SPATIAL MEMORY AND HISTOMORPHOLOGICAL CHANGES INDUCED BY SUBCHRONIC NITROCELLULOSE INHALATION IN MICE: ROLE OF DEXAMETHASONE IN THE CEREBRUM

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ABSTRACT

Introduction: Thinners are toxic substances used as organic solvents. Adolescents (11%) use thinners as inhalants regularly, which is linked to brain damage and learning deficits. This study looked at the effects of thinner inhalation in adult male mice and dexamethasone's anti-inflammatory effects on pyramidal neurons and glia. **Aim**: To investigate dexamethasone's anti-inflammatory effects on nitrocellulose inhalation. **Methods**: Forty mice were used; group one served as a control; group two was exposed to 1200ppm nitrocellulose in a whole-body inhalation chamber for 42 days; group three was injected with 2.5mg/kg dexamethasone twice weekly; and group four received nitrocellulose inhalation daily and dexamethasone twice weekly. Neurobehavioral study for learning and memory was conducted before sacrifice. Brains were harvested and processed for histology and biochemical activities of MDA and IL-6. Analysis of data was done using Graphpad Prism 8.4.3 with level of significance at $P<0.05$. **Results**: Indices of learning and memory in the nitrocellulose group were reduced escape latency and duration spent in the quadrant but not in the control or dexamethasone group (P=0.009). The MDA and IL-6 levels were higher in the nitrocellulose group compared to control and dexamethasone groups (P=0.02; P=0.03, respectively). Density of pyramidal neurons in layer 5 and 6 was significantly lower in the nitrocellulose treated groups compared to control and dexamethasone (P<0.0001). **Conclusion:** Dexamethasone reduced neuronal and glial cell damages in the pre-frontal cortex, accompanied with spatial learning and memory improvement.

Keywords: Health risk, Thinners, Brain injury, Cognitive function, Dexamethasone

INTRODUCTION

Thinners are extremely lethal substances commonly used as organic solvents in industry and household. Nitrocellulose is a chemical used to dilute oil-based paints. It is made up of various aromatic and halogenated hydrocarbons such as dichloromethane, toluene, methanol and acetone (Agin, Hassanian-Moghaddam, Shadnia, and Rahimi, 2016). The detection of these chemicals have been reported in household and workspace environment (WHO, 2005). Its use is on the rise as more industrial workers are exposed to this solvent in painting and lacquering, wood and furniture coating, home decoration and general decorative coating (Jovanovic, Jovanovic, Spasic, and Lukic, 2004; Yilmaz, Kutlu, Canpolat, Sandal, Ayar, Mogulkoc, and Kelestimur, 2001). There is a dearth in records of occupational exposure documentation owing to factors such as sample size that is small, selection bias, unrestrained and/or undetermined co-drug exposures and several solvent exposures (Hannigan and Bowen, 2010).

Inhaling toxic substances seems to be the most popular way of getting exposed to toxic chemicals from volatile solvents, although other routes - direct and indirect oral ingestion are possibilities (Uboh and Ufot, 2013). When inhaled, thinners can also act as psychotropic substances and the euphoria achieved by young sniffers when inhaled has contributed significantly to a global health

concern (Bowen and Cruz, 2012). Long-term use of thinners as inhaled substances is not unconnected with ill-health effects over a long time, allowing damage to the brain and cognitive and behavioral alterations (Malloul, Bennis, Bonzano, Gambarotta, Perroteau, De Marchis and Ba-M'hammed, 2018). After inhaling a thinner, there is a quick diffusion of hydrocarbon ingredients into the blood, this crosses through the blood-brain barrier and may adversely affect the central vervous system (Howard, Bowen, Garland, Perron and Vaughn, 2011).

From a public point of view, nitrocellulose is commonly used in cosmetics as nail-polish preparations and has been known to be a dispersing agent – which could be a nonsurfactant and as a film former (Gottschalck and Breslawec, 2012). As part of other uses, nitrocellulose is a prior-sanctioned food ingredient used in the production of paper and paperboard products in food packaging (Fiume, Berfeld, Belsito, Hill *et al.*, 2016). Another use is the bindery for printing inks and wood coatings.

