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Effect of Formalin Inhalation on the Cerebellum of Adult Male Wistar Rat

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Abstract:

Humans are constantly exposed to chemicals from the environment through inhalation, some of which produce adverse health consequences. This research was done to evaluate the effect of formalin vapor inhalation on the cerebellum of male Wistar rats. Fourteen adult male Wister rats weighing 150-180g were divided into two groups of 7 rats each based on similarity of weight. Group A was the control group and received food and water. Groups B was exposed to 50ml of formalin for 5mins daily in an inhalation chamber for a period of 14 days. On day 15, the rats were weighed and subjected to neurobehavioral studies after which they were sacrificed. The cerebella of two rats from each group were fixed in Bouin's fluid for histological studies. The brains of the remaining five rats were used for antioxidant studies. Results were analyzed using SPSS version 20 and presented as Mean ± SD of 7 rats in each group. Values were significant at $P \leq 0.05$. Percentage increases in weight of rats were also calculated. Our results reveal that the animals in the experimental group were under oxidative stress as MDA levels were significantly higher in the group B compared to the control. There was significant increase in weight of rats across the two groups. However, the percentage of increase was lower in the experimental group B compared to the control. Results of the neurobehavioral studies reveal that there was significant reduction in the muscular strength of rats in the experimental group compared to the control. From our result, we can conclude that formalin vapor inhalation has neurodegenerative effects on the cerebellum of adult male Wistar rats.

Key Words: Cerebellum, formalin, neurobehavioral, inhalation.

Introduction:

Formaldehyde is a colorless, flammable gas, extremely soluble in water; the aqueous solution (about 37% formaldehyde) is called formalin. It is widely used in medical colleges and hospitals, as preservative, disinfectant, embalming solution and in different fields like wood and plastic industries. Although formalin is extensively used in different fields, its toxicity is frequently ignored [1]. Adverse effects of inhaling formaldehyde become more as its concentration increases. The common symptoms from acute exposure to formalin manifest as irritation of the throat, nose, eyes and skin. It can also cause neurophysiologic effects, irritation of upper respiratory tract which can potentially exacerbate asthma symptoms and other respiratory illnesses, dyspnea, coughing, burning of nose, eyes, and pharynx. Chronic exposure can cause bronchitis and pneumonia. When it is swallowed, it can result in sudden death. Some researchers also believe that formaldehyde is a potential carcinogen [2]. This study intends to investigate the effect of formalin inhalation on the cerebellum of male Wistar rats and by extension on motor coordination and muscle strength. The cerebellum is a part of the brain at the back of the skull in vertebrates, which coordinates and regulates muscular activity [3]. In humans, the cerebellum plays an important role in motor control, and it may also be involved in some cognitive functions such as attention and language as well as in regulating fear and pleasure responses [4]. It also contributes to coordination, precision, and accurate timing. It receives input from sensory systems of the spinal cord and from other parts of the brain, and integrates these inputs to fine-tune

motor activity [5]. Damage to the cerebellum leads to disorder in fine movement, equilibrium, posture, and motor learning in humans [5]. The major toxic effects caused by acute formaldehyde exposure via inhalation are eye, nose, and throat irritation and effects on the nasal cavity. Other effects seen from exposure to high levels of formaldehyde in humans are coughing, wheezing, chest pains, and bronchitis [6, 7]. Ingestion exposure to formaldehyde in humans has resulted in corrosion of the gastrointestinal tract and inflammation and ulceration of the mouth, esophagus, and stomach [6, 7]. Acute animal tests in rats and rabbits have shown formaldehyde to have high acute toxicity from inhalation, oral, and dermal exposure [6]. Other animal studies have reported effects on the nasal respiratory epithelium and lesions in the respiratory system from chronic inhalation exposure to formaldehyde [6]. An increased incidence of menstrual disorders was observed in female workers using urea-formaldehyde resins. However, possible confounding factors were not evaluated in this study [6, 7]. A study of hospital equipment sterilizing workers did not report an association between formaldehyde exposure and increased spontaneous abortions. Developmental effects, such as birth defects, have not been observed in animal studies with formaldehyde [6, 7]. Occupational studies have noted statistically significant associations between exposure to formaldehyde and increased incidence of lung and nasopharyngeal cancer. This evidence is considered to be "limited," rather than "sufficient," due to possible exposure to other agents that may have contributed to the excess cancers. Animal studies have reported an increased incidence of nasal squamous cell carcinomas by inhalation exposure [6, 8].

