Environmental Health
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Original Article

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Activity of acetylcholinesterase (AChE) in male albino rats exposed to metal welding fumes in an experimental setting

Ali Sani[1,2*] , Ibrahim Lawal Abdullahi[1] , Sani Ibrahim[1]

[1]Department of Biological Sciences, Bayero University Kano, P.M.B. 3011, Kano, Nigeria
[2]Department of Instrument Science and Engineering, School of Electronic, Information and Electrical Engineering, Shanghai Jiao Tong University, 200240, Shanghai, China

Abstract

Background: There are millions of workers in the world, who engage in activities associated with welding operations but are not classified as full-time metal workers. The present study aimed to determine the activity of acetylcholinesterase (AChE) in blood of laboratory animals exposed to welding fumes.

Methods: Welding fumes were obtained from Kofar Ruwa, Kano by a skilled welder. 130 albino rats were purchased from the Animal Section of Department of Biological Sciences and were divided into 12 groups. They were given doses equivalent to the workers' real life exposure regimes, and 1 group was selected as control group. They were administered intratracheally following anesthetization once weekly for twelve weeks. The rats were euthanized and serum samples were collected. Then, AChE activity was evaluated spectrophotometrically using ELISA kit (Sunlong Biotech Company).

Results: The mean values of AChE ranged from 23.1 to 25.05 ng/mL with the control having a value of 24.7 ng/mL. Thus, there was a decrease in the values of AChE in the blood of treated groups, which was significantly different from the control ($P < 0.05$).

Conclusion: Metal welding fumes negatively affected the AChE by reducing its mean values. This implies that welding fumes possesses neurotoxic effects, which can lead to some neurodegenerative diseases.

Keywords: Acetylcholinesterase, Exposure, Metal welding fumes, Toxicity

Citation: Sani A, Abdullahi IL, Sani I. Activity of acetylcholinesterase (ache) in male albino rats exposed to metal welding fumes in an experimental setting. Environmental Health Engineering and Management Journal 2021; 8(1): 33–38. doi: 10.34172/EHEM.2021.05.

*Correspondence to:
Ali Sani,
Email: asani.bio@buk.edu.ng

Introduction

All around the world, there are many workers who are not regarded as full-time welders though they engage in activities related to welding. Around 800 000 workers are regarded as full-time welders globally, and approximately 410 040 workers are regarded as welders, cutters, solderers, and brazers in the United States alone (1). The process of welding involves the vaporization of the metals and oxides of an electrode or wire that is consumed during the process to release fumes/dusts. Particulates are generated from excessive condensation of the vapours which are comprised of different metal oxides that are related to the electrode composition (2). The process by which high-temperature metal vapours are transformed into primary particles is called nucleation (3). Metal works are found all over the states landscape and regarded among the major economic activities in the area. Many individuals are involved in the occupation without knowing the hazards and risks associated with it. In addition, there is less or no regulation and control to checkmate the proper conduction of the activities in order to safeguard the health of the people and environment at large. So far, the toxicity and associated implications that result from metal fumes and particles exposure are largely unattended. There is also no information about the severity length of the problem in the urban population of Kano, Nigeria, while it can broaden the scope of environmental health issues in urban population of Kano, Nigeria. Therefore, the present study aimed to determine the activity of acetylcholinesterase (AChE) in blood of laboratory animals exposed to welding fumes.

Materials and Methods
Collection of welding fumes

Open front cubicle that resembles a chamber was used to collect the produced fumes. The volume of the chamber is 1 m³. A skilful metal worker performed the manual metal welding (shielded manual metal arc welding) process using a stainless steel hard surfacing electrode (Hyundai Welding electrode low hydrogen E 7018 3.2 mm) and the

fumes were dusted on a 0.2-µm nuclepore filters. A large amount of fumes were collected just before the start of the study. Furthermore, the obtained fumes were suspended subsequently in distilled water and sonicated for 1 minutes (4).

Experimental design

In this study, 130 male Albino rats (*Rattus norvegicus*) with a weight between 210 to 250 g were collected from the Animal Section, Department of Biological Sciences, Bayero University, Kano, and divided into 13 experimental groups (n=10 per group) (5). The animals were kept at an animal house in Aminu Kano Teaching Hospital located in Kano, Nigeria. The experimental design used for this study was randomized block design. The animals were acclimatized for 14 days prior to treatment.
Housing and feeding conditions for experimental animals

The animal house had restricted access and was free of pathogens and other extraneous factors. The animals were put in cages and a mark was made on their tails for identification.