The pre-frontal cortex and the hippocampus, two main regions in the brain for processing memory and performing learning and cognitive function have been studied extensively, and reported to be adversely affected by inhalant chemical agents including thinners (Malloul, Bennis, Bonzano, Gambarotta, Perroteau, De Marchis and Ba-M'hammed, 2018). The underlying mechanisms suggested have been varied; oxidative stress is one of the pathophysiology proposed to cause the damage in neurons and glia population. Dexamethasone has been reported to be a steroid capable of reducing inflammation by imitating anti-inflammatory hormones synthesized in the body. It does this by reducing the immune system of the body.

It has been reported that dexamethasone could dampen the oxidative stress related to inflammation in the brain tissue. We therefore examined the inflammatory role of dexamethasone on subacute nitrocellulose inhalation on the pyramidal neurons in layer 5 and 6 of the cerebral cortex and pyknotic index in CA1 and CA3 of the hippocampus (Malloul, Bennis, Bonzano, Gambarotta, Perroteau, De Marchis and Ba-M'hammed, 2018; Shokunbi, Olopade, Femi-Akinlosotu and Ajiboye, 2020).

METHODS

Forty animals were purchased for this study (10 served as control and 30 served as experimental animals). Physically, the animals that inhaled nitrocellulose in an enclosed chamber showed signs of hypoxia and rushed for fresh air in the 5-minute break they have. Swiss mice (age 8 weeks old, male, body weight 20-25g) were obtained and placed in the Central Animal House, Bowen University. The mice were acclimatized for 7 days under 12-h/12-h light dark with water and mice feed *ad libitum*. The mice were properly taken of according to the regulations issued by Bowen University Teaching Hospital Research Ethics Committee. Suffering of animals was brought to the minimum by all efforts made. All measures on animal handling were permitted by the Ethical Review Board of Bowen University with approval number BUTH/REC-1020.

Animal grouping and treatments

The arrangement of animal grouping and treatment is indicated in Figure 1. Animals were acclimatized for a week and were later placed in the following groups: (1) Control mice exposed to fresh air; (2) Mice who were exposed to 1200ppm of nitrocellulose daily for 6 weeks; (3) Mice who were injected 0.01ml of 2.5mg/kg dexamethasone twice weekly; (4) Mice who were exposed to 1200ppm of nitrocellulose daily and 0.01 ml of 2.5mg/kg dexamethasone twice weekly.

Paint Thinner Inhalation

Whole-body inhalation chamber made with plexiglass walls was used for the exposure as earlier described by Bowen, Wiley and Balster, 1996. The animals were put at the bottom of the chamber, with a lid at the top and a known quantity of liquid thinner was introduced into the filter paper located at the bottom of the platform. The conversion factor used was (1µl~1.5ppm). Sub-chronic inhalation entails 1hour daily inhalation of 1200ppm of thinner over 42 days. There was exposure to the same dose of thinner for 2x15 minutes separated by 5 minutes with the animals being returned to their initial cage. After another 15 minutes exposure to thinner, the animals were introduced to neurobehavioral test – Morris Water Maze. Control animals were not placed in the whole-body inhalation chamber but were given access only to fresh air. Exposure to paint thinner via inhalation took place in the morning between 9:00 and 11:00am to evade any circadian variations in the bodily activities of the animals, likewise the behavioral tests were done early in the day (between 9:00am and 12 noon).

Neurobehavioral Test
Morris water maze tests

To study the outcome of nitrocellulose on memory functions, we embarked a behavioral study (n=7/group) using Morris water maze (MWM) task. This assessment was carried out to test hippocampal-dependent spatial learning and memory. It includes a circular pool of water (109cm in diameter, 48cm in height) containing water to depth of 17.5cm which was made opaque by adding milk. A round escape platform was located underneath the water (10cm in diameter and 20 cm in height), this was put 1cm beneath the water surface and placed at the middle of one quadrant. The mouse learns to locate the hidden escape platform using visual cues. The pool was separated into four quadrants and labeled north, south, east and west. The hidden platform was positioned in the north-west quadrant. Each mouse was placed in the pool and was expected to locate the platform. Inability to find the platform after 1 minute means the mouse will be led to it. This was carried out for 6 days with two trials each day. The length of time each mouse took to locate the platform (escape latency) was noted as a measure of its learning capability. The removal of the platform from the pool was done on the 6^{th} day and the duration in the escape platform quadrant was recorded as a test of memory (Ajiboye, Olopade, Femi-Akinlosotu and Shokunbi, 2024).

