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Research Article

Effects of Mosquito Coil Smoke Inhalation on Spatial Memory in Mice

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Summary: Mosquito coil (MC) is widely used to repel mosquitoes in order to prevent malaria in many malaria-endemic countries. Although we are fully aware and concerned about carbon monoxide (CO) and its toxicity, exposure to CO from common, though occult sources like MC smoke is often overlooked. Equally, the adverse health effects, especially to the brain, are usually underestimated. This work was aimed at assessing the effects of exposure to CO from MC smoke inhalation on spatial memory in mice. Sixteen, adult, male, mice, were randomly assigned to either the experimental or the control group; each having 8 mice. The experimental group was exposed to the MC smoke (Wavetide, China) that was allowed to burn inside the gas chamber (75 cm x 50 cm x 50 cm) for 15 minutes, daily, for 14 days. Digital CO meter (*PCMM05 Pyle*) was used to measure the amount of CO and Barnes maze protocol to assess the spatial memory. Our results indicate that burning MC for 15 minutes produced up to 312 *parts per million* (*ppm*) of CO and raised the blood carboxy-hemoglobin (*COHb)* level by 15.8%. This is higher than the *WHO* recommended limit (<100 mg/m^3 or 87 *ppm* for 15 min.) of CO exposure and the %*COHb* level of <2%. Mosquito coil smoke was also associated with impaired spatial memory. However, the dose and duration of exposure did not significantly affect weight gain in the mice. Although widely used to prevent malaria, MC could serve as a potential source of CO and other neurotoxins that could be harmful to the brain; the use and toxicity of which is mostly overlooked even by the public health professionals.

Keywords*:* mosquito coil; carbon monoxide; carboxyhemoglobin; neurotoxicity; learning and memory

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INTRODUCTION

Although there was a general reduction in the global burden of malaria as shown by the 21% decrease in the incidence and mortality among the populations at risk; there were disproportionately higher malarial cases (90%) and increase in about 92% of malarial death (Organization, 2016). Nigeria alone contributes the highest burden of global malaria morbidity (25% of global malaria cases) and deaths (30% of global malaria deaths) (Arowolo, 2016). The WHO agenda to end malaria in Nigeria identified vector control as one of the key facts to tackle malaria. Among the many control strategies used by the people of Asa local government area of Kwara state in Nigeria, the use of mosquito coil (MC) was the second most popular (15.4%) (Salihu & Sanni, 2013).

Mosquito coil is a coiled substance having one or more insecticides that slowly burns to emit smoke that can drive mosquitoes away (S. H. Garba, Shehu, & Adelaiye, 2007). The most common active ingredients of MC are various pyrethrins (0.3 – 0.4% of the coils mass), organic fillers such as sawdust, binders such as starch gel, and synergist such as piperonyl butoxide (PBO). They are effective against many genera of mosquitoes including Aedes, Anopheles, and Mansonia (Krieger, Dinoff, & Zhang, 2003). Sub-micrometer particles and gaseous irritants such as the aldehydes, sulfates and polycyclic aromatic hydrocarbons released when MC is burning evaporate together with the insecticide (Weili Liu et al., 2003).

Mosquito coils are mostly used by people in the night when they retire to their bedrooms to sleep. Some people burn it in an enclosed room, and later extinguish it and open the windows before they sleep; While some will keep it burning throughout the night when they are asleep. In either case, people (pregnant women, children and the elderly) get exposed to the MC smoke for quite a number of hours in the night. Some rooms may be well or poorly ventilated, especially during the cold season when people use to close most of their windows and doors for warmth. The effectiveness of MC is usually more when used indoors or areas with limited ventilation, however, it is always advised to maintain good ventilation to avoid toxicity.

