Effect of Chloroform Inhalation on the Hippocampus of Adult Male Wistar Rat

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Abstract. Chemicals used in laboratories may pose health threats to individuals handing them. This work was carried out to evaluate the effect of chloroform inhalation on the hippocampus of Wistar rats. Fourteen male Wistar rats weighing 150-180g were divided into two groups of 7 rats each. Group A was the control group and Group B was the experimental group which was exposed to 50ml of chloroform for 5minutes daily in an inhalation chamber for 14 days. Neurobehavioral study was done before and after exposure and the rats were sacrificed. Two rat brains were fixed in Bouin's fluid for histological studies and the other five for antioxidant studies. Results were analyzed using SPSS and values were significant at $P \leq 0.05$. Results reveal the animals in group B were under oxidative stress compared to those in control. Although rat weight increased in the 2 groups, the percentage increase was lower in the experimental group B compared to control. Histological analysis showed varying degrees of necrosis in the hippocampus of group B rats. Neurobehavioral studies revealed significant increase in the time to discover the escape platform compared to control. We therefore infer that chloroform inhalation has neurodegenerative effects on the hippocampus of Wistar rats.

Key words: hippocampus, chloroform, neurobehavioral, inhalation

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 (Stone et al., 2007). Chloroform can be produced with common household liquids including sodium hypochlorite solution (chlorine bleach) missed with liquid chemicals such as acetone, butanone, ethanol, or isopropyl alcohol (Washburn and Anderson, 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 (Sidhu, 2003). Chloroform is also used to extract and purify penicillin (Baeder and Hofmann, 1991). However, chloroform is no longer recommended for use in these products. One of the reasons is continuous scientific evidences implicating chloroform as toxic to different organs and systems of the body, including the nervous system. Research has shown that exposure to high concentration of chloroform vapor may produce central nervous system effect such as staggered gait, slurred speech, and at very high concentrations may result in rapid unconsciousness and death due to respiratory failure (Chilcott et al., 2007). 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 practicals. This study therefore intends to investigate the effect of chloroform inhalation on the hippocampus of male Wistar rats.

The hippocampus is associated with the formation of memory. As such, this study will also consider the effect of exposure to chloroform on the formation and retention of memory.

The hippocampus is a bilateral structure, located beneath the neocortex, on the basal medial surface of the temporal lobes. It extends from the amygdala to the septum along the temporal lobes (Mai et al., 2003; Huang, 2011). The axis from the amygdala to the septum, along the temporal lobe defines the septotemporal axis of the hippocampus (Kuhn et al., 2015). It receives its main afferents from the entorhinal cortex and sends efferents to other areas of the limbic and extra-limbic systems like the fornix and temporal neocortex. The hippocampus and entorhinal cortex represent an important memory center of the brain (Kilbum et al., 1989).

The major toxic effects caused by acute chloroform exposure via inhalation are dizziness, irritated grainy feeling, sneezing and coughing. Other effects seen from exposure to high levels of chloroform in humans are shortness of breath, loss of balance or coordination and Death (McKenzie et al., 2012; WHO, 1989). 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 (Gomall et al., 1949; Sayorwan et al., 2012).

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 for one week before exposure. The rats were feed with rat chow and were provided with water throughout the duration of the experiment ad libitum. Rats were handled according to global best practices. Analytic grade chloroform manufactured by May and Baker Ltd 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 2 min : 409.2, 3 min : 455.9, 4 min : 495.6, 5 min : 495.6.

After acclimatization, all the rats were exposed to Morris water maze test to test for memory. This was done on day 8 following acclimatization. Group A rats (control) were not exposed to any chemical. Group B rats were exposed to 50ml of chloroform 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 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 22, the animals were subjected to Morris water maze test, as was done before the exposure, after which the rats were sacrificed by cervical dislocation and their brains harvested. Five of the brains were introduced into phosphate buffer solutions and centrifuged at 10,000rpm to separate the supernatants from the residues. The supernatant was used to test for antioxidant parameters; malondialdehyde (MDA), super oxide dismutase (SOD), Catalase (CAT) and reduced glutathione (GSH).

The other two brains from each group were collected and fixed in Bouins fluid for histological 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. 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

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*

Results were presented as Mean ± Standard deviation of 7 animals.
* Indicated statistical significance at 95% confidence level ($P \leq 0.05$).

Animals all showed weight increase between the time of commencement of exposure and the time prior to sacrifice. Differences were statistically significant at 95% confidence level ($P \leq 0.05$).

