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Research Article

Brain Oxidative Status in Mice after Smoke Exposure

Kurawa M.I[1], Magaji R.A[2], Magaji M.G[3], Yusuf T[2]., Ya'u M[4], Daneji U.A[4]

[1]*Department of Human Physiology, College of Health Sciences, Bayero University, Kano, Nigeria*
[2]*Department of Human Physiology, Ahmadu Bello University, Zaria, Nigeria*
[3]*Department of Pharmacology and Therapeutics, Ahmadu Bello University, Zaria, Nigeria*
[4]*Department of Biochemistry, College of Health Sciences, Bayero University, Kano, Nigeria*

ABSTRACT

Although endogenous Carbon monoxide (CO) is beneficial, exogenous CO exposure may cause increased oxidative stress in other organs. Here, we investigated the effect of sub-chronic exposure of mice to CO from three common sources (cigarette, mosquito coil, and generator) on the brain. 32 mice, weighing 20 – 25g were recruited into one of the four groups. Each group was exposed to either of the 3 smokes for 15 minutes, daily, over 14 days. Digital CO meter was used to measure the amount of CO in the gas chamber (75 cm x 50 cm x 50 cm). Malondialdehyde, glutathione levels and superoxide dismutase activity were measured in the brain. Exposure to generator fumes produces the highest CO (1000 ppm), followed by cigarette smoke (356 ppm) and then MC smoke (304 ppm). Mice that were exposed to the generator fumes had a relatively higher level of MDA (0.04 ɲMol/mL), however, it is not significantly different from that of other groups. There was significant oxidative stress in the cigarette group due to the high SOD activity (2.36 µ/mgprotein) and also in the MC group due to the low GSH level (132.34 mg GSH/ gprotein). The significant oxidative stress observed in the cigarette and MC groups couldn't have been due to CO alone, some specific constituents of cigarette and MC could have aggravated the problem. Brief, daily exposure to CO from the 3 sources for 14 days was associated with significant oxidative stress that could affect the normal functions of the brain.

Keywords: *carbon monoxide, cigarette smoke, generator exhaust fumes, mosquito coil smoke, oxidative stress*

*Author for correspondence: *E-mail*: *mikurawa2012@gmail.com*; Tel. +234 8034280587

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INTRODUCTION

Carbon monoxide (CO) is among the most common and widely distributed air pollutants in the world. It is a colorless, odorless, tasteless and non-irritating gas that is poorly soluble in water (H2O) and slightly lower in density than air (Austin et al., 2002). It can be produced endogenously in the body, or exogenously from incomplete combustion of any carbon containing material. Common sources in Nigeria includes but are not limited to motor vehicles exhaust, gasoline-powered generators, kerosene stoves, wood burning, mosquito coil, and cigarette smoke (Ayodele *et al*., 2007).

Automobile exhaust is the main source of CO outdoors, while bio-fuel is the main source indoors (Prockop and Chichkova, 2007). Typically, exhaust fumes from any combustion engine contain unburned hydrocarbons, nitrogen oxides, carbon dioxide, water, and minute quantities of CO depending on the efficiency of the combustion system. Mosquito coils are slowly burning, coiled substance containing one or more insecticides that is used as mosquito repellent (Garba, 2007). The most common active ingredients of mosquito coils are various pyrethrins (0.3 – 0.4% of the coils mass), organic fillers, binders, and synergist (Peshin *et al*., 2009). Burning any mosquito coil however produces CO. Mosquito coil is used by many people, on daily basis, throughout the night, and often in poorly ventilated rooms to repel mosquitoes. Cigarette, on the other hand contains dried leaves of tobacco plant, Nicotiana tabacum. The chief ingredient in tobacco is the alkaloid, nicotine which is a central nervous system (CNS) stimulant.