Generally, CO is produced when burning any carbon-containing substance, depending on the availability of oxygen. Carbon monoxide is considered

to be a highly toxic gas to virtually every organ in the body by either decreasing the oxygen supply to the tissue (hypoxia) (Stewart, 1975), producing reactive oxygen species (ROS) (Hennekens et al., 1996) or via many other unproven mechanisms (Zhang & Piantadosi, 1992). The highly active organs like the brain and heart are mostly affected due to their high energy demand and low energy reserve (Henry et al., 2006). The brain is extremely sensitive to decrease in oxygen supply, as a result, it suffers energy failure only a few minutes after an interruption in oxygen supply (Erecińska & Silver, 2001). This may lead to reversible or permanent tissue damage (Choi, 1983). Acute exposure to mild or moderate amount of CO may result to mild central nervous system (CNS) symptomatology like headache, dizziness, weakness, nausea, confusion, disorientation, and visual disturbances that may often be ignored or considered as part of the daily life chores (Myers, Snyder, & Emhoff, 1985; Raub, Mathieu-Nolf, Hampson, & Thom, 2000). Chronic exposure, however, may cause subclinical symptoms like chronic fatigue, affective conditions, emotional distress, memory deficits, and difficulty in walking (Weaver, 2009). This could be one of the many reasons why CO is considered "the silent killer".

Although a lot of studies were conducted on the toxicities of MC smoke, however, they concentrated more on the effects of major constituents like pyrethrins and its toxicity to organs other than the brain (S. Garba, Adelaiye, & Mshelia, 2007; WK Liu & Wong, 1987). Carbon monoxide is seldom mentioned in the gaseous products of MC. Most CO poisonings are due to acute exposure from fire accidents or exhaust fumes inhalation; however, chronic exposure was fully documented (Townsend & Maynard, 2002; Wilks, Tomashefski, & Clark JR, 1959). While acute poisoning is so glaring and can easily be diagnosed, however, chronic mild exposure is mostly overlooked and may lead to sub-clinical symptoms which can be very difficult to attribute to CO poisoning alone. A substantial amount of CO can be produced by MC especially when used in the poorly ventilated environment.

The aim of the study was therefore to mimic the chronic, mild exposure to CO from MC in humans. Since MC smoke toxicity to other organs is well known, we choose to evaluate its effects on the brain. In the setup, we created a partially ventilated gas chamber similar to poorly ventilated bed rooms used by our people for exposing the animals. Instead of the average of 8 hours of night sleep for humans, we choose a minimal period of exposure that people can get exposed to MC smoke per day, which is about 15 minutes. Our assumption is that if this brief, daily exposure to CO from MC smoke for just 14 days can affect spatial memory in the exposed mice, then an average of 8 hours of daily exposure throughout our lives can have tremendous consequences on our brain health.

MATERIALS AND METHODS

Sixteen, adult, male mice were recruited for the study. They were housed in the animal room for about a week prior to the start of the study in order to acclimatize with the environment. Animals were maintained under natural day and night atmospheric conditions of the savannah region (Kano state, Nigeria). The temperature ranges from 27 - 30^0C throughout the period of the study. They were fed with laboratory animal feed and water *ad libitum*. They were also handled in accordance with the *Ahmadu Bello University* animal use and care guideline. The animals were categorized into either the experimental or control groups (8 mice each) using the simple randomization method. The experimental group was exposed to *MC* smoke (*Wavetide*, *Xiaoshan Yunshi*, China) that was made to burn inside the partially ventilated gas chamber (75 cm x 50 cm x 50 cm) for 15 minutes, daily, for 14 days. The control group was also placed in the gas chamber for 15 minutes, daily, for 14 days, however, they were not exposed to the MC smoke. The exposure was in the mornings (8-9 am). Digital *CO* meter (*PCMM05*, *Pyle*) was used to measure the amount of *CO* produced when the *MC* was burning inside the gas chamber. The peak daily dose of *CO* attained within the period of exposure were recorded. Environmental temperature and that inside the gas chamber were also recorded during each exposure sessions.

Measurement of body weight: on the first and last days of the study, the weights of all the animals were measured and recorded in order to assess the weight gain.

Screening for motor coordination deficits: There is a general requirement for motor strength and coordination before a reliable assessment of cognitive behavior can be made; therefore, all the animals were screened for motor coordination deficits before recruitment into the study (Stanley et al., 2005). The balance beam test (Beam walk), which was used here, is a useful measure of motor coordination and balance deficits that can be used to show gross or subtle motor coordination and balance deficits. It consists of 100 cm long 12 mm and 6 mm flat beams resting on two poles which are 50 cm above the surface of a table. A black escape box is attached to one end of the beam at the finish point. Mouse was forced to move away from the aversive stimulus (60 watt light bulb) at the starting point of the beam towards the escape box; during which the latency and hind-feet slips were recorded as a measure of motor coordination and balance (Carter,

Morton, & Dunnett, 2001; Southwell, Ko, & Patterson, 2009).