Table 2: Percentage changes in rat weights

Group	Initial weight	Final weight	Percentage weight gain
A	162.86	227.14	16.70%
B	177.14	212.86	9.00%

On further investigation into the percentage increase in rat weight, we observed that the percentage increase in weight in the experimental group B was less (9%) when compared to the control group with 16.7 % 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	0.26 ± 0.06**	0.62 ± 0.07**	1.27 ± 0.23**	6.32 ± 0.11*

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. Super oxide 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 Morris water maze test

Group	Initial	Final
A	3.87 ± 0.42	0.37 ± 037
B	3.32 ± 2.75	9.65 ± 4.17*

Our results reveal that it look lesser time for animals in group A (control) to locate the stage at the final test than it was at the onset although the difference was not statistically significant. However, Table 4 reveals that it took animals in the experimental group B more than tripple the time to locate the stage at the end of the period of exposure than it took them before exposure.

Result of Histological Studies

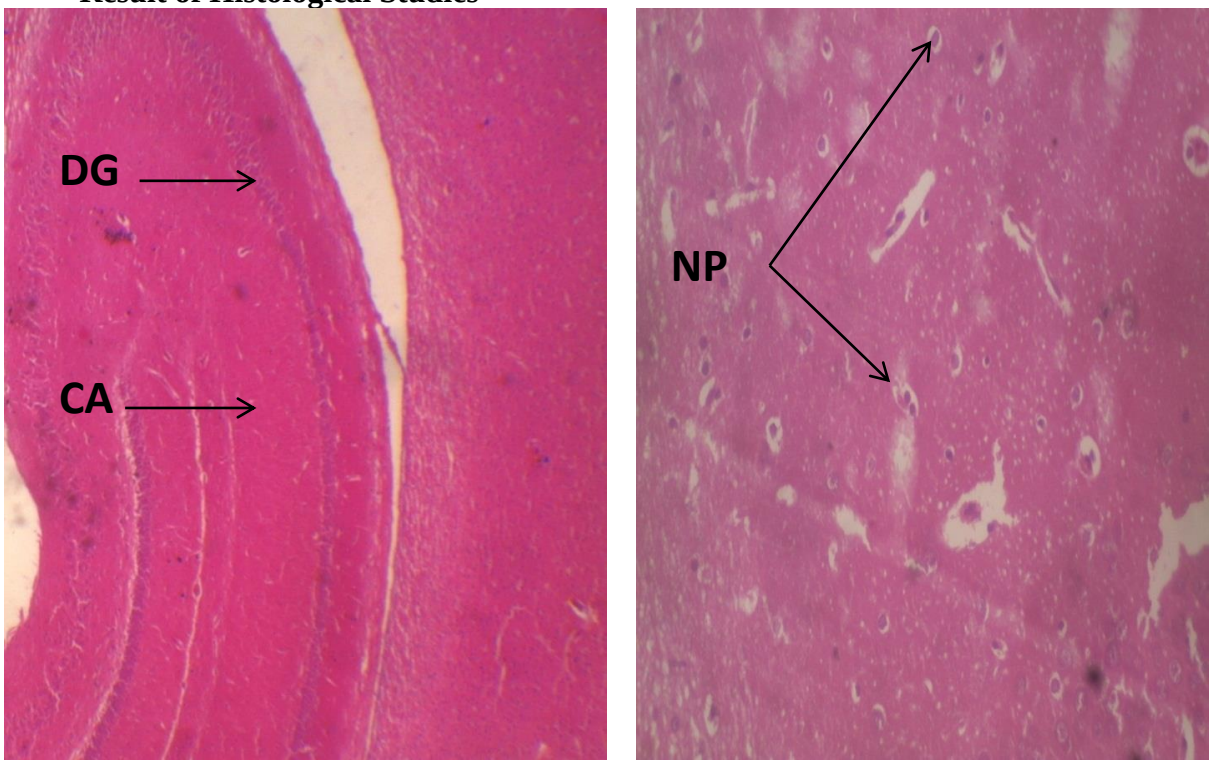

Plate 1: Representative photomicrograph of rat hippocampus in group A (control) showing the dentate gyrus (DG) and the cornu ammonus (CA) at lower magnification. At higher magnification we see normal pyramidal cells (NPCs) with clear nuclear outline.