Materials and Methods:

Fourteen (14) 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 in the animal house of the department of anatomy, Madonna university Elele campus, Rivers state for the period of one week before exposure. The rats were feed with rat chow and were provided with water ad libitum throughout the duration of the experiment. Analytic grade formalin manufactured by BDH Chemical Ltd Poole England was used for this study. A gas meter was used to determine the extent of gas released at various durations of exposure as follow: for 2mins: 821.0ppm, 3mins: 880.0ppm, 4mins: 870.1ppm and 5mins: 965. 8ppm. The group A which served as the control group were not exposed to any chemical. Group B was exposed to 50ml of formalin poured into a beaker and placed in a glass inhalation chamber for 5minutes daily for 14 days. For the purpose of exposure, the rats were put in well ventilated plastic cages which were in turn placed within the inhalation chamber. After exposure each day the animals were transferred back to their cages. The exposure period lasted for 14 days. On day 15 the animals were subjected to hanging wire test (neurobehavioral studies) after which the rats were sacrificed by chloroform sedation and their brains harvested. Five of the brains were introduced into phosphate buffer solutions and refrigerated for antioxidant studies. The other two brains from each group were collected and fixed in Bouins fluid for histopathological studies. The weight of each rat was taken before the commencement of exposure using a sensitive digital weighing balance and was repeated on the last day of exposure (day 14). The mean body weight for each group was determined for each group, analyzed and compared using the students T-test. Data were expressed as Mean ± SD. Difference were considered significant at $P \leq 0.05$.

Results and Discussion:

Table 1: Changes in Weight of Rats

Group	Initial weight	Final weight
A	162.86± 4.90	227.14 ± 28.70*
B	146.43 ±17.00	180.43± 8.14*

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

* Indicated statistical significance at 99% confidence level ($P \leq 0.01$).

Animals all showed weight increase between the time of commencement of exposure and the time prior to sacrifice. Differences were statistically significant at 99% confidence level ($P \leq 0.01$).

Table 2: Percentage changes in rat Weights

Group	Initial weight		Final weight		Percentage
	Weight	Percentage	Weight	Percentage	Weight gain
A	162	41.65 %	227	58. 35%	16. 70 %
B	146	44.77 %	180	54.50 %	10. 42%

On further investigation into the percentage increase in rat weight, we observed that the percentage increase in weight in the experimental group B was reduced (10.42%) as compared to the

control group with 16% weight increase within the same period.

Table 3: Showing antioxidant result.

Group	MDA	SOD	CAT	GSH
A	0.12 ± 0.01	1.70 ± 0.14	3.04 ± 0.32	6.96 ± 0.46
B	0.18 ±0.01*	0.57 ±0.16**	1.48 ± 0.26**	5.80 ±0.43**

Our result shows that the animals in the experimental groups were under oxidative stress as levels of MDA were significantly higher in the experimental group compared to the control. Supraoxide dismutase (SOD), Catalase (CAT) and reduced glutathione (GSH) levels were highly significantly lower in the experimental group compared to the control.

Table 4: Result of Hanging wire test

Group	Initial (in minutes)	Final (in minutes)
A	2.52 ± 035	2.73 ± 0.62
B	3. 69 ± 0.67	1.19 ± 1.33**

Our results as presented in table 4 above indicates that the duration for the hanging wire test in group A (control) was slightly higher at the end than the initial duration although the difference did not attain statistical significance. The results of the experimental group B which inhaled formalin for 2 weeks showed highly significant reductions in the duration of the hanging wire test in the rats at the end of the experiment as compared to the values before exposure to formalin vapor (initial).