Assessment of learning and memory: The Barnes maze was made up of a circular platform (122 cm in diameter) with 40 equally spaced holes (5 cm diameter; 3.5 cm between holes; 2 cm from the edge) along the perimeter and was elevated 90 cm above the floor. There was a small dark recessed "goal box" (28 x 14 x 18 cm) located under one of the holes where mice can escape the aversive stimulus (bright light) and hide. Charts were pasted on the walls to act as visual cues and were kept permanently in their positions throughout the days of the study. A video camera was positioned about 150 cm above the platform to record the activities (Barnes, 1979).

The protocol involved an adaptation and 4 days of training (acquisition/ learning) that was followed by a probe/ retention test on the 5th day of the study. During the acquisition task, the number of primary errors (total number of head deflections into incorrect holes before reaching the target hole), total errors, primary latency (time taken to locate the target hole for the first time), and path length (total length of the path to locate the target hole) were measured by the experimenter. On the 5th day, however, number of pokes/ errors (total number of head deflections into incorrect holes), latency, and path length to reach the virtual target hole were measured.

The search strategies used by mice can be grouped in to 3 different categories. Direct (Spatial) search strategy involves moving directly to the target hole or to an adjacent hole before visiting the target. In a mixed search strategy, the target hole searches were separated by crossing through the center of the maze or an unorganized form of search. In the serial search method, the first visit to the target hole was preceded by visiting at least two adjacent holes in a serial manner; either clockwise or counter-clockwise direction. The strategies were observed by the experimenters during the study and cross-checked with the video recordings at the end of every day's trials.

Assessment of *carbon monoxide* level in blood: About 2.5 *ml* of blood was collected in test tubes containing ethylenediaminetetraacetic acid (EDTA, potassium salt), 1.5 *mg/mL* of blood. Measurement of blood *carboxyhemoglobin* (*COHb)* is a principal biomarker for assessing exposure to *CO* by spectrophotometric method (Ernest & Carol, 1984).

Statistical analyses

Data obtained from the study were expressed as means ± *standard error of the mean* (*SEM*), or as medians and interquartile ranges. Depending on nature and the characteristics of the data, either parametric or non-parametric analysis was employed to analyze the results, followed by an appropriate post hoc test where necessary. For all evaluations, values of $p \leq 0.05$ were considered to imply statistical significance. Microsoft Office Excel version 2013 and statistical package for social scientist (SPSS) version 22.0 software were used in analyzing the data.

RESULTS

The mice in both groups gained weight during the period of the study as shown by the significant increases in the body weight between the initial and final body weights in the MC smoke exposed group (p=0.017) and the control (p=0.012) group (Table 1). There was no significant difference (p=0.681) between the temperature inside the gas chamber and that of the environment (Table 2).

Table I.
Effects of mosquito coil smoke on body weight

Group	*n*	Body weight (g)	Variable	p-value	Z	Percentiles		
						25th	Median	75th
Mosquito coil	8	19.7	Initial Body weight	0.017*	-2.38	15.60	18.55	24.15
		25.6	Final Body weight			21.13	25.45	30.15
Control	8	20.9	Initial Body weight	0.012*	-2.52	15.33	16.85	17.65
		27.5	Final Body weight			19.00	22.00	25.00

Wilcoxon Signed Ranks Test, n=8, p≤0.05, * indicates statistical significance, and its absence indicates insignificance.

Table 2.
Temperature variation between the gas chamber and the environment

Location	*n*	Mean ± SE	Standard Deviation	p-value
Gas chamber	14	27.8^{0}C ± 0.366	1.369	0.681
Environment	14	28.0^{0}C ± 0.363	1.359	

Independent-Samples T-Test, *n*=14, *p*≤0.05, * indicates statistical significance, and its absence indicates insignificance.

Table 3
Screening for motor coordination deficit in the animals

Groups	*n*	Variable	p-value	Z	Percentiles		
					25th	Median	75th
Control and Mosquito coil	8	Left Foot Slip (LFS)	0.52	-.64	0.00	0.00	1.00
		Right Foot Slip (RFS)	0.44	-.77	0.00	0.00	1.00
	8	Latency	0.92	-.11	6.00	6.50	8.00

Mann-Whitney U-test, n=8, p≤0.05, * indicates statistical significance, and its absence indicates insignificance.

