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Oxidative Stress after Acute Exposure of Mice to Exhaust Fumes

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ABSTRACT

This study assesses oxidative damage as a result of acute exposure of mice to carbon monoxide (CO) from exhaust fumes of gasoline powered generator (TIGER, TG950, Suzhou Tiger Power Machine Co., Ltd., China). Thirty six mice were divided into 3 exposure groups and each group subdivided into either control group, which was exposed to room air, or an experimental group that was exposed directly to the fumes for 30 minutes, 1 hour and 2 hour periods in a partially enclosed gas chamber before the neurobehavioral tests. Elevated plus maze (EPM) was used to assess learning and memory. Biomarkers of oxidative stress, specifically malondialdehyde (MDA), superoxide dismutase (SOD), and glutathione peroxidase (GPx) were estimated in the serum using standard kits from Northwest Life Science Specialties Ltd. Carbon monoxide (CO) monitor (Amprobe, CM100) was used to record the dose of CO in parts per million (ppm). The dose of 100 – 150 ppm of CO exposure was maintained throughout the study. The result in general suggests decreased ability of the exposed mice to learn and also recall the learned behaviour. There were also significant increases in the MDA, SOD and GPx in the experimental group when compared to their controls. Our results suggest that acute exposure to CO could be responsible for the significant oxidative damage and impaired learning and memory observed in the experimental mice. Therefore oxidative stress could serve as yet another mechanism of CO toxicity aside hypoxia.

Keywords: *Carbon monoxide, Learning and memory, Neurobehaviour, Oxidative stress*

INTRODUCTION

Carbon monoxide (CO) is one of the most common and widely distributed air pollutants in the world. It is a colorless, odourless, tasteless and non-irritating gas that is poorly soluble in H_2O and has a slightly lower density than air (WHO 2000). It was used by the Greeks and Romans in the ancient times to execute criminals.

Automobile exhaust is the main source of CO outdoors, while bio-fuel is the main source indoors (Leon and Rossitza 2007). Typically, exhaust fumes contain unburned hydrocarbons, nitrogen oxides, carbon dioxide, water, and minute quantities of CO depending on the efficiency of the combustion system. However, CO is the most toxic among the contents and is studied in detail. In Nigeria, the most important sources are motor vehicles, gasoline-powered generators, kerosene stoves, wood burning and cigarette smoke (Ayodele et al. 2007). CO emanating from burning wood alone can raise the average indoor level (5 ppm) by about 1000 times (USEPA 1991). Ambient levels of CO were found to be much higher than WHO standards in Ibadan and

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Kano cities of Nigeria (Ayodele et al. 2007; Sunny et al. 2008).

Although there is high level of awareness and monitoring standards in the developed countries, the incidence of mortality and morbidity from CO exposure is similar worldwide (James et al. 2000). Despite efforts in prevention, CO intoxication remains frequent, severe, and too often overlooked (Molitor, 1997). Carbon monoxide is considered a major air pollutant both in and outdoors (Ajayi and Dosunmu 2002) resulting from increased use of bio-fuel as a major source of energy for transportation, cooking, and electricity generation. In addition, petrol which is frequently used here generates more CO (28 times) than diesel (Campbell 2009).

Neurological symptoms are the most frequent symptoms in any case of CO poisoning. Poisoning occur mostly due to acute exposure to high amount of CO, however, poisoning from chronic exposure is usually subclinical and largely undocumented (Weaver et al. 2007). Symptoms of chronic CO poisoning like chronic fatigue, affective conditions, emotional distress, memory deficits, and difficulty in walking are usually subtle and very difficult to diagnose because they mimic that of other common illnesses like flu; it may a times be overlooked as part of the daily life chores (Penney 2008). This could be one of the many reasons why CO is considered “the silent killer”.