At the end of the behavioral test, cervical dislocation was the method used to sacrifice the mice, their brains were harvested and postfixed in 10% formalin. Coronal sections of the brains were obtained anterior to the optic chiasm. Homogenate of brain tissue was obtained and processed for oxidative stress markers - IL-6 and MDA (Pamungkas, Kalanjati, Abdurachman, Aditya, Nasution, Syamhadi, 2023; Rambung, Kalanjati, and Abdurachman, 2022), with details explained elsewhere (Niraula, Witcher, Sheridan, Godbout, 2019; Halifeoglu, Canatan, Ustundag, Ilhan and Inanc, 2000).

Tissue processing

The fixed brains were processed for hematoxylin and eosin (H&E) to determine the histomorphology and microscopic density in the cerebral cortex and hippocampus subregions (Shokunbi, Olopade, Femi-Akinlosotu and Ajiboye, 2020). Five brain specimens were selected from each group for quantitative analyses. The slides were examined using DM 500 digital light microscope (Germany), and images were taken with Leica ICC50 E digital camera (Germany). For each sample, average

numbers of pyramidal neurons and glia were quantified by counting six fields for counting in the prefrontal cortex and three fields for counting the intact neurons versus pyknotic cells in CA1 and CA3 of the hippocampus using a standardized square of 160,000µm2 with a computerized image analyzer (Image J/Micro-Manager 1.4) from H&E photomicrographs. The pyknotic index was calculated as previously described (Taveira, Carraro, Cataloa, Lopes, 2012).

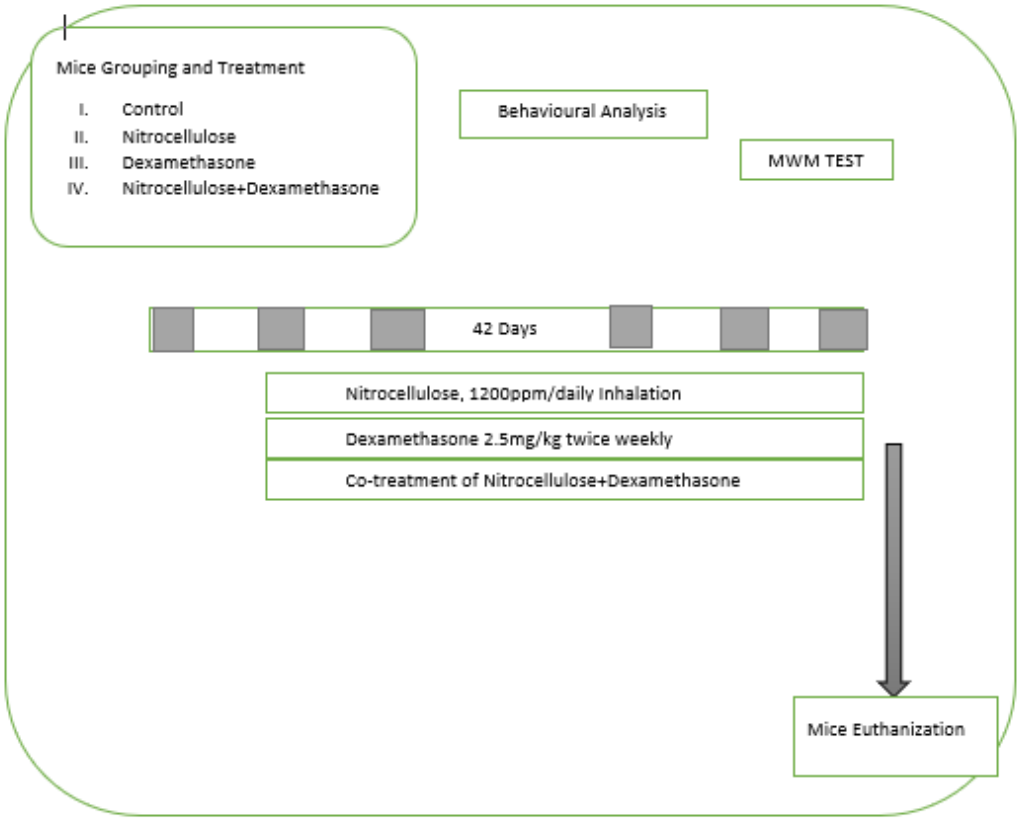

Figure 1. Scheme of the experimental schedule. (i) Control mice exposed to air for 42 days. (ii) Mice who were exposed to nitrocellulose 1200ppm for 42 days. (iii) Mice treated with dexamethasone 2.5mg/kg twice weekly for 42 days (iv) Mice with 1200ppm of nitrocellulose daily and cotreated with 2.5mg/kg twice weekly for 42 days. After behavioral analyses on the 42nd day, the adult mice were eutanized and further subjected to histological analysis.