Exposure to indoor pollution alone was suggested to be responsible for nearly 2 million deaths in developing countries and contributed about 4 % of the global burden of disease (Gall et al., 2013). Higher indoor and outdoor CO levels beyond the WHO recommended limits were recorded in some of the major cities of Nigeria (B, 2013). There is still paucity

of reported data on the incidence of acute or that of chronic CO poisoning in Nigeria. The few suspected cases of acute CO poisoning brought to hospitals were grossly underestimated (Augustine, 2012).

Most of the effects of CO toxicity were considered to be due to induction of hypoxia; however, a lot of other effects identified could not be explained by the hypoxia alone. Therefore, oxidative stress and other mechanisms were also considered as yet other mechanism of toxicity of the gas. Oxidative stress occurs all over the body at a rate based on the metabolic activity of the organs (Halliwell and Poulsen, 2006). The relatively high metabolic rate of the brain and reduced capacity to regenerate makes it more susceptible to insult and less likelihood for repair; thus, making every insult on the brain tissue a permanent neurologic deficit that may linger on for life. Among the body organs also, brain is particularly susceptible to free radical attack because it has less antioxidants and generates more oxidative by-products per gram tissue than any other organ (Valko et al., 2005). Although brain contributes only about 2% of the body's weight, it utilizes up to 20% of the oxygen consumed by the body (Beltrán FA, 2012). There is a very high content of iron in some areas of the brain which favors production of more reactive oxygen species (ROS) (Andersson et al., 2002). The brain is also rich in lipids that can act as a potential target for lipid peroxidation (Halliwell et al., 1992).

With this level of vulnerability, the brain should have an efficient antioxidant system in order to avoid oxidative damage. However, the brain contains only low to moderate activities of superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) when compared to either liver or kidney (Dringen, 2000). This research aimed at determining the level of MDA, glutathione and SOD activity in mice's brain tissue after brief CO exposure from 3 common sources.

MATERIALS AND METHODS

Animals and groupings: Thirty-two, male mice weighing between 20 - 25 g were used for the study. Animals were maintained under natural day and light atmospheric conditions. The temperature ranges between 24 – 32^{O}C throughout the period of the study. They were fed with standard laboratory animal feed and tap water *ad libitum.* They were also handled in accordance with the Ahmadu Bello University Animal Use and Care Guidelines. The animals were randomly categorized into 4 exposure groups, each having 8 mice. Group 1, 2, and 3 were exposed to CO from mosquito coil smoke (MCS) (Wavetide, Xiaoshan Yunshi, China), cigarette (Aspen) smoke (CS) and exhaust fumes of gasoline-powered generator (TIGER, TG950, 220v/240v) respectively; while group 4 served as the control. Cigarette and mosquito coil were burnt inside the partially ventilated gas chamber (75 cm x 50 cm x 50 cm) for the duration of the exposure, while the exhaust fume was delivered into the gas chamber through a delivery pipe. The control groups were however maintained in the laboratory away from the place of exposure, which was done in the morning hours.

Procedure: Digital CO meter (PCMM05, Pyle) was used to measure the amount of CO inside the gas chamber. After 14 days of exposure (15 minutes, daily), the animals were humanely sacrificed, head was decapitated, and the brain tissue harvested were weighed and put in an ice-cold 100 mM phosphate buffer solution (pH 7.4; 1 g of tissue/ 9 mL). It was later minced, ground in a cold glass mortar and the homogenate (10% w/v) was then centrifuged at 3,000 rpm for 15 minutes. The supernatant was transferred into plain sample bottles and stored at a temperature between 4^{0}C – 80C before analysis of oxidative stress markers.