In terms of temperature, the two groups were maintained under similar conditions throughout the study period. The mean daily *CO* exposure was 312 *ppm* for the *MC* group and 2 *ppm* for the control group (Figure I). The *COHb* was 15.4% and 1.8% for the *MC* and control groups respectively (Figure 1).

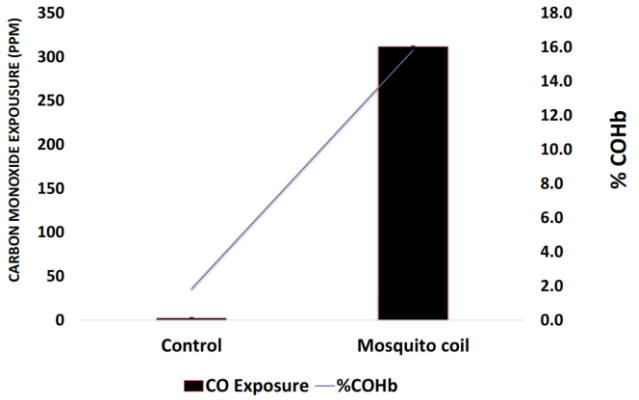

Figure 1:
Carbon monoxide exposure in relation to the percentage carboxy-hemoglobin

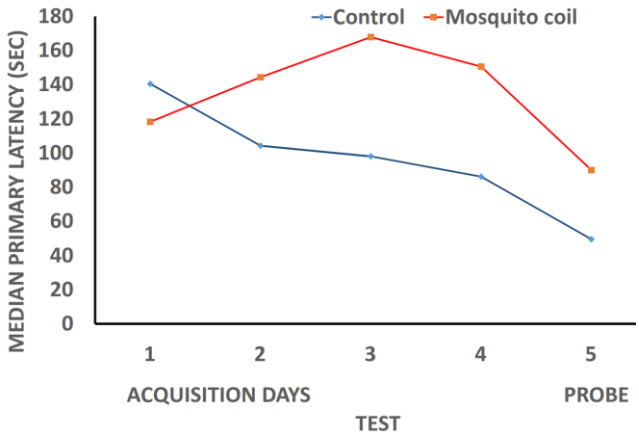

Figure 2:
Changes in the primary latency to reach the target hole**.**
Control: Friedman test indicates significant decrease in the latency [χ^2 (4) = 16.715, p = 0.002] that occurred between D1 and D5 (p=0.01) after post hoc comparison. Mosquito coil: There is a significant change in the latencies [χ^2 (4) = 14.065, p = 0.007], however, no significant difference was observed after post hoc comparison. p≤0.05, n=8.

All the mice were screened for possible motor coordination and balance deficit prior to the start of the study. There were no statistically significant differences in terms of the left foot slip (LFS) (p=0.52), right foot slip (RFS) (p=0.44), and Latencies (p=0.92) between the two groups (*MC* and control) (Table 3). They were all free from any gross motor coordination and balance deficit that might have affected their performance in the Barnes maze tasks.

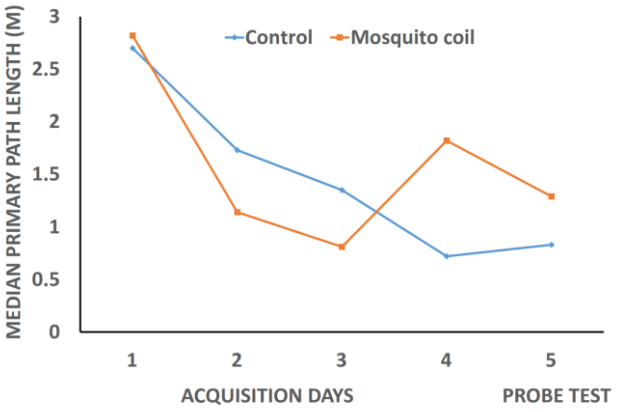

Figure 3:
Changes in the primary path length to locate the target hole.
Control: Friedman test indicates significant [X2(4) =11.190, p=0.025] decrease in the primary path length, however, no significant differences were observed between the daily primary path lengths after post hoc comparison. Mosquito coil: There was no significant [X2(4) =5.800, p=0.215] difference between the daily changes in the primary path lengths. n=8, p≤0.05.