Statistics

Data from the neurobehavioral tests and tissue sections were statistically analyzed as quantitative using Graphpad Prism version 8.4.3 for Windows (Software Mackier, San Diego, California) and presented as means ± standard error of means. Sample means were generated and used after the normality test, and were compared among all the groups using one-way ANOVA or Kruskall-Wallis test with confidence interval set at 95% and level of significance fixed at 0.05.

RESULTS

In the Morris water maze test, all the animals had reduced escape latencies with each successive trial to locate the hidden platform. Learning was reduced in the nitrocellulose group (12.05 ± 1.43)s compared to control (25.19 ± 4.084)s (**P = 0.0077). However, no difference was seen in the other groups. Retention of memory was taken as the time used in the experimental quadrant that had the escape platform. The time spent by nitrocellulose mice (10.86 ± 1.1)s in the quadrant of study was significantly lesser than the controls (20.00 ± 2.769) (**P =0.0089).

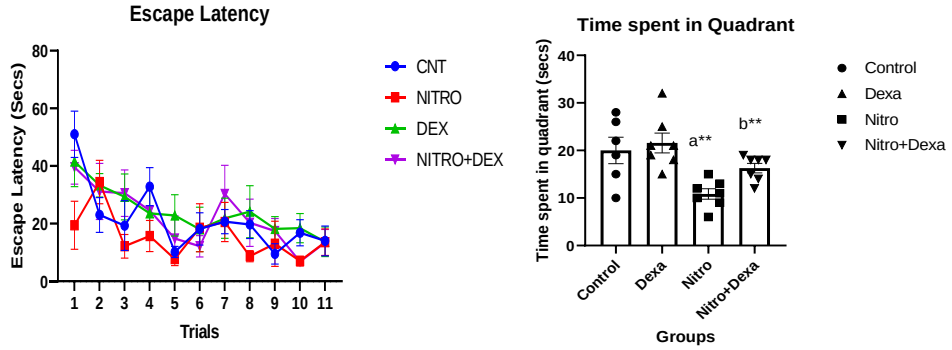

Figure 2. A. The escape latency of control and the experimental mice, control (n=6), nitrocellulose (n=7), dexamethasone (n=7), nitrocellulose + dexamethasone (n=7). Control vs. nitrocellulose (**P = 0.0089). **B**. The measures of memory retention during the probe trial by the control, nitrocellulose, dexamethasone and nitrocellulose + dexamethasone. (**P<0.05) a = control vs nitrocellulose, b = nitrocellulose vs dexamethasone.

Table 1 presents the results of MDA expression in the Swiss albino mice model exposed to nitrocellulose. The median values of the control, nitro-, dexa- and nitro + dexa groups are 0.02612, 0.06648, 0.04846 and 0.03101, correspondingly. A significant difference in MDA expression was observed between the four groups (P = 0.0242). In Table 2, the result of Mann-Whitney Test of IL-6 expression in the cerebral cortex, (P = <0.0001) is presented.

Table 1. Level of MDA in the cerebrum of Swiss mice

Group	Mean ± SEM	Median	Normality Shapiro-Wilk (P)	One-way Anova (*P*)
Control	0.0298 ± 0.0074	0.0261	0.5301	0.0242*
Nitrocellulose	0.0778 ± 0.0087	0.0665	0.1429	
Dexamethasone	0.0527 ± 0.0151	0.0485	0.9165	
Nitrocellulose + Dexamethasone	0.0363 ± 0.0068	0.0310	0.0168	

Routine staining of the brain tissue was done using hematoxylin and eosin (H&E) stain to evaluate the survival of pyramidal neurons and glia in layers 5 and 6 of the prefrontal cortex. There was comparison between the density of neuron cell and glial cell nuclei. Neurons have a round type nucleoli that are noticeable with basophilic cytoplasm. Astrocytes have rounded, round type nucleoli while the oligodendrocytes have round chromatin type with nucleoli not very visible, the microglia cell nucleus appears ovoid or planar (Machin, Kalanjati, Abidah, Sugianto, Susanto and Firdha, 2024).

