,43,44,45,46]. We have also demonstrated that formalin inhalation causes derangement of lipid profile, serum proteins and liver enzymes in rats [47-48].

There is a paucity of data in the literature on the inhalational effects of formalin on renal profile. This study was undertaken to assess the effects of formalin inhalation on renal function of albino wistar rats.

2. MATERIALS AND METHODS

2.1 Experimental Animals

Thirty apparently healthy mature male Albino wistar rats were used for this study. They were purchased from the Animal house of the University of Nigeria, Nsukka. They were 10 weeks old and weighed about 100-160 g. The rats were kept in metal rat cages of wired mesh by the sides, in a controlled environment and allowed to acclimatise in a trial room temperature of 24± 1°C for 2 weeks. The animals were fed with portable water and normal rat chow *ad libitum* while the experimental animals in addition, also had formalin exposure. The animals were weighed before and after the experiment.

2.2 Ethical Consideration

All rats were handled according to the care and use of laboratory animals as provided by the Institutional Animal, Medical Ethics, Bio-safety and Bio-security Committee (IAMEBBC), (NO.33/320/IAMEBBC/IBSC). The research protocol was approved by the Ethics Committee of Imo State University, Owerri.

2.3 Formalin Exposure

Iron cages containing animal subgroups were kept in the Anatomy dissecting halls of medical students, during the usual dissecting sessions in Anatomy laboratory of Imo State University, Owerri. Formalin vapour in the cadavers which were preserved with 10% formalin in the dissecting hall was directly inhaled by the rats just like the medical students, anatomist and the laboratory attendants at different time durations.

Group A (Control group) – No inhalation of Formalin.

Group B - Inhalation of Formalin for 2hr /day for 4 weeks.

Group C - Inhalation of Formalin for 4hr/day for 4 weeks.

Group D - Inhalation of Formalin for 6hr/day for 4 weeks.

Group E – Inhalation of Formalin for 8Hr/day for 4 weeks.

The experiment lasted for four weeks. At the end of every 2weeks, four rats were randomly picked from each group , anaesthetised with chloroform and blood samples collected by cardiac puncture in plain sample bottles. The blood samples were centrifuged at 10,000rpm for 10 minutes, serum was collected for the analysis of sodium, potassium, chloride, bicarbonate, urea and creatinine. Analyses were performed with spectrophotometer using commercial kits.

Electrolytes: The electrolytes such as Sodium, Potassium, and Chloride were analysed using the centrifuge-MSE Centour, electric water bath, spectrophotometer-spectronic 20D, and Ion Selective Electrode (ISE) module-Humalyte. This operates basically like a photometer. The potential generated by the ion of interest in the test sample is measured by the selective electrode and compared with that of a standard solution in which the concentration of the ion of interest is known.

Creatinine: Creatinine + Picric acid forms Creatinine-Picrate complex, which is an alkaline orange red complex with Picric acid. The absorbance of this complex is proportional to the creatinine concentration in the sample [49].

Urea: Urea is hydrolysed in the presence of water and urease to produce Ammonia and

Carbondioxide. In a modified Berthelot reaction, the Ammonium ions react with hypochlorite and salicylate to form a green dye. The absorbance increases at 578nm equivalent to the concentration of urea in the sample [50-51].

2.4 Statistical Analysis

The data generated from the study were cleaned, coded, entered into Excel sheet and later analysed using IBM-SPSS software version 21. Descriptive statistics and Student's t-tests were performed and results expressed as Mean±SEM(Standard error of mean). In all cases, the difference was considered statistically significant when p-value was ≤0.05.

3. RESULTS

The results are presented as mean±SEM and in tables.

The mean weight of the experimental rats increased significantly in groups A and B(P <0.05). No significant change in weight was noted in group C rats exposed for 4 hrs daily when compared with the pre-exposure weight (p>0.05). Rats in groups D and E had statistically significant weight reduction in their final post-exposure weights compared with their pre-exposure levels(p <0.05). Table 1 show the weight variations of the rats in this study.

Table 2 show other physical signs observed in the rats following exposure to formalin inhalation. Rats in groups A and B displayed no significant changes skin colour, feeding habits and neurological signs. Yellow discoloration of the skin, reduced feeding habits, and reduced level of consciousness (dizziness), altered movements and hair loss were noted in groups C,D & E when compared with control group. These physical changes were more severe in group E rats exposed for 8 hrs everyday.