There was significant (p=0.01) decrease in the latency from day 1 (D1) to D5 of the acquisition period in the control group; however, it increases gradually throughout the acquisition period in the MC group (Figure 2). The control animals gradually learn to locate the target hole without wasting time as the training continues (Figure 3). There was a significant (p=0.025) decrease in the primary path-lengths (PPL) over the acquisition days in the control group (Figure 3). Although there was a decrease in the PPL in the MC group, however, it was not significant (p=0.215) and associated with an increase in the PPL after D3 (Figure 3). The number of primary errors made to locate the target hole over the acquisition period decreases significantly in both the control (p=0.000) and MC groups (p=0.001) (Figure 4).

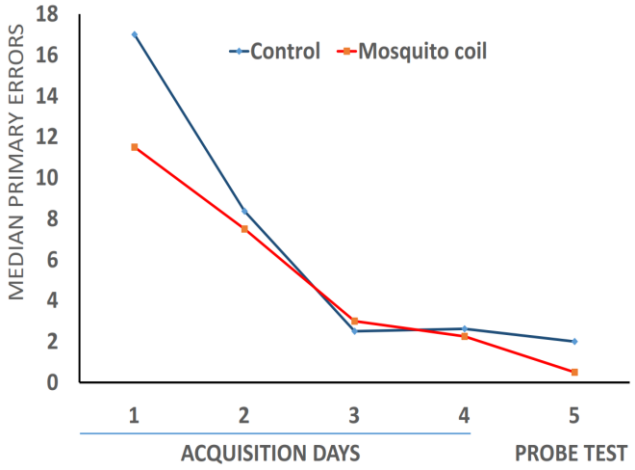

Figure 4:
Changes in the number of errors made to locate the target hole. *Control: Friedman test indicates significant decrease in the errors [X2(4) =22.248, p=0.000] between D1 and D3 (p=0.01) after post hoc comparison. Mosquito coil: Friedman Test indicates significant [X2(4) =18.854, p=0.001] decrease in the errors that occurred between D1 and D5 (p=0.000) after post hoc comparison. n=8, p≤0.05.*

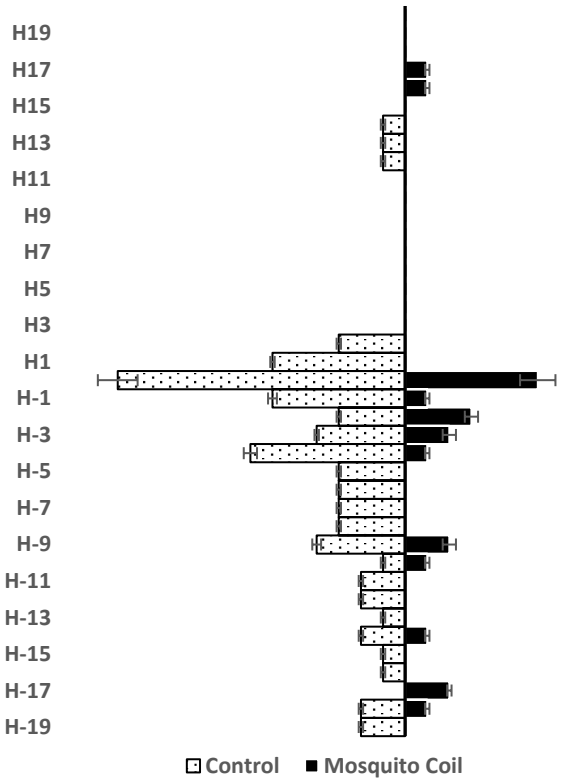

Figure 5: Higher preference for the virtual target hole in the control and mosquito coil groups on the probe test day. *Mann-Whitney U-test,* there was no significant (p=0.102) difference in the preference of the virtual target hole between the control and mosquito coil group. *n=8, p≤0.05*, * indicates statistical significance, and its absence indicates insignificance.

Although mice in both groups prefer the virtual target hole on the probe test (Figure 5), however, the choice of strategy to locate the target hole during the acquisition and probe test days was more of "mixed" in the MC group (Figure 7) as compared to the increase in the "direct" strategy exhibited by the control group (Figures 6).