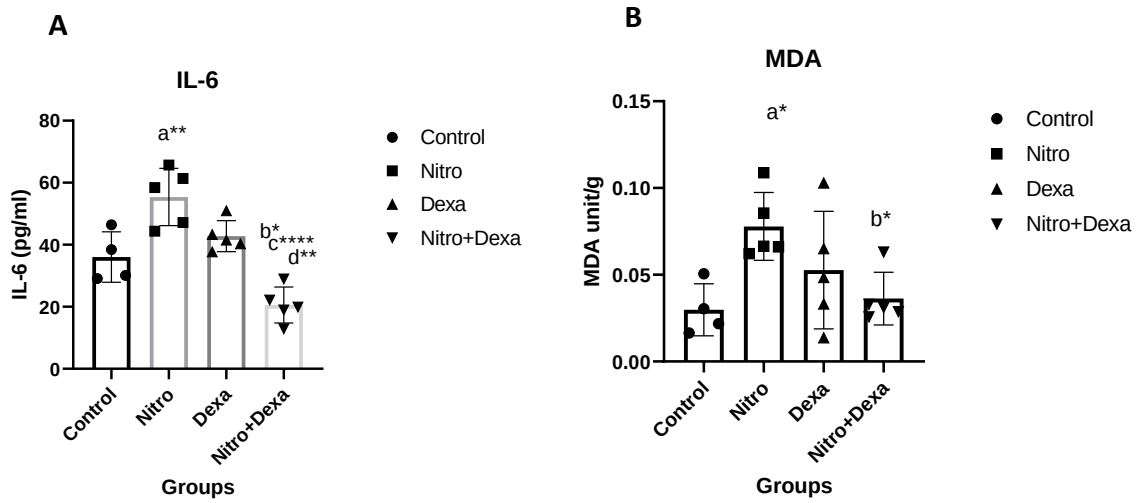

Figure 3. A. Bar chart showing the level of IL-6 in the control, nitrocellulose, dexamethasone and nitrocellulose + dexamethasone (*P<0.05) a = control vs nitrocellulose, b = control vs nitrocellulose + dexamethasone, c = nitrocellulose vs nitrocellulose + dexamethasone, d = dexamethasone vs nitrocellulose + dexamethasone. **B**. MDA in the control, nitrocellulose, dexamethasone and nitrocellulose + dexamethasone (*P<0.05) a = control vs nitrocellulose, b. nitrocellulose vs nitrocellulose + dexamethasone.

Table 2. Result of Mann Whitney U Test of IL-6 expression in the cerebral cortex of Swiss Albino mice

Group 1	**Group 2**	***P***
Control	Nitro	0.0317*
	Dexa	0.2857
	Nitro +Dexa	0.0159*
Nitro	Dexa	0.0317*
	Nitro + Dexa	0.0079**
Nitro + Dexa	Dexa	0.0079**

Table 3. Pyramidal cell nuclei count in layer 5 and 6 of the cortex

Group	Mean ± SEM	Median	Normality Shapiro-Wilk (P)	One-way Anova (*P*)
Control	16.12 ± 0.4055	16.00	0.9732	< 0.0001*
Nitrocellulose	8.476 ± 0.1970	8.40	0.3850	
Dexamethasone	17.16 ± 0.6975	17.02	0.1572	
Nitrocellulose + Dexamethasone	14.35 ± 0.3246	14.22	0.7126	