Table 3 shows that serum concentrations of sodium, potassium, urea and creatinine increased significantly with increasing duration of formalin inhalation exposure in the test groups C to E when compared with the control(p<0.05). Only the animals exposed for 8 hours (Group E) experienced statistically significant increase in the serum values of chloride and bicarbonate

Table 1. The mean weight of the rats following exposure to formalin inhalation

Groups	Average Weight before experiment (g)	Average Weight after experiment (g)
Group 1- Control	120.4±0.03	172.4±0.13®
Group 2	121.5±0.09	165.5±0.08®
Group 3	119.2±0.03	122.0±0.06
Group 4	120.1±0.15	101.3±0.16®
Group 5	119.8±0.21	93.1±0.24®

° Significant level at p <0.05

Table 2. The Physical changes observed on the rats following formalin inhalation

	Group 1 Control	Group 2 Two weeks exposure 2 hrly/day	Group 3 Four weeks exposure 4 hrly/day	Group 4 Six weeks exposure 6 hrly/day	Group 5 Eight weeks exposure 8 hrly/day
Findings	-No skin changes. -Normal feeding habits.	No skin changes. -Normal feeding habits	-No skin changes -Mild reduction in feeding habits immediately after exposure	-Mild yellow discolouration of skin -Reduced feeding habit immediately after exposure -Drowsy and sluggish movements	-Deep yellow discolouration of skin and fur -Reduced feeding habit immediately after exposure -Drowsy and sluggish movements -Hairs fall off on slight manual manipulation

Table 3. Effects of Formalin inhalation on renal indices after 2 weeks

Group	Sodium	Potassium	Chloride	Urea	Creatinine	Bicarbonate
A (Control)	122.06±1.15	4.78±0.33	95.80±3.17	28.88±0.77	1.01±0.06	22.67±0.88
B (Exposed for 2 hrs daily)	121.16±3.34	5.01±0.16	96.13±3.43	29.88±0.69	1.17±0.25	22.85±0.84
C (Exposed for 4 hrs daily)	124.53±0.52®	6.00±0.38®	96.67±3.78	30.74±1.03®	2.52±0.09®	23.22±0.39
D (Exposed for 6 hrs daily)	125.28±1.07®	6.77±0.32®	96.72±3.38	32.37±0.48®	2.80±0.19®	23.80±1.16
E (Exposed for 8 hrs daily)	126.10±1.42®	8.25±0.27®	97.21±0.49®	34.47±0.59®	3.18±0.46®	26.14±0.98®

® Significant level at p<0.05

Table 4. Effects of Formalin inhalation on renal indices after 4 weeks

Group	Sodium	Potassium	Chloride	Urea	Creatinine	Bicarbonate
A (Control)	124.00±1.15	5.60±0.36	105.54±3.28	27.37±0.35	1.05±0.13	22.67±1.45
B (Exposed for 2 hrs daily)	124.87±1.14	6.13±0.20	107.33±0.62	28.76±1.49	1.27±0.12	23.08±1.29
C (Exposed for 4 hrs daily)	127.77±3.12®	7.07±0.35®	112.08±4.65®	32.97±1.18®	2.65±0.14®	24.82±0.93®
D (Exposed for 6 hrs daily)	130.67±1.48®	7.75±0.19®	118.02±5.86®	35.16±0.81®	3.22±0.09®	25.63±1.37®
E (Exposed for 8 hrs daily)	132.44±1.89®	9.75±0.84®	120.15±1.47®	38.50±2.32®	4.34±0.18®	28.40±1.47®

® Significant level at p <0.05

when compared with the control($p < 0.05$). Animals exposed for 8 hrs per day had the greatest impact of the inhalation effects of formalin in this study. Rats exposed for 2 hrs only had no significant difference compared with the control.

Table 4 shows that rats in the test groups C to E had statistically significant increase in the serum levels of sodium, potassium, chloride, bicarbonate, urea and creatinine when compared with the control($p<0.05$). The animals in group E had the greatest impact of formalin inhalation compared to the other test groups. No significant difference was observed between animals in group B and the control.

4. DISCUSSION

Humans are variously exposed to formalin, not only in our homes, the open environments and work places but also in our learning institutions. Globally, medical education is rigorous, expensive and full of challenges. Environmental challenges such as exposure to formalin inhalation during dissecting sessions have not been adequately addressed. This study was undertaken to assess inhalational effects of formalin on rats' physical characteristics and renal profile during dissecting sessions of medical students in a Nigerian University.

The formalin-exposed rats exhibited some physical signs such as reduced feeding habits, drowsiness, sluggish movement, weight loss, yellow discolourations of the skin and hair loss when compared with the control. These findings are in tandem with earlier studies [52-55]. Neurobehavioural impairments have been reported in many formalin studies [56-60].

The results of this study also revealed statistically significant duration dependent increase in serum concentrations of sodium, potassium, urea and creatinine in the formalin exposed rats when compared with the control group.