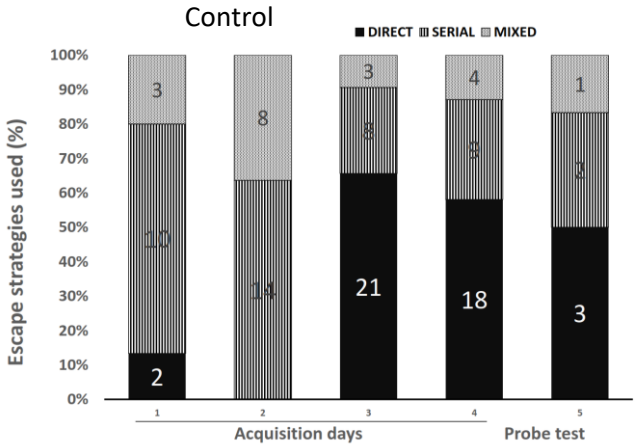

Figure 6:
The preferred escape strategy used by mice to locate the target hole in the control group.

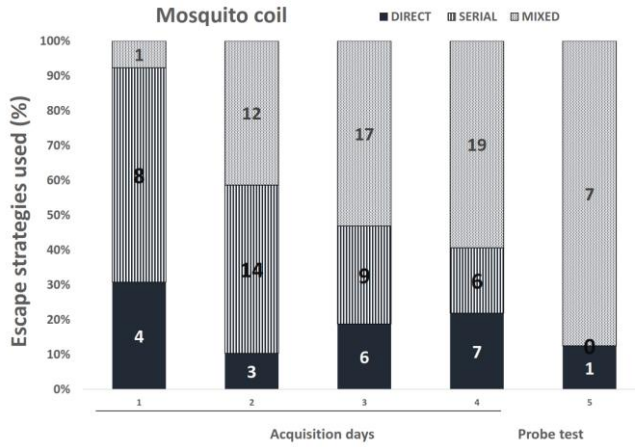

Figure 7:
The preferred escape strategy used by mice to locate the target hole in the mosquito coil group.

DISCUSSION

Body growth is a marker of health, and growth can be objectively assessed through measurement of organ and body weights. During the study period, an initial body weight of all the animals was taken to provide the baseline measure of the weights of the animals. On the final day of the study, another body weight was taken to provide an objective measure of the growth status. Under normal circumstances, and being that the studied animals had no restriction to either water or their feeds, we expect a normal weight gain, with a significant difference between the initial and final body weights. All the animals had significant weight gain over the period of the study. Although there was normal growth in our study, however, many studies of CO poisoning, especially during pregnancy were found to be associated with increased risk of fetal death, developmental disorders, and chronic cerebral

lesions (Raub et al., 2000). Salam and colleagues (2005) also found perinatal exposure to CO as a major risk factor for low birth weight and intrauterine growth retardation (Salam et al., 2005). The normal weight gain observed in our result was not unexpected and could be explained by the short duration of exposure (15 min.), smaller CO dose (312 ppm), and exposure to adult mice. To buttress our point, exposure to a higher dose of CO (up to 1000 ppm) equally lead to 25% mortality and poor growth in those animals that survived (data not shown).

In order to maintain both the control and experimental groups under similar conditions aside from the difference in the treatment; the environmental temperature of the two groups were monitored throughout the period of the study. Our results showed that the temperature inside the gas chamber during the exposure did not differ significantly with the temperature outside the gas chamber, nor was it different from that of the control groups' environment, throughout the study period. We can conclude that both groups were maintained under similar conditions of temperature throughout the period of the study, and any observed behavior couldn't have been caused by the variations in the temperature.

The CO exposure observed in the control group was within the world health organization (WHO) recommended ambient levels (<2.5 ppm); however it was beyond the WHO recommended limit in the MC smoke exposed group (<100 mg/m3 or 87.1 ppm for 15 min) (Carratu et al., 1999). The %COHb was also lower and within the non-smoker, ambient range (<2%) in the control group. However, that of the MC smoke exposed group was much higher than the WHO recommended limit; rather, it falls within the chronic smoker range (>9 ppm). From the literature, patients who were mildly exposed to CO (<1 %COHb) usually had intact memory functions equivalent to that of the control group and were even better in some areas like learning, word recall, and quality of learning by Buschke's verbal memory testing (Deschamps, Geraud, Julien, Baud, & Dally, 2003). Although the mean of the %COHb recorded in our control was up to 1.8%, it was still within the non-smoker range and does not pose much toxicity risk. Such mild exposures were even found to enhance learning by the same author (Kurawa, 2016).