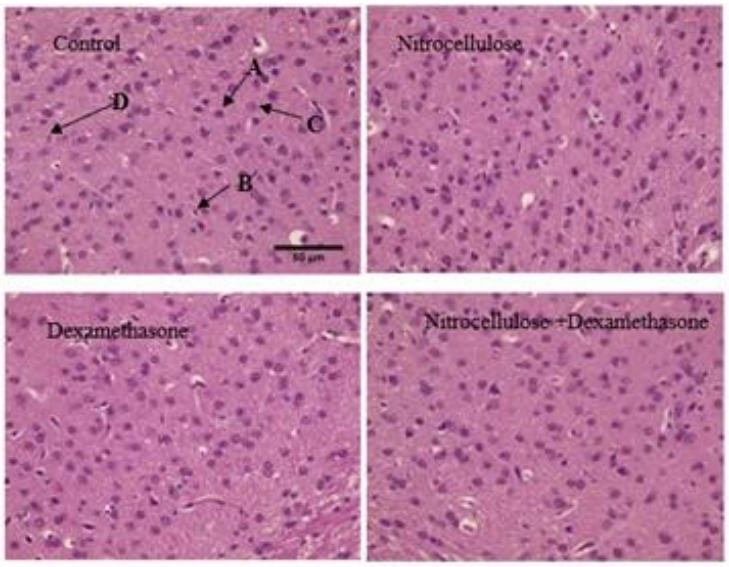

Figure 4. Representative sections (H&E) showing neuron cell nuclei numbers and glial cell numbers in layer 5 and 6 of prefrontal cortex (PFC) of cerebral cortex between control, nitrocellulose, dexamethasone and nitrocellulose + dexamethasone. Pyramidal neurons and glia (astrocytes, oligodendrocytes, microglia) were counted: A – pyramidal neuron; B – microglia; C – oligodendrocyte; D – astrocyte.

The density of pyramidal neurons in layer 5 and 6 of the PFC is significantly decreased in the nitrocellulose group (8.47 ± 0.1970) compared with control (16.12 ± 0.4055) (P<0.0001). Administration of dexamethasone twice weekly for 42 days at a dose of 2.5kg body weight caused an increase in the quantity of neurons (P= 0.0093) (see Tables 1 and 2). Glial cells increased in the nitrocellulose group (7.02 ± 0.1707) due to gliosis and inflammation caused by oxidative stress induced by nitrocellulose inhalation and probably due to response of glia to central nervous system (CNS) damage, unlike what is seen in the control group (5.91 ± 0.2725). There was a significant difference in the glia nuclei number between control with nitrocellulose and control with nitrocellulose + dexamethasone (P=0.0136 and 0.0494) respectively.

Table 4. The *Posthoc* Tukey Test of neuron cell nuclei number in layer 5 and 6 of the cerebral cortex

Group 1	Group 2	*P*
Control	Nitrocellulose	<0.0001*
	Dexamethasone	0.3775
	Nitrocellulose + Dexamethasone	0.0554
Nitrocellulose	Dexamethasone	<0.0001*
	Nitrocellulose + Dexamethasone	<0.0001*
Dexa	Nitrocellulose + Dexamethasone	0.0020*

The H&E stained hippocampus of the animals revealed a well arranged pyramidal neurons with large neurons and active prominent nucleoli in control group, whereas the stained hippocampus of the nitrocellulose group showed more neurons with scattered nuclei showing abnormal clumping of chromatin in CA1 and CA3 subregions. However, this was not statistically significant (P = 0.4602 and 0.1038) respectively. Figure 5 and 6 show no difference in the total quantity of neurons found in the CA1 (72.90 ± 10.41) and CA3 (48.95 ± 5.001) of nitrocellulose group compared with the CA1 and CA3 in control animals (82.68 ± 6.725; 62.00 ±4.6075) respectively.

Table 5. Glial cell nuclei numbers in layer 5 and 6 in the cerebral cortex of Swiss mice

Group	Mean ± SEM	Median	Normality Shapiro-Wilk (P)	One-way Anova (*P*)
Control	5.91 ± 0.2725	5.99	0.1851	0.0009***
Nitrocellulose	7.02 ± 0.1707	7.01	0.9145	
Dexamethasone	6.19 ± 0.2876	6.25	0.4583	
Nitrocellulose + Dexamethasone	4.95 ± 0.2803	5.19	0.0387	

Examination of hippocampus of control mice showed a well layered arrangement of the hippocampal pyramidal neurons with well-defined nuclei and few darkly stained, shrunken pyramidal neurons in the CA1 and CA3. The pyramidal neurons in CA1 and CA3 of the hippocampus in nitrocellulose groups were interspersed with more pyknotic neurons (Figure 5 and 6). However, after counting these neurons, the density of these neurons showed no statistical difference in the pyknotic indices between all the groups (both with P≥0.05).