Malondialdehyde (MDA) and activities of superoxide dismutase (SOD) and glutathione (GSH) were used to assess the level of oxidative stress in the brain homogenate. Analysis of MDA was based on its reaction with thiobarbituric acid (TBA), forming an MDA-TBA2 adduct that absorbs strongly at 532 nm (Ohkawa et al., 1979). The SOD assay kit (WST-1 method) with catalog No: BC0020, 96T from Elabscience Company was used. The kit adopts the xanthine oxidase (hydroxylamine method) to measure SOD activity. For GSH, reagent with catalog No: BC0051 purchased from Elabscience Company was used for the analysis. The reaction was based on the development of a relatively stable yellow colour when Ellman's reagent was added to sulfhydryl compounds. The chromophoric product was then read at 412 nm in a spectrophotometer (Srivastava and Beutler, 1970).

Our data did not pass the usual parametric assumptions; therefore, non-parametric equivalents were used to analyze the data followed by an appropriate post-hoc test. Values of $p \leq 0.05$ were considered statistically significant, and Microsoft Excel version 2013 and Statistical Package for Social Scientist (SPSS) version 22.0 software were used for the analyses.

RESULTS

Carbon monoxide exposure: Highest dose of up to 1000 parts per million (ppm) of CO was produced by the generator exhaust fumes, followed by cigarette smoke (356 ppm) and then mosquito coil (304 ppm) (Figure 1). The pair wise comparison indicates significant differences between all the groups, except between mosquito coil and cigarette groups ($p=0.705$). The dose of CO produced from all the 3 sources were by far higher than the WHO recommendations.

GSH: Highest GSH level was found in the control (251.44 mg GSH/ gprotein) than any other group (MC=132.34, C=145.58, and Gen. =149.98 mg GSH/ gprotein) (Figure 2). The Difference was however found to be significant ($p=0.036$) only between the animals exposed to mosquito coil smoke and control group. Lowest level of GSH found in the mosquito coil group may indicates oxidative stress, because it is normally consumed during neutralization of reactive oxygen species (ROS) produced.

SOD: Higher SOD activity was found in the animals exposed to cigarette smoke (2.36 µ/mg protein), followed by the generator (2.31 µ/mgprotein), mosquito coil (2.28 µ/mgprotein), and then control (1.62 µ/mgprotein) group [Figure 3]. The difference between the cigarette exposed group and the control was found to be statistically significant ($p=0.022$). Higher SOD activity observed in the cigarette group may indicates oxidative stress.

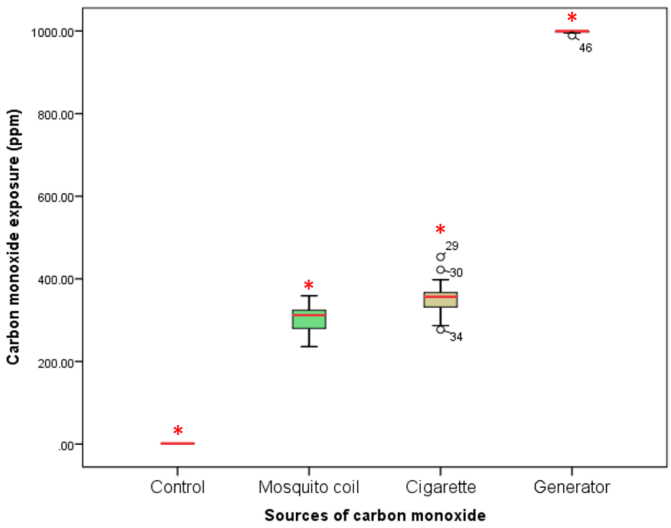

Figure 1
Amount of carbon monoxide produced in relation to the source during the exposure. Kruskal-Wallis Test indicates significant (X^2=49.65, p=0.000) differences between all the groups [control and MC (p=0.048), control and cigarette (p=0.000), control and generator (p=0.000), MC and generator (p=0.000), and between cigarette and generator (p=0.048)] after Bonferroni adjustment, except between MC and cigarette groups (p=0.705); n=8, $P \leq 0.05$. * indicates statistical significance, and its absence indicates insignificance