The balance beam test is a useful measure of motor coordination and balance deficits. It can be used to show gross or subtle motor coordination and balance deficits. All the mice were screened for possible motor coordination deficits prior to the start of the study. They were found to be free of any gross motor coordination or balance deficit, and they were fit to undergo the neurobehavioral test. Parameters like the primary latency (PL), primary error (PE), primary path-length (PPL), number of pokes per hole on the probe trial day, and the pattern and kinds of strategies used by mice to locate either real or virtual target holes (VTH) were considered in order to assess learning and memory behavior. The expectations here were significant decreases in the time taken to reach the target hole for the first time (PL), the distance covered before it locates the target hole for the first time (PPL), and the number of errors made before locating the target hole for the first time (PE), as the animal acquire more training over the 4 days of acquisition period; with the least PL, PE, and PPL expected on the probe test day (D5), when all the animals were expected to have mastered the location of the target hole using the visual cues. The target hole, which was closed on D5, should have the highest number of pokes followed by the adjacent holes. This indicates recall of the actual location of the target hole (TH).

The pattern of the strategies exhibited by mice during the acquisition period will indicate whether the animals were learning to locate the target hole as the number of training increases over the days or not. The use of the "serial" form of search strategy initially, followed by a switch to the "direct" form of strategy to locate the target hole is the most appropriate pattern that indicates gradual and progressive learning. The preference for the "mixed" form of search strategy will, however, indicate utter confusion and impaired learning (during the acquisition period) or impaired recall (on the probe test day/ D5). The earlier the animals switch from the use of other strategies to the use of the "direct" strategy indicates faster/ enhanced learning abilities. On day 5, the "direct" strategy should be more preferred/ favored than any other strategy if at all the animals have learned and been able to recall the actual location of the target hole. It appears that neurobehavioral tests can be relied upon in the assessments of subtle neurologic dysfunctions resulting from CO poisoning (Rajiah & Mathew, 2011).

There was significant, progressive decline in the primary latency (Figure II) and also a sharp decline in the primary error (Figure IV) during the acquisition period in the control group. They were able to achieve perfection as early as D3 of the acquisition period; this indicates how fast they were able to learn the location of the maze. In D5, the virtual target hole had the highest number of pokes, followed by the adjacent targets (Figure V). The sharp switch from the use of serial and mixed strategies, to the use of direct strategy on the second day of acquisition period indicate fast learning ability of the animals in the control group (Figure VI). From the literature, patients who were mildly exposed to CO (<1 %COHb) usually had intact memory functions equivalent to that of the control group and were even better in some areas like learning, word recall, and quality of learning by Buschke's verbal memory testing. Attention was also found to be

better in the patients, in whom visual reaction time was shorter than in controls (Deschamps et al., 2003). Although the mean of the % COHb recorded in our control was up to 2% (Figure I), it was still within the non-smoker range and does not pose much toxicity risk. Such mild exposures were even found to enhance learning by the same author (Kurawa, 2016).

Contrary to what was obtained in the control group, the animals in the MC group had impaired learning ability throughout the acquisition period, as shown by the almost horizontal line graph of the primary latency (Figure II). They, however, had a significant gradual decrease in PE over the acquisition days (Figure IV). Although there was more preference to the virtual target hole on D5 (Figure V), the pattern of the choice of strategy during the acquisition period and on the probe test day clearly indicates confusion (Figure VII). In both the acquisition period and probe test, mice in this group nonspecifically tried their luck in locating the target hole, with total disregard to the use of the visual cues. The pattern of the primary path length exhibited by the MC group also clearly shows the gross impairment of memory (Figure III). Patients with CO poisoning usually exhibited impaired memory, attention, and executive functions in many other studies (Penney, 2007).