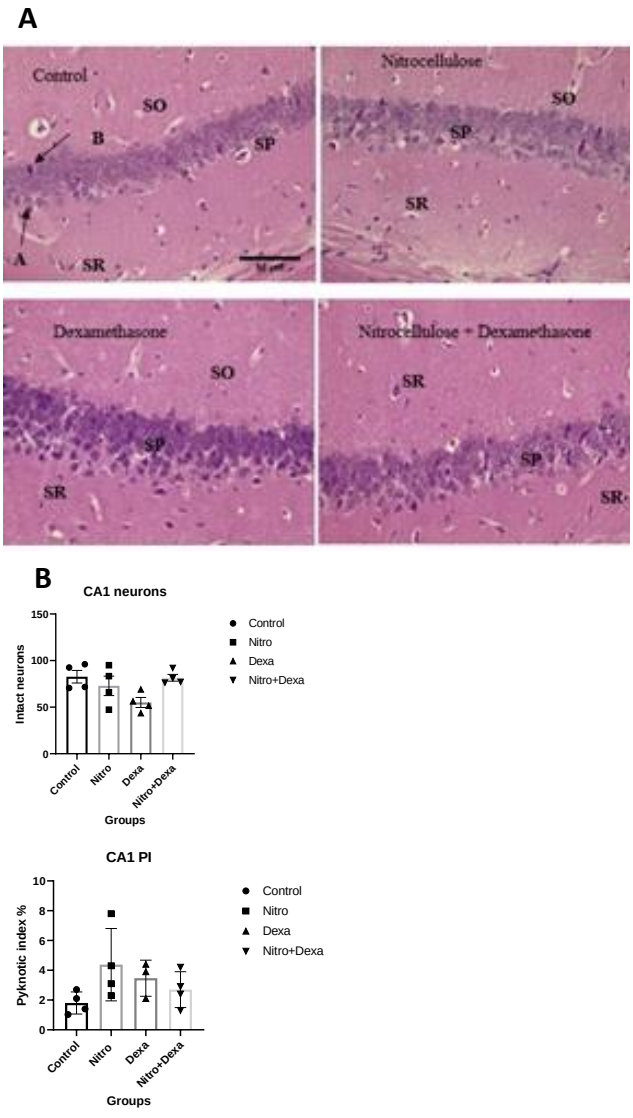

Figure 5. Representative H&E sections of CA1 of mouse hippocampus in control, nitrocellulose, dexamethasone and nitrocellulose + dexamethasone. Normal pyramidal neurons in the stratum pyramidalis (SP) are indicated by arrows, while dark reactive and dark pyknotic pyramidal (arrowheads) neurons are scattered in the nitrocellulose group. Stratum oriens (SO), stratum pyramidalis (SP), stratum radiatum (SR). Scale bar for all figures 50µm. x 40 Magnification. B. Bar chart showing intact neurons and pyknotic indices (PI) of pyramidal neurons in the CA1 region of the hippocampus in control, nitrocellulose, dexamethasone and nitrocellulose + dexamethasone.