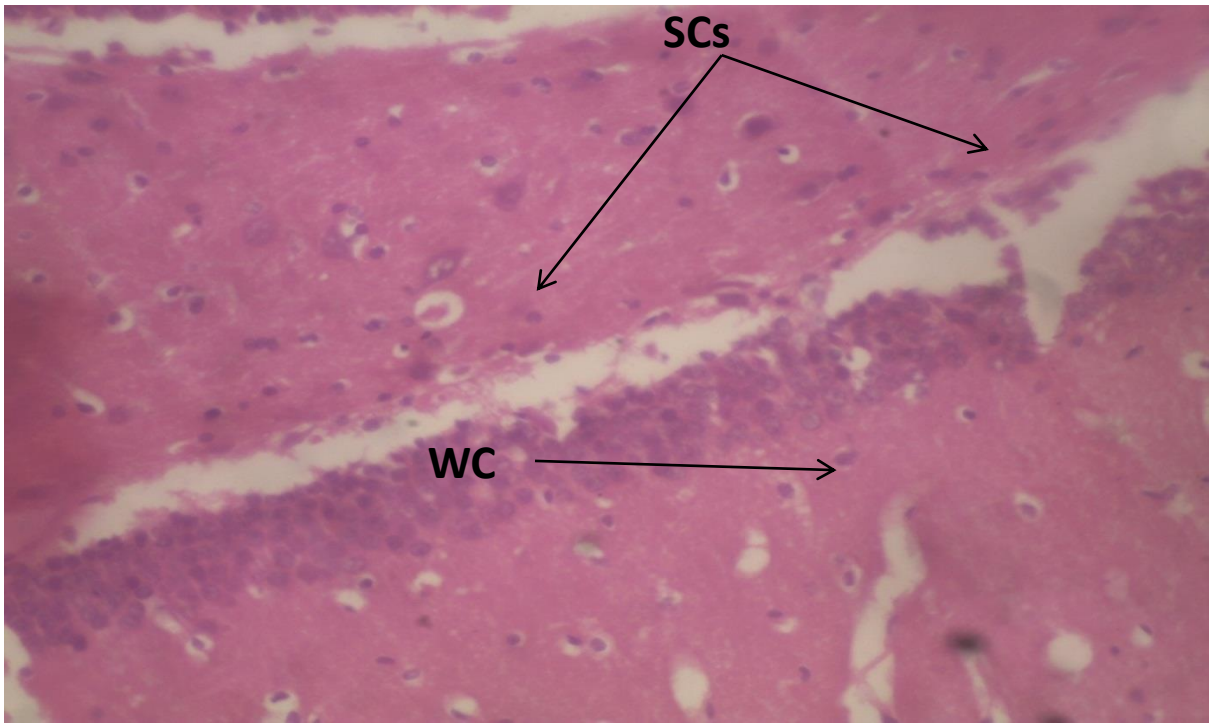

Plate 2: Representative photomicrograph of rat hippocampus in group B showing shrunken cells (SCs) and wide cellular gaps (WCGs)

These results show that chloroform exposure for 5 minutes each day for 14 days caused significant weight decrease at the end of the period of exposure when compared to the initial weight. The percentage increase in weight was lower (9%) compared to that of the control group (16.70%). This makes the impression that with time, constant exposure to chemical will affect rat weight which is line with Aguwa et al, (2018). However, physical observation reveals that despite the reduction in weight, the rat in group B all looked healthy and unaffected compared to those in the control group. The result of our antioxidant studies reveal that the rats exposed to chloroform 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) and in confirmation to the work of Aguwa et al. (2018) 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 (Tongnit et al., 2004). So we may imply from the findings of this study that persons regularly exposed to chloroform in their line of duty or during may constantly be under oxidative stress. This no doubt may lead to debilitating health consequence in the near future when the body's immune system has been overpowered. The hippocampus belongs to the limbic system and is involved in emotion and memory. Any impairment on the hippocampus will most likely negatively affect memory, ability to remember as well as emotional stability of the affected animal. The Morris water maze test is a neurobehavioral study that tests for memory among other things. Our results as presented in Table 4 reveals that there were significant reductions in the values of the Morris water maze test at the final reading when compared to the initial readings in groups B animals that were exposed to chloroform 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 Morris Water Maze test between the experimental group and the control. In the final readings however, we see highly significant reduction in escaping time for the group B rats. This may be a sign of gradual but steady loss of cells involved in building and storing memory. This observation is further supported by the histological slides of the hippocampus shown in Plate 1. There we see for the control group a normal histological framework of the hippocampus of rats (a X100) with the three distinct layers : showing the dentate gyrus (DG) and the cornu ammonus (CA) at lower magnification. At higher magnification we see normal pyramidal cells (NPCs) with clear nuclear outline. On examination, the hippocampus of rats in group B showed shrunken cells (SCs) and wide cellular gaps (WCGs). This together with the results of the neurobehavioral and antioxidant studies gives strong indication of hippocampus impairment which may lead to motor dysfunction in the near future.

Conclusion

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

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