The impaired learning and memory in the MC group could probably be due to the exposure to CO (up to ≈ 312 ppm) from the MC smoke. Although CO is not the only constituent of MC smoke, and may not be the only toxin responsible for the impaired memory, however, the result is comparable to that of many cases of CO poisoning. It is also worth noting that most CO poisoning doesn't involve CO in isolation; rather, together with many other toxic gases and substances as is always the case in fire accidents. It is important to recall that the duration of exposure rather than the dose plays an important role in determining the level of %COHb in the body and ultimately the toxicity.

Carbon monoxide poisoning impaired memory and attention, and also causes severe white matter damage (WMD) that was associated with Parkinsonism-like features that abate after 13 months later (Sohn, Jeong, Kim, Im, & Kim, 2000). 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, Weaver, & Kesner, 1993). Significant atrophic changes in the fornix were associated with significant decline in verbal memory; however, visual memory, processing speed and attention, and or concentration did not decline (Kesler et al., 2001). Neuropsychological tests such as memory, new learning ability, attention and concentration, tracking skills, visuomotor skills, abstract thinking, and visuo-spatial, planning, and processing were all impaired after CO poisoning (Amitai, Zlotogorski, Golan-Katzav, Wexler, & Gross, 1998). The recall and recognition memory were significantly impaired in patients who suffered bilateral hippocampal damage and temporal-parietal atrophy after CO poisoning (Bastin et al., 2004).

In most of our communities, cigarette smoking is considered antisocial behavior because it goes against most of the cultures, therefore seriously rejected; however, the use of MC is not stigmatized. Therefore the hazard posed by MC could by far be more dangerous when compared to cigarette smoke. On average, we found that burning either cigarette (Aspen brand) or MC inside the chamber for 15 minutes produces similar concentrations of CO (cigarette ≈347ppm and MC≈312ppm). Therefore; based on the dose of CO produced, families that uses MC in the night will be ≈28 times much more affected by the toxicity of CO (assuming they were exposed for 7 hours equivalent to the average duration of a night sleep), when compared to cigarette smoking which usually last no more than 15 minutes. It is also important to realize that cigarette smokers exhale most of the smoke after inhalation; some even use filters, and mostly smoked in an open space where there is enough ventilation. In contrast, most people use MC indoors to concentrate the smoke; with very poor ventilation, especially during the cold season. Liu et al., (2003) was able to establish that exposure to a single MC was equivalent to burning 75-137 cigarettes in terms of the particulate matter (PM 2.5) and also equivalent to burning 51 cigarettes in terms of formaldehyde exposure (Weili Liu et al., 2003).

The level of community awareness on the hazards of cigarette smoke is quite high; however, that of MC and other commonly ignored indoor air pollutants is quite low even among the high socioeconomic class (Niphadkar et al., 2009). Salvi and colleagues (2016) observed that simply opening the windows and door when MC burns in the night, decreases the CO and PM2.5 levels by 50%. This clearly shows the importance of health education in the fight against MC hazards in our community (Salvi et al., 2016). Nandasena and colleagues (2010) concluded that indoor air quality and pollution may be considered a neglected public health problem in Sri Lanka just like any other developing nations (Nandasena, Wickremasinghe, & Sathiakumar, 2010). Animals that were exposed to MC smoke similar to that of humans for just 60 days, had a lower body weight, histopathological lesions in the respiratory tract, and elevated levels of liver enzyme activities (Ayorinde, Oboh, Otubanjo, Alimba, & Odeigah, 2014). Nephrotoxicity (S. H. Garba et al., 2007), other organs damage (Taiwo, Nwagbara, Suleiman, Angbashim, &

Zarma, 2008) and neurobehavioral changes (Patel, Gupta, & Oswal, 2012) were all documented in MC smoke exposure. Significantly higher frequencies of chromosome aberrations were identified in MC smoke-exposed animals by Vences-Mejía and colleagues in 2012 (Vences-Mejía et al., 2012).

The aim of this study was to mimic the usual human exposure to CO from mosquito coil and the attending neurotoxicity especially in relation to learning and memory process that is often overlooked. Our results indicate that exposure to MC smoke could impair spatial memory. Although there are many other constituents of MC, CO is considered one of the most toxic and the pathology observed here were comparable to that of many other CO poisoning studies. Exposure to CO from common but neglected sources like MC should be considered serious because it can impair learning and memory ability depending of course on the dose, duration of exposure, and other peculiar factors in the subject and the source. Therefore avoiding, or limiting exposure is a key to better health.

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