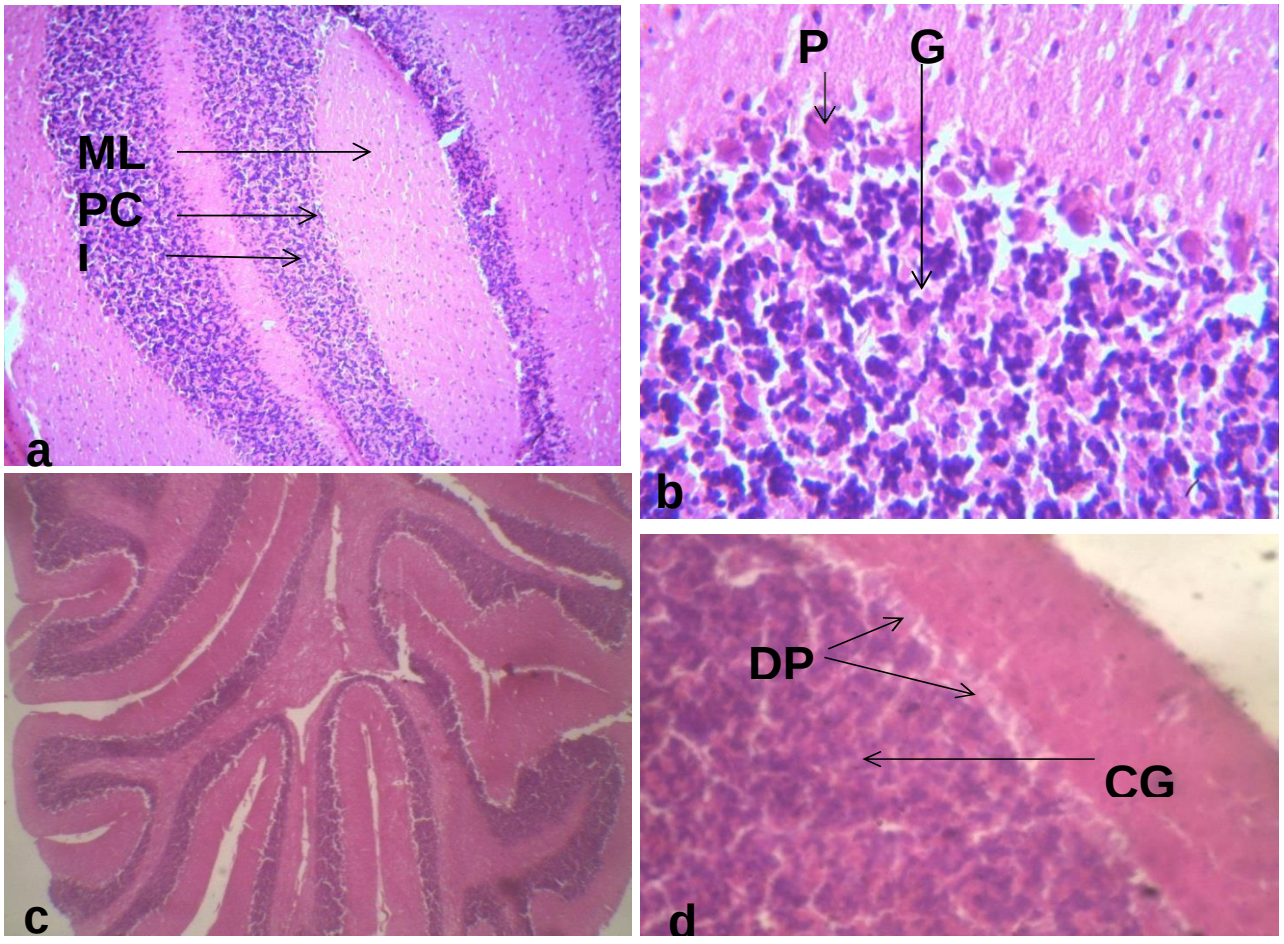

Plate 1: Representative photomicrograph of rat cerebellum. in group A (a, b) and group B (c, d). *a* show normal cerebellar cytoarchitecture (x100); molecular layer (ML), Purkinje cell layer (PCL), and granular layer (GL); *b* shows purkinje cell (pc) and granular cell (GC) (x400). In C and D, we see signs of intense cell death. DPC represents degenerating purkinje cells most of which have lost their nuclei and other nuclear materials. CGCs represent congestive granule cells.