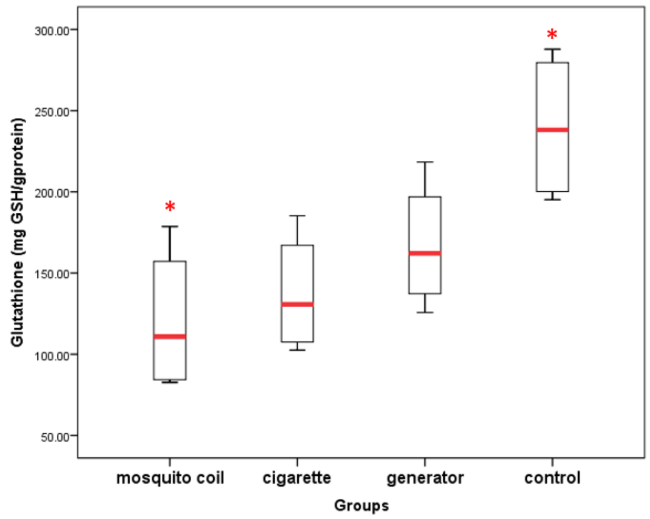

Figure 2:
Effects of carbon monoxide exposure on the levels of glutathione in the brain. Kruskal-Wallis Test indicates significant difference (X^2=8.54, p=0.036) observed between mosquito coil and control group after Bonferroni adjustment. * indicates statistical significance, and its absence indicates insignificance, n = 8, $P \leq 0.05$.

MDA: Higher MDA was found in the generator (0.04 ɲMol/mL) group, followed by the control (0.03 ɲMol/mL), and then mosquito coil and cigarette groups (Figure 4). Malondialdehyde is a bye product of lipid peroxidation; therefore, high level indicates high oxidative stress. Although not significantly different (p=0.343), high MDA level in the generator group may indicate minimal oxidative stress

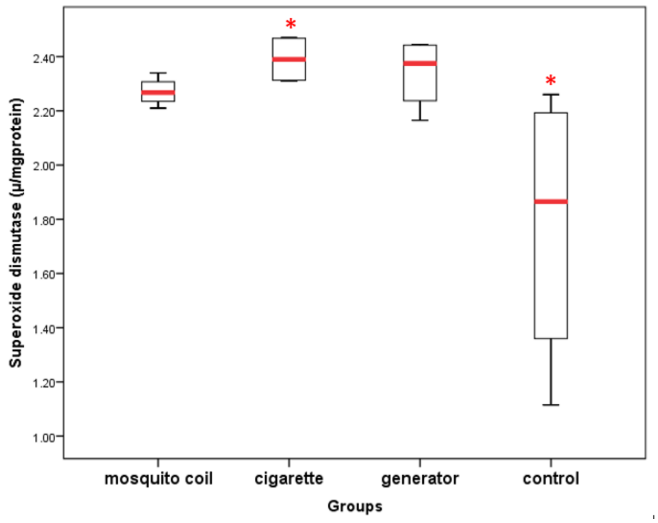

Figure 3:
Effects of carbon monoxide exposure on the activity of superoxide dismutase in the brain. Kruskal-Wallis Test indicates significant difference (X^2=9.04, p=0.029) observed between the cigarette and control group after Bonferroni adjustment (p=0.022). * indicates statistical significance, and its absence indicates insignificance, n = 8, $P \leq 0.05$.