Although CO is not among the normal respiratory gases, its physico-chemical similarities with oxygen (O_2) allow it to be transported through the airways and across alveolo-capillary membranes in a similar way. Minute quantity (0.4 - 0.7%) of CO is produced endogenously in the body from catabolism of haemoglobin (Hb) and acts as physiologic smooth muscle relaxant, neurotransmitter and cytoprotector against oxidative stress (Ryter et al. 2004; USEPA 2011). However, any additional exposure beyond the physiological level may lead to high level of COHb which will cause impaired O_2 delivery to organs especially the highly metabolic ones like the brain and the heart. Although tissue hypoxia is the main mechanism of CO toxicity, some other effects like the “delayed neurological syndrome” cannot be explained by hypoxia alone. Therefore oxidative injury induced by reactive oxygen species (ROS), free radicals, and neuronal nitric oxide is currently being considered as the possible molecular mechanism of CO poisoning; however the relevant mechanism of this injury is yet to be fully understood (Sumeyya et al. 2014).

MATERIALS AND METHODS

Thirty six adult mice weighing between 18 - 32 g were obtained from animal house of the Department of Pharmacology and Therapeutics of Ahmadu Bello University, Zaria. Animals were maintained at room temperature, fed on standard feed and allowed access to tap water *ad libitum*. They were allowed to acclimatize with the environment for at least two weeks before commencement of the study. Animals were then divided into three groups with 12 mice each for the 30 minutes, 1 hour and 2 hours of exposure. Each of the three exposure groups was further subdivided into control and experimental groups containing 6 mice each. The experimental groups were directly exposed to exhaust fumes of the generator for 30 minutes, 1 hour and 2 hour periods before the neurobehavioral test while the control groups were exposed to room air. Animals were treated and handled in accordance with the Ahmadu Bello University Research policy.

Gasoline powered generator (TIGER, TG950, 220v/240v) manufactured by Suzhou Tiger Power Machine Co., Ltd., China served as the source of CO. Cages of the experimental animals were placed into an improvised gas chamber that allowed partial ventilation to fresh air. It measured 150 x 100 x 100 cm. The exposure was similar to that adopted by Samuel and Micha (2007), in which human subjects were accidentally exposed to a gasoline-powered generator (5 kW, 3000 U/min) directly adjacent to a long stable where they were sleeping.

A CO monitor (Amprobe, CM100) was also placed inside the gas chamber in order to record the dose of CO in parts per million (ppm). It can measure CO in the range of 0 – 999 parts per million (ppm); with error resolution of 1 ppm and accuracy of +/- 15% at 100 – 500 ppm. The meter has a screen and a backlight for operation in the dark. The dose of 100 – 150 ppm of CO exposure was maintained throughout the study by adjusting the positions and the direction of the exhaust fumes of the Generator depending on the wind direction.

Assessment of Learning and Memory

Elevated plus maze was used to assess long term memory (Itoh et al. 1990). On the first day (learning task) a mouse was placed at the end of one of the open arms, facing away from the central platform. Latency for the mouse to enter one of the closed arms was recorded for a maximum period of 90 seconds. Following entry into an arm, the animal was allowed to explore the apparatus for 30 seconds, and 24 hours later, the second trial (retention test) was performed. Mice appear unwilling to venture into the open arms of the maze because of a general aversion to open spaces and to height; this induced learning responses in the animal (Lister, 1990). After each trial, the maze was thoroughly cleaned with methylated spirit (95% ethanol and 5% methanol) and allowed to dry before the next trial. The time taken for a mouse to move from the starting point of the open arm to any of the closed arms was measured (in seconds) to indicate learning (first day) and memory (second day) (Itoh et al.1990).

Assessment of Biomarkers of Oxidative Stress

At the end of the neurobehavioral tests, mice were sacrificed and blood samples obtained by cardiac puncture and used for quantitative estimation of biomarkers of oxidative stress, specifically malondialdehyde (MDA), superoxide dismutase (SOD), and glutathione peroxidase (GPx). Kits were purchased from Northwest Life Science Specialties Ltd. The analysis of MDA was based on the reaction of MDA with thiobarbituric acid (TBA), forming an MDA-TBA2 adduct that absorbs strongly at 532 nm. The analysis of SOD was based on monitoring the auto-oxidation rate of haematoxylin as originally described by Martin et al. (1987), with modifications to increase robustness and reliability. While analysis of GPx was based on adaptation of the method of Paglia and Valentine (1967).