Our results show that although the rats exposed to formalin for 5 minutes each day for 14 days showed significant weight increase at the end of the period of exposure when compared to the initial weight, the percentage increase in weight was lower (10.42%) compared to that of the control group (16.70%). This makes one fact clear; although the people being exposed to these chemicals may initially not show any signs of negative health manifestations, the changes may be very gradual and unnoticeable at the initial time and may take a long period to manifest. This was also obvious from the physical observation as the rats in the formalin group all looked shrunken compared to the control group. The result of our antioxidant studies reveals that the rats exposed to formalin vapor were under oxidative stress. This was obvious as malondialdehyde (MDA) level which is an indicator of lipid peroxidation was highly significantly increased in the experimental group compared to the control. This is in line with the works of Gornall et al, (1949) [10], which show that oxidative stress leads to a rise in MDA levels in rats. Furthermore, the antioxidant enzymes which are responsible for mopping up free radicals generated by lipid peroxidation (SOD, CAT and GSH) were all significantly depleted in the experimental group compared to the control. Oxidative stress has been associated with many chronic diseases some of which are incurable, including cancer, diabetes and high blood pressure [11]. The fact that these animals were under oxidative stress could mean that this may be the regular state of health of workers who are constantly exposed to these chemicals. This no doubt may lead to debilitating health consequence in the near future when the body's immune system has been overpowered. The cerebellum is a part of the hindbrain that among other things are involved in motor coordination [6]. Any impairment on the cerebellum will most likely negatively affect movement, muscle strength as well ascoordination and balance in the affected animal. The hanging wire test is a neurobehavioral study that tests for muscle strength among other things. Our results as presented in table 4 reveals that there were significant reductions in the values of the hanging wire test at the final reading when compared to the initial readings in groups B animals that were exposed to formalin vapor. The values were initially taken at the commencement of the experiment after acclimatization. No significant difference was observed in the initial values of the hanging wire test between the experimental group and the control. In the final readings however, we see highly significant reduction in suspension time on the hanging wire for the group B rats. This was a striking observation as the animals still looked healthy and active. This may be a sign of an underlying cerebellar dysfunction. This observation is further supported by the histological slides of the cerebellum shown in plate 1. There we see a normal histological framework of the cerebella ofrats in the control group (a X100) with the three distinct layers of the cerebellar cortex: molecular layer (ML), purkinje cell layer (PCL) and the granular layer (GL). Higher magnification (b X400) reveals the giant purkinje cells in the purkinje cell layer with prominent nuclei and nucleoli. On examination of the cerebella of rats in group B we see signs of cell death and faint nuclear outline of the purkinje cell. There are signs of nuclear destruction (pyknosis as well as Karyorhexis). The granule and basket cells were also atrophied and more sparsely dispersed in the molecular layer. This together with the results of the neurobehavioural and antioxidant studies gives strong indication of cerebellar impairment which may lead to motor dysfunction in the near future. A chief technologist and supervises the preservation of our cadavers suddenly resigned due to the emergence of several health problems. First the eye sight was impaired. Next it was difficulty in movement. It took a lot of struggle just to walk around within very close distances. At a point when he could no longer put up, he resigned from the job. It was this observation that stimulated our interest in this study. Also, we see that the young men who took over the job always have red eyes and a drowsy outlook.

Conclusion:

We therefore conclude from our work that exposure to formalin vapor for as little as five minutes daily may lead to cerebellar impairments which may produce deleterious health consequences.

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