The serum levels of chloride and bicarbonate appreciated significantly compared with control

only in those exposed for 8 hr/day in the first two weeks and later in all test groups except those in group B after 4 weeks. Increased concentrations of urea and creatinine in particular, are very important markers of nephrotoxicity following formalin exposure [47,56,61,62-65].

Toxic effects of formaldehyde (FA) have been observed in almost every system in the body including the renal system, nervous system, gastrointestinal system and the skin. Several experimental and clinical studies have attempted to explain the various ways formalin exerts its nephrotoxic effects.

Firstly, oxidation of FA to formic acid is catalysed by many enzymes including NAD-dependent dehydrogenase formaldehyde, xanthine oxidase, catalase, and peroxidase. Increased production of these enzymes for the detoxification of FA has been associated with increased concentration of urea [64-65]. FA exposure has also led to an increase in serum levels of urea, creatinine and reduced urine production [46,51]. Increased serum values of creatinine and urea strongly suggest renal failure due to exposure to formalin [62,63,65-69].

Secondly, evidences abound that prolonged exposure to FA can cause profound histopathological and biochemical alterations in renal tissues. Exposure to FA has been associated with impaired glomerular patterns, thickened tubular and glomerular basal membranes, congestion of intratubular vessels, vacuolisation and dilatation of distal tubules, glomerular and tubular degeneration and renal papillary necrosis [7,42,54]. In addition, prolonged exposure to FA can lead to degeneration and necrosis of proximal tubule of the kidney and consequently impaired urinary system [7,29,67].

Thirdly, reactive oxygen species(ROS) at low concentrations, have very important physiological functions in the body especially cellular processes. However, in high amounts, they can cause deleterious changes in cell components such as proteins, lipids and DNA [70,71] and oxidative stress. Generation of ROS occurs in normal cellular respiratory processes and can be potentiated in the presence of exogenous chemicals such as FA [4,70,71]. FA can break the antioxidant defense mechanisms in the kidneys, leading to formation of oxidative stress [7,66]. Furthermore, toxicity of FA exposure has been associated with oxidative damage in kidney tissues [4,6,42,72].

Fourthly, the kidney has been described as one of the most sensitive organs to inflammation and a very important source of chemokines and cytokines in the tubular epithelium probably due to its close contact with high blood flow [73]. Several studies have demonstrated that prolonged exposure to formalin can induce many pathophysiological conditions, including inflammatory diseases by interfering in the level of T $CD3^+$ cells, natural killer (NK) cells, TNF, IL-6 and IL1-b [74-76]. The reported renal damage following exposure to FA has been accompanied by local inflammatory processes which promote the infiltration of cells mainly conducted by chemokines. And an increase in the levels of chemokines such as CCL2, CCL3, and CCL5 has been associated with influx of cells including monocytes, lymphocytes, and eosinophils into tissues and fluids [77-78]. In addition, some studies have demonstrated high concentrations of CCL2, CCL3 and CCL5 in the renal parenchyma of animals exposed to FA. Elevated levels of these chemokines have been associated with worsening renal inflammatory injury [69,79-82].

Fifthly, the increase in serum urea concentration among the test group animals might be due to the dehydration effects of formalin exposure. Formalin, a known dehydrating agent, can induce haemoconcentration in the rats leading to increase in urea levels [66,83].

This study also revealed elevated serum levels of some electrolytes including sodium, potassium, chloride and bicarbonate in the formalin exposed rats. These findings are in agreement with earlier works [56,67,84-85]. The exact mechanism for the effect of formalin on serum electrolytes is unclear. However, the following may explain the effects:

Formalin exposure has been linked with serious histopathological and biochemical derangements in renal tissues that may lead to oliguria, anuria, and renal failure [53,62,82]. The above reported anomalies are often associated with electrolyte alteration including Na^+, K, Cl^-, HCo_3 [56,65,66].

The increase in serum electrolytes with exposed rats may also be due to the dehydration and thirst effects of formalin [56,66]. Dehydration stimulates ADH release with attendant increased osmolarity and serum electrolytes.

5. CONCLUSION AND RECOMMENDATIONS

This study showed that Formalin had negative impacts on both physical characteristics and renal profile of albino wistar rats. We, therefore, recommend that medical students and lecturers, as well as those using Formalin, should have time-limited exposure, well-ventilated rooms, use of less toxic chemicals for embalming as well as a restriction to the utilisation of this chemical as a food preservative, and use of personal protective equipment during procedures.

CONSENT

It is not applicable.

ETHICAL CONSIDERATION

All rats were handled according to the care and use of laboratory animals as provided by the Institutional Animal, Medical Ethics, Bio-safety and Bio-security Committee (IAMEBBC), (NO.33/320/IAMEBBC/IBSC). The research protocol was approved by the Ethics Committee of Imo State University, Owerri.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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