RESULTS

When transfer latencies of the control and experimental groups were compared in the three exposure periods, only the 30 minutes and 1 hour exposures showed significant change with p values of 0.023 and 0.001 respectively, at $P < 0.05$. Although latency of the experimental group dropped to 13.4 ± 1.5 seconds during the 30 minutes of CO exposure, that of 1 hour exposure period increased to 73 ± 8.3 seconds when compared to their controls (28.6 ± 5.2 sec.) and (21.8 ± 4.4 sec.) respectively (Figure 1).

There were significant increases in the TLs of the experimental group during the 30 minutes (48.2 ± 12.8 sec.) and 2 hours (66.8 ± 15.3 sec.) of exposure when compared to the controls (13.6 ± 1.9 sec.) and (22.6 ± 6.6 sec.), respectively (Figure 2). There were significant increases in the MDA (2.63 ± 0.06), SOD (1.83 ± 0.09) and GPx (44.40 ± 1.16) of the experimental groups when compared to their controls (2.26 ± 0.07), (1.57 ± 0.08) and (38.70 ± 1.19) respectively (Figure 3).

DISCUSSION

In this study, there was significant reduction in the mean transfer latency (TL) of the experimental group during the 30 minutes CO exposure in Day 1. It was proper to suggest that 30 minutes partial exposure to CO at concentration ranging from 100 - 150 ppm may enhance the ability of mice to learn new behaviour. Our finding is in support of the opinion of Boehning et al. (2003) who suggested that CO together with nitric oxide (NO) could act as gaseous-neurotransmitters in the central nervous system (CNS) under physiologic conditions; but as the exposure increased, poisoning may occur (Mannaioni et al. 2006).

Increased deterioration of the recall capacity was found to be directly proportional to the duration of CO exposure. Previous data suggested specific toxicity of CO on memory functions in animals and also delayed neuronal death in areas involved in memory process (Piantadosi et al. 1997; Nabeshima et al. 1991). Equally Katoh et al. (1990) reported a 17% decrease in pyramidal cells in the CA1 region of mice, who received pure CO compared to normal controls. Similarly, CO poisoned subjects were found to have impaired ability to remember new temporal, linguistic, and spatial information while previous knowledge for temporal, linguistic, and spatial information was intact (Hopkins et al. 1993). Though 30 minutes of partial CO exposure enhanced the learning/acquisition task, the same 30 minutes exposure produced significant deterioration in the memory/recall task after 24 hours. It can therefore be deduced that any CO exposure may impair recall in mice.

Although tissue hypoxia is the main mechanism of CO toxicity, some other effects like the "delayed neurological syndrome" cannot be explained by hypoxia alone. Therefore oxidative injury induced by reactive oxygen species (ROS), free radicals, and neuronal nitric oxide was considered to be the possible molecular mechanism of CO poisoning; however the relevant mechanism of this injury is yet to be fully understood (Sumeyya et al. 2014).