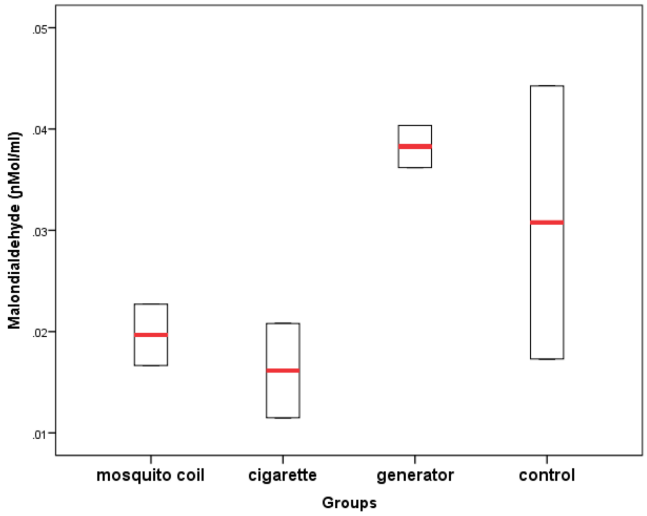

Figure 4:
Effects of carbon monoxide exposure on the levels of Malondialdehyde in the brain. Kruskal-Wallis Test indicates no significant difference ((X^2=3.33, p=0.343) among all the groups. * indicates statistical significance, and its absence indicates insignificance, n = 8, $P \leq 0.05$.

DISCUSSION

Carbon monoxide that is produced either from within or outside the body may lead to oxidative stress through the production of reactive oxygen species (ROS). The amount of ROS produced depends on the local concentration of CO, location of heme proteins, and also specific oxidation-reduction (redox) reactions (Piantadosi, 2008). Glutathione (GSH) is an important antioxidant, especially in the brain. Because of the "thiol" group, GSH is capable of neutralizing the damaging effects of most ROS by reducing the disulfide

bonds formed within cytoplasmic proteins to cysteines and serving as an electron donor. In the process, glutathione is converted to its oxidized form, glutathione disulfide (GSSG). Hydroxyl free radicals are usually produced in the brain after hypoxia, these free radicals can either be diminished or accelerated after CO poisoning (Piantadosi et al., 1995). The free radicals produced in the body were believed to be the key to brain aging and neurodegeneration (Poon et al., 2004).

Highest GSH activity found in the control group was expected because of minimal amount of ROS and also oxidative stress in the control group. However, because of higher ROS as a result of severe oxidative stress in the mosquito coil group, the level of GSH was low. The severity of oxidative stress was also higher in the mosquito coil group followed by the cigarette and generator group respectively. High level of oxidative stress observed in the mosquito coil group couldn't have been solely due to CO; likely, some constituents of mosquito coil might have aggravated the condition. Least level of oxidative stress was observed in the generator group, although they were exposed to the highest dose of CO. This is likely because concentration of CO alone may not be responsible for all the oxidative stress observed.

Pyrethroids in the mosquito coil was found to induce oxidative stress and alter the oxidation status in different organ systems of rats (El-Demerdash, 2011). Although they exhibit low level of toxicity and are rapidly metabolized, they act mainly on the nervous system (Soderlund et al., 2002). Analysis of room air sampled after mosquito coil usage showed a maximum concentration of allethrin (0.0120 ppm) within 30–45 min of use (Ramesh and Vijayalakshmi, 2001). Pyrethroid compounds were found to cause oxidative stress in the male reproductive tract of rats (Issam et al., 2011). Increased oxidative stress markers were observed in the brain and liver of rats exposed to cypermethrin (El-Demerdash, 2011). Increased lipid peroxidation was specifically observed in the brain, liver, and kidney of rats exposed to mosquito repellant smoke (Gupta et al., 1999). There was significant increase in thiobarbituric acid reactive substances and reduction in the activities of SOD, catalase, and glutathione-S-transferase in the liver after prallethrin exposure (Mossa et al., 2013). There was significant increase and decrease in the level of MDA and GSH respectively after exposure to mosquito coil (Thirumurugan et al., 2015).