The room temperature and relative humidity were about 22°C (± 3°C) and 30%, respectively. Also, the light-dark cycle of 12 hours was used in this study. The rats were fed with a conventional laboratory diet and water *ad libitum*. Ethical clearance for the study was obtained from College of Health Sciences Research Ethics Committee (CHS-REC), Bayero University, Kano, Nigeria (6).

Preparation of the test substance

The dose administered in this study followed a real-time workers exposure schedule, which was adopted from the study of Sani and Abdullahi (5). It was presented as follows:

A simulation was used to evaluate the lung burden of metal workers with various working regimes (hours/day) (7,8). The factors that were considered for the simulation include:

Fume concentration (5 mg m^{-3}, threshold limit value for welding fumes), human minute ventilation volume (20 000 mL min^{-1} × 10^{-6}m^{3}mL^{-1}), daily duration of exposure (no. of h/day × 60 min h^{-1}), and efficiency of deposition (15%) (9,10).

With regard to the above-mentioned elements, the daily burden of metal workers for several hours per day was obtained by the following calculations:
1.   Metal worker daily burden (2 h/day) = fume concentration (5 mg/m^3) × human minute ventilation volume (20000 mL/min × 10^{-6} m^3/mL) × exposure duration (2 h/day × 60 min/hr) × deposition efficiency (15%) = 1.8 mg
The surface area of alveolar epithelium (rat = 0.4 m^2; human = 102 m^2) was used as dose metric (11). Rat daily burden of exposure was obtained to be 0.0070 mg. Then, similar exposure in rats for 3, 5, 10, and 20 years were obtained to be 7.66, 12.77, 25.55, and 51.10 mg, respectively,

considering 365 days as a year. The concentrations were administered over 12 weeks.
2.   Metal worker daily burden (4 h/day) = fume concentration (5 mg/m^3) × human minute ventilation volume (20000 mL/min × 10-6 m^3/mL) × exposure duration (4 h/day × 60 min/h) × deposition efficiency (15%) = 3.6 mg
The surface area of alveolar epithelium (rat = 0.4 m^2; human = 102 m^2) was used as a dose metric (11). The daily burden for rat exposure was obtained to be 0.0141 mg. Then, such exposure regimes in rats for3, 5, 10, and 20 years were obtained to be 15.44, 25.73, 51.46, and 102.93 mg, respectively, considering 365 days as a year. The concentrations were administered over 12 weeks.
3.   Metal worker daily burden (8 h/day) = fume concentration (5 mg/m^3) × human minute ventilation volume (20000 mL/min × 10-6 m^3/mL) × exposure duration (8 h/day × 60 min/h) × deposition efficiency (15%) = 7.2 mg

The surface area of alveolar epithelium (rat = 0.4 m^2; human = 102 m^2) was used as dose metric (11). The daily burden for rat exposure was obtained to be 0.0282 mg. Then, such exposure regimes in the rats for 3, 5, 10, and 20 years were obtained to be 30.88, 51.46, 102.93, and 205.86 mg, respectively, considering 365 days as a year. The concentrations were administered over 12 weeks.

Table 1 shows the working concentrations of metal welding fumes administered to the albino rats over a period of 12 weeks. Each concentration was administered per animal per week (5).

The samples of the metal fumes were prepared in sterile saline, and then, were sonicated for 1 minutes to have equal distribution of the fumes throughout the solution. The samples were prepared weekly prior to administration. Rats were anaesthetized with ketamine (0.1 mL/100g b.w I.P), and following the loss of consciousness, the animals were intratracheally instilled with the respective dose per animal once a week for 12 weeks. Control groups received 200 µL sterile saline via intratracheal route after anaesthesia (12).

Collection of blood samples

The experimental animals were euthanized a week after the last 12th week of administration. Blood samples were collected from the jugular vein into a plain bottle and taken to the laboratory. However, the blood samples were centrifuged to obtain the serum for the analysis (12,13).

Table 1. Working values for test substances administered to the animal groups used in the present study

Groups		
Group IA: 0.64 mg	Group IIA: 1.29 mg	Group IIIA: 2.57 mg
Group IB: 1.06 mg	Group IIB: 2.14 mg	Group IIIB: 4.27 mg
Group IC: 2.13 mg	Group IIC: 4.29 mg	Group IIIC: 8.56 mg
Group ID: 4.26 mg	Group IID: 8.58 mg	Group IIID: 17.16 mg

Determination of acetylcholinesterase activity

The activity of AChE was determined spectrophotometrically using ELISA kit (Sunlong Biotech Company).