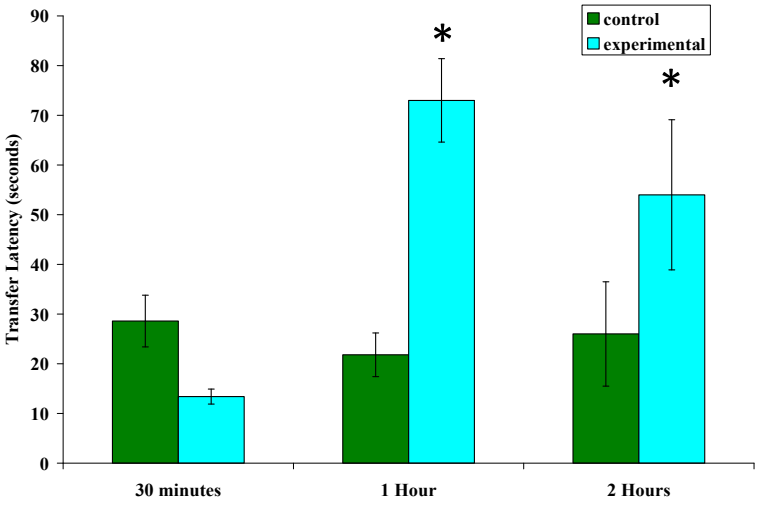

Fig. 1: Transfer latencies of the control and experimental groups during the Acquisition/ learning test (Day-1). * = significant, n= 6

Even though the brain contributes only about 2% of the body's weight, it utilizes up to 20% of the oxygen consumed by the body due to its high metabolic demands (Clarke and Sokoloff, 1999). The brain is rich in lipids that can act as a potential target for lipid peroxidation (Halliwell and Gutteridge 1990). The high iron content of some areas of the brain also favours production of more reactive oxygen species (ROS)

(Anderson and Root 2004). With this level of vulnerability, 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 (Schulz et al. 2000). This in turn makes the brain much more vulnerable to oxidative damage.

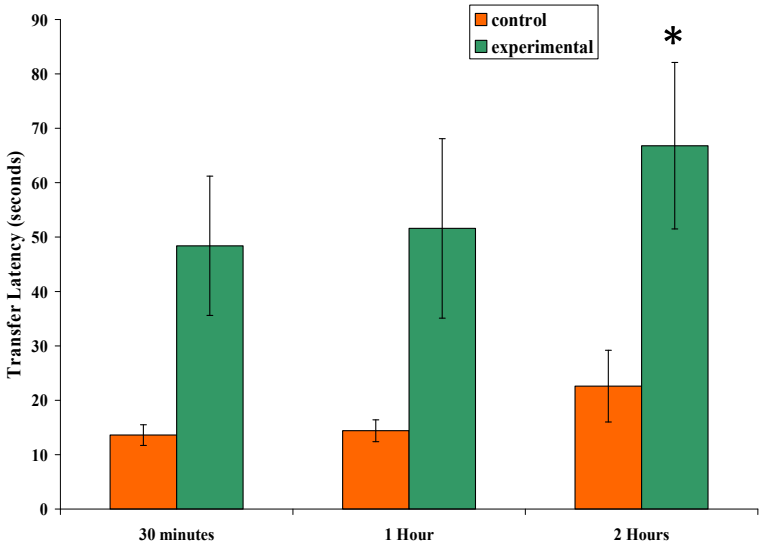

Fig. 2: Transfer latencies of the control and experimental groups during the recall/ memory Task (Day-2). * = significant, n= 6

Our results suggest that acute exposure to CO could be responsible for the significant increase in the MDA, SOD and GPx. Our findings is in line with that of Jing and Claude (1992) who also implicated oxidative stress to be the main cause of significant CO-mediated neuronal injury. Products of lipid peroxidation were found to increase by 75% over the base-line values 90 minutes after CO exposure at a concentration sufficient to cause unconsciousness (Thom 1990). In line with this, Kudo et al. (2001) suggested that the CO-induced lipid peroxidation and neuronal injury could be independent of hypoxia but dependant on the temperature. Nitric oxide-derived oxidants were found to be involved in CO-mediated oxidative stress within the vascular compartment and that plasma levels could be useful in assessing level of neurological damage (Stephen et al. 1997). There was also a 10-fold increase in nitrotyrosine in the brains of CO-poisoned rats and platelets were thought to be responsible for most of the production in the early phase of exposure (Ischiropoulos et al. 1996). Although the WHO recommended level of exposure to CO is less than 100 ppm, exposure to 50 - 100 ppm was found to increase hydrogen peroxide (H_2O_2) production in the lungs of rat (Thom et al. 1999). Chronic exposure to as low as 25 ppm of CO was found to cause significant increase in both SOD-1 and SOD-2 in the cerebeller cortex of the CO-poisoned pups (Lopez et al. 2009). Total oxidant status (TOS) and carboxyhaemoglobin (COHb) levels were found to be significantly increased in CO poisoned patients, however TOS, oxidative stress index (OSI) and COHb levels were reduced immediately after treatment. Measurements of the TOS, total antioxidant status (TAS) and OSI levels were then proposed by Havva (2011) to be useful markers of severity of CO poisoning.