Superoxide dismutase is an enzyme that alternately catalyzes the dismutation/ separation of the superoxide (O_2^-) radical into either ordinary molecular oxygen (O_2) or hydrogen peroxide (H_2O_2). Thus, acting as an important antioxidant defense especially in the brain. High level of SOD observed here indicates high level of oxidative stress in the cigarette smoke-exposed group. This level of oxidative stress was also thought to be due to some of the constituents of cigarette that could have aggravate the condition. Very high levels of superoxide and other ROS were thought to be derived from cigarette combustion (Mazzone et al., 2010). Maternal cigarette smoke exposure was associated with mitochondrial dysfunction, neuronal cell damage, and increased markers of hypoxia, inflammation, and oxidative stress in both dams and offspring brains of rat (Chan et al., 2016). An increase in the levels of superoxide, TBARS and protein carbonyl were observed in the hippocampus of rats exposed to cigarette smoke (Tuon et al., 2010). Rats exposed to cigarette smoke showed significant increase in the MDA levels with concomitant decrease in SOD, catalase (CAT), glutathione peroxidase (GPx), glutathione reductase (GR), and glutathione-S-transferase (GST) (Ramesh et al., 2015). Chronic exposure to as low as 25 ppm of CO was found to cause a significant increase in both SOD-1 and SOD-2 in the cerebellar cortex of the CO poisoned pups (Lopez et al., 2009). Total oxidant status (TOS) and carboxy-hemoglobin (COHb) levels were also found to be significantly increased in CO-poisoned patients, however TOS, oxidative stress index (OSI) and COHb levels were reduced immediately after treatment (Kavakli et al., 2011).

Malondialdehyde is a product of lipid peroxidation of polyunsaturated fatty acids found in abundance in the plasma membranes. The degree of lipid peroxidation can be estimated by the amount of MDA produced in the tissue. Reactive oxygen species can easily degrade polyunsaturated lipids, forming MDA. The MDA produced is equally reactive, and causes toxic stress in cells by the formation of covalent protein adducts. When MDA reacts with deoxyadenosine and deoxyguanosine in DNA, it forms DNA adducts. It was conclusively agreed that the unifying factor in determining toxicity and carcinogenicity for most metals such as iron (Fe), is the generation of ROS and nitrogen species (Valko et al., 2005). In our own case here, both Fe and NO are strongly associated with CO; this may lead to vicious cycle of CO toxicity in the brain.

Although highest MDA level was recorded in the generator group, the differences between the 4 groups were not significant (Figure 4). Brief exposure to CO (15 minutes) might not be enough to produce high amount of CO in the body to cause any significant oxidative stress. Our assumption can be confirmed from the Coburn-Forster-Kane (CFK) estimates where the %COHb observed in the generator group that was exposed to ≈ 1000 ppm was just 18% instead of the expected 61% that was predicted by Coburn, (1965) in his CFK model (Coburn et al., 1964). Because mice have a high respiratory rate (80-230 breaths/ min.), excess CO in the blood circulation can easily be washed out of the body before it reaches the brain in toxic levels to cause any significant oxidative stress in the neurons. We can also assume that the anti-oxidant system in the brain effectively try to neutralize the little oxidation caused by the exposure. Products of lipid peroxidation were found to increase by 75% over the baseline values 90 minutes after CO exposure at a concentration sufficient to cause unconsciousness (Thom, 1990).

Nitric oxide production and perivascular nitration in the brain after CO poisoning were key events contributing to brain oxidative stress in any CO poisoning (Fan et al., 2016). In another study, leukocytes were also found to be the key players responsible for the development of biochemical changes in the brain following CO poisoning (Otterbein et al., 2000). Derangement in the biochemical parameters was observed after sub-acute inhalation of mosquito repellent mat vapor for 3 hours daily; either for 15 or 30 days (Karthikeyan et al., 2006). Carbon monoxide-mediated delayed neuron damage was also associated with elevation of lipid peroxidation and reduction of anti-oxidative status; a process

that could explain the impaired memory due to neuronal toxicity (Wang et al., 2009).

There was significant oxidative stress associated with exposure to either cigarette or mosquito coil smoke. Presence of very high levels of superoxide and ROS in the cigarette, and also pyrethroids in the mosquito coil were thought to aggravate the oxidative stress rather than CO; because, highest levels of CO exposure was found in the generator group but had only minimal level of oxidative stress.

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