Statistical analysis

Relevant statistical tools were employed to analyze data or results obtained from the study. Means of various parameters were analyzed statistically to check for statistical differences by ANOVA using SigmaStat version 3.5. Confidence interval was taken at 95% and statistical significant level was considered at $P < 0.05$.

Results

The mean values ranged from 23.1 to 25.05 ng/mL with the control having a value of 24.7 ng/mL. The values of AChE decreased across the groups IA, IB, IC, and ID, which was statistically significant ($P < 0.05$) (Table 2). Similarly, the values of AChE decreased across the groups IIA, IIB, IIC, and IID, which was statistically significant ($P < 0.05$) (Table 2). All the values are less than the control value of 24.7 ng/mL. In addition, the values of AChE decreased across the groups IIIA, IIIB, IIIC, and IIID, which was statistically significant ($P < 0.05$). All the values are less than the control value of 24.7 ng/mL as presented in Table 2.

Discussion

AChE is an important enzyme in the central nervous system that helps promote the transmission and catalysis of acetylcholine, which is a neurotransmitter in cholinergic synapses. It is related to the regulation of outgrowth and survival of neurons and intracellular calcium levels (14). Therefore, AChE inhibitors are crucial in the management of neurodegenerative disorders such as Alzheimer's disease (15). The activity of AChE is mostly evaluated in the prediction of neurotoxicity induction as is the case with high manganese (Mn) exposure. Acetylcholine is transported to the nerve ends via axonal transport and released into the synaptic space (16).

The suppression of AChE leads to the accumulation of AChE in the synapses of nerve muscles and creates abnormal results, most importantly, higher activity of muscle tissues (17).

Table 2. Mean values of AChE (ng/mL) in blood of experimental rats exposed to metal fumes

Test animal groups	I	II	III
A	25.05	24.03	23.63
B	24.58	23.91	23.40
C	24.45	23.57	23.10
D	23.63	23.075	21.90
Control	24.25	24.25	24.25
P value	<0.05	<0.05	<0.05

One of the metals present in the welding fumes is Mn. It acts as an antioxidative enzyme cofactor of Mn superoxide dismutase or AChE that functions in the production and transfer of neurotransmitters (18,19).

In addition, many studies have reported a modification in AChE activity resulting from Mn substitution and increased accumulation of AChE in the brain. Thus, it causes a reduction in the control of muscle and nerve. In addition, neurotoxin effects which seems to be under the effect of pollutants are related to changes in normal behaviours (20). The accumulation of metals taken that enter the body through different routes at various levels in organs and tissues was reported. Those metals that possess some physiologic functions are stored and could be thrown out after joining in some metabolic ways in the living body. Toxic stored metals could damage enzyme structure (21).

Lead (Pb) and zinc (Zn) were revealed to inhibit AChE activity in the brain tissue of zebrafish (*Danio rerio*) (22). As reported in the present study, the levels of AChE decreased by exposure to metal fumes. The inhibition under the effect of Pb could be caused by binding the metal to the functional groups of proteins like imidazole, sulfhydryl, and carboxyl (23). There was emergence of catalytic activity loss in functional groups (24). It showed a significant inhibition of AChE in the brain and muscle tissues following sublethal administration of cadmium (Cd) to zebrafish (25). The same result was obtained on *Mugil cephalus* and zebrafish after exposure to Cd, Iron (Fe), copper (Cu), Pb, and Zn (26), which is consistent with the results of the present study. Mercury (Hg), Cd, Pb, and arsenic (As) are among the toxic heavy metals whose accumulation could lead to disorders of central nervous system, low energy generation, and damage to the composition of most important organs (27). Excessive exposure to pollutants could lead to slow and progressive physical and mental problems such as neurodegenerative process of Alzheimer's disease and Parkinson's disease (28).

Such heavy metals and organophosphates are renowned inhibitors of AChE which can alter its efficiency (22,29). This has been supported by the findings of the present study where exposure to the welding metal fumes inhibits AChE by reducing its levels as seen in Table 2.