There is also evidence that there is a connection between increased ROS and loss of neurons during the progression of neurodegenerative diseases like Parkinson's disease (PD), Alzheimer's disease (AD) and amyotrophic lateral sclerosis (ALS) (Anderson and Root 2004). In another study also, chronic oxidative damage was linked to age-related neurodegenerative diseases (Anderson and Root 2004). Increased levels of nitrotyrosine, a permanent marker of $ONOO^-$ attack on proteins, and of 4-HNE (the most cytotoxic product of lipid peroxidation) were demonstrated in AD, PD, ALS, and other neurodegenerative diseases (Pacher et al. 2007). If it is true that CO poisoning causes oxidative stress which is linked to neurodegenerative diseases and that protection from exposure to CO cannot be guaranteed in our daily lives, then we can speculate that CO together with other pro-oxidants could be responsible for these neurodegenerative diseases seen in the elderly.

CONCLUSION

Oxidative stress may serve as yet another mechanism of CO toxicity aside hypoxia and could be responsible for the significant impairment in the long term memory observed. Although the gas is beneficial under physiological conditions, a lot is yet to be known concerning its chronic, subclinical toxicities. The significant oxidative damage observed is in line with previous findings and could pave way for understanding the pathogenesis of neurodegenerative diseases seen mostly in the elderly. People should therefore be aware of the existence of subclinical CO toxicity and avoid frequent exposure in order to safe guard their health as long term neurological sequelae were recorded in previous studies.

Conflict of Interest

None declared.

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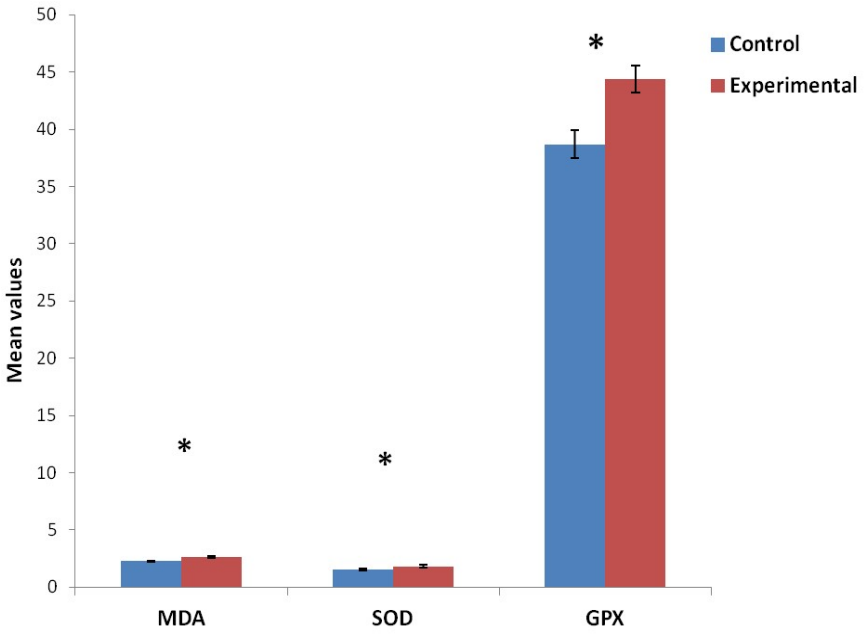

Fig. 3: Mean values of MDA, SOD, and GPx in the control and experimental groups
* = significant, n= 6

REFERENCES

Ajayi, A. and Dosunmu, O. (2002) Environment hazards of importing used vehicles into Nigeria. Proceedings of International Symposium on Environmental Pollution Control and Waste Management, Tunis (EPCOWM'2002): 521-532.

Anderson, S.A. and Root, T. W. (2004) Investigation of the effect of carbon monoxide on the oxidative carbonylation of methanol to dimethyl carbonate over Cu+ X and Cu+ ZSM-5 zeolites. Journal of Molecular Catalysis A: Chemical. 220(2): 247-255.

Ayodele, J.T., Adekiya, A.O. and Yakubu, I. (2007) Carbon monoxide as indoor pollutant in Kano Metropolis. Journal of Applied Science and Environmental Management. 11(3): 27-30.

Boehning, D., Moon, C. and Sharma, S. (2003) Carbon monoxide neurotransmission activated by CK2 phosphorylation of haeme oxygenase2. Neuron. 40: 129-137.

Campbell, M.G. (2009) Diesel exhaust fumes, an overview. Carbon Monoxide Headquarters Report. Retrieved November 13, 2015 from http://campbellmgold.com; 02 102009/1.

Clarke, D.D. and Sokoloff, L. (1999) Intermediary metabolism. Substrates of cerebral metabolism. Basic Neurochemistry: Molecular, Cellular and Medical Aspects. 6th ed. Bethesda, Maryland, USA. National Center for Biotechnology Information.

Halliwell, B. and Gutteridge, J.M.C. (1990 Role of free radicals and catalytic metal ions in human disease: An overview. Methods Enzymology. 186:1-85.

Havva, S.K. (2011) Oxidative stress increases in carbon monoxide poisoning patients. Human and Experimental Toxicology. 30(2): 160-164.

Hopkins, R.O., Weaver, L.K. and Kesner, R.P. (1993) Long term memory impairments and hippocampal magnetic resonance imaging in carbon monoxide poisoned subjects. Undersea and Hyperbaric Medicine: 20(4).

Ischiropoulos, H., Beers, M.F., Ohnishi, S.T., Fisher, D., Garner, S.E. and Thom, S.R. (1996) Nitric oxide production and perivascular tyrosine nitration in brain after carbon monoxide poisoning in the rat. Journal of Clinical Investigation, 97: 2260-2267.

Itoh, J., Nabeshima, T. and Kameyama, T. (1990) Utility of an elevated plus maze for the evaluation of memory in mice: effects of narcotropics, scopolamine and electroconvulsive shock. Psychopharmacology. 101: 27-33.

James, A. R., Monique, M. N., Neil, B. H. and Stephen, R. T. (2000) Carbon monoxide poisoning - a public health perspective. Toxicology. 145:1-4

Jing, Z. and Claude, A.P. (1992) Mitochondrial oxidative stress after carbon monoxide hypoxia in the rat brain. Journal of Clinical Investigation. 90: 1193-1199.

Kudo, R., Adachi, J., Uemura, K., Maekawa, T., Ueno, Y. and Yoshida, K. I. (2001). Lipid peroxidation in the rat brain after CO inhalation is temperature dependent. Free Radical Biology and Medicine, 31(11), 1417-1423.

Leon, D.P. and Rossitza, I.C. (2007) Carbon monoxide intoxication: An updated review. Journal of the Neurological Sciences. 262(1-2): 122-130.

Lopez, I.A. Acuna, D., Beltran-Parrazal, L., Amarnani, A., Cortes, M. and Edmond, J. (2009) Evidence for oxidative stress in the developing cerebellum of the rat after chronic mild carbon monoxide exposure (0.0025% in air). BMC Neuroscience. 10: 53.

Mannaioni, P.F., Vannacci, A. and Masini, E. (2006) Carbon monoxide: the bad and the good side of the coin, from neuronal death to anti-inflammatory activity. Inflammation Research, 55: 261-273.

Molitor, L. (1997) A 45-year-old woman with flu symptoms. Journal of Emergency Nursing. 23: 83-84.