There is an interaction between heavy metals including Hg/Pb and the causes of neurodegenerative diseases. Such metals could accumulate in the brain which by crossing blood-brain barrier can induce oxidative stress and may lead to neurodegenerative diseases such as Alzheimer's disease and Parkinson's disease (27). Hence, cholinergic system is responsible for manifestations of behaviour in animals (30). It has been reported that Pb has caused impairments of AChE system, and subsequently, cognitive problems in animals exposed to Pb. The inhibition of AChE is one of the early biomarkers of exposure to environmental toxins in humans and is associated with

adverse health effects on the nervous system after exposure to metals and insecticides such as organophosphate (31). The basic concept of monitoring and applying inhibition of AChE and its subsequent monitoring is applied and used as a biological marker for assessing and real-time detection of exposure to hazardous compounds in the occupational setting and environmental medicine (32). Exposure to Pb obviously reduces the red blood cells (RBCs) and activity of AChE in plasma and has a negative association with blood Pb concentration. The reduction in RBC and plasma AChE, as reported by the present study, shows damages to the cholinergic activity, which leads to neurotoxicity in the Pb-exposed workers (33). Many studies have reported that AChE activity is decreased following the exposure to free radicals and inhibitory effect of Pb on AChE (33,34). The mechanism is that Pb shows stronger affinity for free-SH groups in enzymes and proteins whose binding could affect their function, resulting in cholinergic receptors desensitization and effectiveness in the action of transmission (35). According to a study by Reddy et al, the change in the activity of Ach and AChE can progress after withdrawal of Pb exposure (36). Probably as a result of inhibition of AChE activity by Pb in synaptosomal cortex, hippocampus, and cerebellum regions of the brain. However, their susceptibility to Pb exposure depends on the maturation and formation of differences (37). Exposure to Pb at physiologically relevant concentrations causes relative inhibition of AChE activity in the midbrain, and subsequently, defects of neurobehaviour and disruption of AChE at specific region and acetylcholine receptors (38).

Hg, Cd, and Pb have been observed to disrupt the activity of AChE in the central nervous system of the red swamp cray fish after exposure to sub-lethal concentrations of the metals affected (39). Therefore, it can be suggested that oxidative damage in the zebrafish brain has resulted in a significant reduction in AChE activity but has not affected the pattern of gene expression. High concentrations of Hg can directly inhibit AChE activity in vitro experiments whereas lower doses of this metal cannot alter the enzyme activity (22). After a short-term exposure to Cd, AChE activity in the brain of adult rats has been inhibited with additional induced oxidative stress (39).

Metals, carbamates, and organophosphates have revealed inhibition of AChE. However, some studies have recently suggested that some metals can activate AChE during acute exposure (40). Hypothetically, it has been demonstrated that metals could interact with AChE receptors during acute exposure by influencing their binding capacity, which ultimately, leads to increased AChE (40). Similarly, initial increase after exposure was observed in groups exposed to lower doses of metal welding fumes in the present study. It has been reported that metals, carbamates, and organophosphates inhibit AChE (41,42).

Though there are reports that described inhibition of AChE by metals under acute exposure, some suggest otherwise. Zatta et al have revealed an elevation of AChE in rats administered Al orally (43). Some studies have explained the effect of stimulation by Pb in rats and oligochaetes (42,44). Also, Gallegos et al have described that Hg (0.4 mg/L) leads to a significant increase in the activity of esterase in *Callianas sathyrrena* (45). The increased activity of AChE might also be associated with an up-regulation of AChE gene, due to an initial inhibitory effect of metals (40). Gallegos et al have reported an increase in the activity of AChE in rat's brain that were exposed to 10 mg/kg Pb, after 30 minutes, though there was a sharp reduction in this activity after 24 and 72 exposure hours (45). Hence, the initial increase in the activity of AChE after exposure to metals might be a feedback to acute toxicity. However, a reduction would be expected after excessive exposure periods. Perhaps after chronic exposure, metals would be able to inhibit AChE, as proposed and observed in the present study.

Conclusion
According to the results, the metal welding fumes negatively affected the AChE by reducing its values in blood of treated groups, which was significantly different from the control group ($P < 0.05$). This implies that welding fumes have neurotoxic effects which can lead to some neurodegenerative diseases.

Ethical issues
The authors certify that the data collected during the study are as stated in the manuscript, and no data from the study has been or will be published separately elsewhere.

Competing interests
The authors declare that they have no conflict of interests.

Authors' contributions
All authors contributed and involved actively in the problem analysis, experiments design and execution, data collection and analysis, manuscript preparation and approval.

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