Martin, J.P., Dailey, M. and Sugarman, E. (1987) Negative and positive assays of superoxide dismutase based on hematoxylin autoxidation. Archives of Biochemistry and Biophysics. 255:329-336.

Nabeshima, T., Katoh, A. and Ishimaru, H. (1991) Carbon monoxide-induced delayed amnesia, delayed neuronal death and change in acetylcholine concentration in mice. Journal of Pharmacology and Experimental Therapeutics, 256: 378-384.

Pacher, P., Beckman, J. S. and Liaudet, L. (2007) Nitric oxide and peroxynitrite in health and disease. Physiological Reviews. 87(1): 315-424.

Paglia, D.E. and Valentine, W.N. (1967) Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. Journal of Laboratory and Clinical Medicine. 70: 158-169.

Piantadosi, C.A., Zhang, J., Levin, E.D., Folz, R.J. and Schmechel, D.E. (1997) Apoptosis and delayed neuronal damage after carbon monoxide poisoning in rat. Experimental Neurology. 147: 103-114.

Penney, D.G. (2008) Chronic carbon monoxide poisoning: a case series. In Penney, D.G. (Ed) Carbon monoxide poisoning. Boca Raton, FL: CRC Press. 551-67.

Ryter, S.W., Morse, D. and Choi, A.M. (2004) Carbon Monoxide: To boldly go where NO has gone before. Science's Signal Transduction Knowledge Environment. 230: RE6.

Samuel, H. and Micha, M. (2007) Prospective study of accidental carbon monoxide poisoning in 38 Swiss soldiers. Swiss Medical Journal. 135: 398-406.

Schulz, J. B., Lindenau, J., Seyfried, J. and Dichgans, J. (2000) Glutathione, oxidative stress and neurodegeneration. European Journal of Biochemistry. 267(16): 4904-4911.

Stephen, R.T., Melissa, K., Donald, F. and Harry, I. (1997) Release of glutathione from erythrocytes and other markers of oxidative stress in carbon monoxide poisoning. Systemic Oxidative Stress in CO Poisoning. 1424-1432.

Sumeyya, A., Serpil, E., Nuri, I., Safa, C., Mehmet, K., Fatma, U., Senol, D. and Omer, A. (2014) The role of reactive oxygen species and oxidative stress in carbon monoxide toxicity: An in-depth analysis. Redox Report. 19: 180-189.

Sunny, O.B., Olatunde, O.M., Isaiah, O.O. and Mynapelli, K.C.S. (2008) Ambient carbon monoxide and carboxy-haemoglobin levels in Ibadan City, Nigeria: A source of health inequality between developed and developing nations? Journal of Environmental Health Research. 7:1.

Thom, S.R. (1990) Carbon monoxide-mediated brain lipid peroxidation in the rat. Journal of Applied Physiology. 68: 997-1003.

Thom, S.R., Fisher, D., Xu, Y.A., Garner, S. and Ischiropoulos, H. (1999) Role of nitric oxide-derived oxidants in vascular injury from carbon monoxide in the rat. American Journal of Physiology. 276: 984-992.

WHO (2000) Chapter 5.5 Carbon monoxide; United States Air Quality Guidelines (AQG). World Health Organization (WHO) Regional Office for Europe, Copenhagen, Denmark, Second Edition.

United States Environmental Protection Agency (USEPA) (2011) Automobiles Emissions: An Overview. In Air and Radiation; Fact Sheet OMS- 5; 400-F-92-007.

United States Environmental Protection Agency, USEPA (1991) Carbon monoxide. Washington, DC. Air Quality Criteria (AQC), Office of Research and Development. Publication no. EPA-600/B-90/045F.

Weaver, L.K., Valentine, K.J. and Hopkins, R.O. (2007) Carbon monoxide poisoning: risk factors for cognitive sequelae and the role of hyperbaric oxygen. American Journal of Respiration and Critical Care Medicine. 176: 491-497.