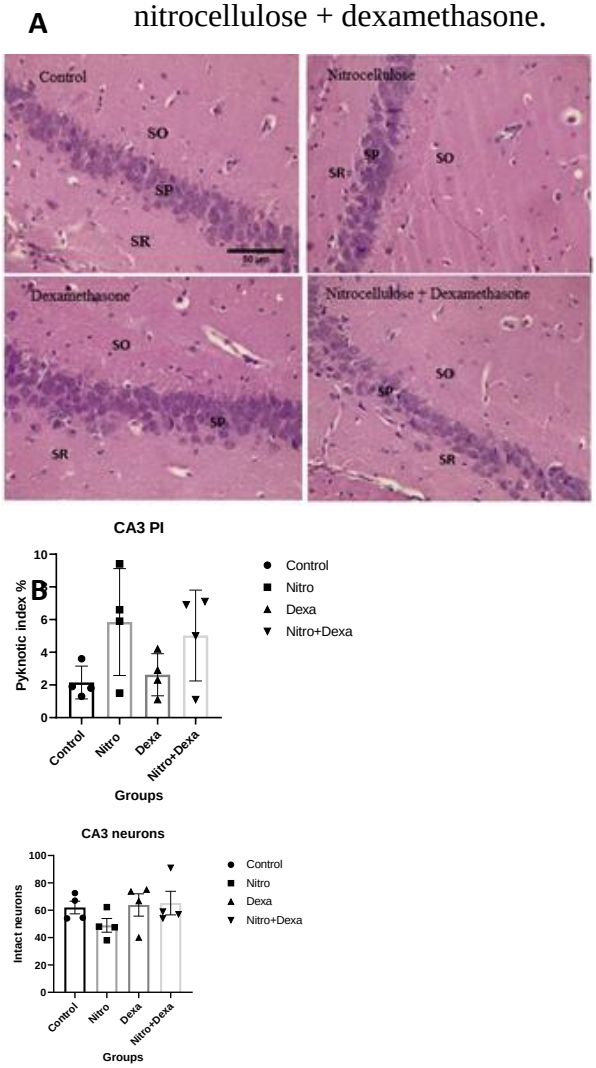

Figure 6. A. Representative HE sections of CA3 of mouse hippocampus in each group. Arrows: Normal pyramidal neurons in the stratum pyramidalis (SP), arrowheads: dark pyknotic pyramidal nuclei in Stratum oriens (SO), stratum pyramidalis (SP), stratum radiatum (SR). Scale bar is 50µm (400x magnification). B. Pyknotic indices (PI) of pyramidal neurons in the CA1 region of the hippocampus in group 1-4, respectively.

Table 6. The *Posthoc* Tukey Test of glial cell nuclei number in layer 5 and 6 of the cerebral cortex

Group 1	Group 2	*P*
Control	Nitrocellulose	0.0434*
	Dexamethasone	0.8695
	Nitrocellulose + Dexamethasone	0.0877
Nitrocellulose	Dexamethasone	0.1554
	Nitrocellulose + Dexamethasone	0.0005***
Dexa	Nitrocellulose + Dexamethasone	0.0235*

DISCUSSION

Inhalant abuse is a global problem, with an increased occurrence in communities that are marginalized. Everyone is affected and this poses an important health and psychosocial challenge (Malloul, Bennis, Bonzano, Gambarotta, Perroteau, De Marchis and Ba-M'hammed, 2018). Several chemicals are found in inhalants, this includes nitrous oxide, volatile anesthetics, volatile solvents and alkyl nitrites (Beckley and Woodward, 2013). Toluene is the most commonly used chemical and has received widespread attention in recent studies (Cruz, Rivera-Garcia and Woodward, 2014). Aromatic hydrocarbons used commonly as abused solvents by artisans to spray, as paint thinner necessitated this research. Adult male mice were exposed to inhaling paint thinner – nitrocellulose using previously reported protocol (Fifel and Bennis, 2014). Exposure of mice to paint thinner at the behavioral and cellular level was investigated on the prefrontal cortex and the hippocampus, especially CA1 and CA3. Data on the consequences of these chemicals on the hippocampus are scarce despite plenty literature highlighting dysfunctional cortical and subcortical brain regions as a result of exposure to volatile solvents (Beckley and Woodward, 2013).

Prolonged (subchronic) thinner exposure on hippocampal-related functions like learning and memory were assessed. Previously, it has been stated that short-term contact with inhalants on adult animals led to several changes in hippocampal-related behaviors following treatment (Fifel and Bennis, 2014), our data showed that 42 days after subchronic nitrocellulose inhalation, there was a deficit in spatial learning and memory. This deficit suggested that learning was not achieved in the nitrocellulose group and dexamethasone at the dose (2.5kg/body weight) used did not seem to significantly aid learning and memory consolidation. Accordingly, we reported the incidence of oxidative stress in the nitrocellulose group as shown by the increase in MDA and IL-6 levels in the cerebral cortex. A significant reduction in both pyramidal neurons and glia in the nitrocellulose compared to controls was observed. However, there was no difference in the hippocampal neurons and pyknotic indices found in both CA1 and CA3 subregions.

In another study with a subchronic treatment within a period of 6 weeks where mice exposure to thinner was daily, elements of depressive-like behavior emerged according to modifications in memory/learning related functions as evidenced by step-through passive avoidance

task (SPAT) and object recognition test (ORT). These findings agree with a similar study in rats in exhibited cognitive deficits in the SPAT and Morris water maze after thinner exposure treatment for 45 days (Malloul, Bennis, Bonzano, Gambarotta, Perroteau, De Marchis and Ba-M'hammed, 2018; Fifel and Bennis, 2014; Baydas, Ozvern, Akdemir, Tuzcu and Yasar, 2005a, b). There is a significant damage in learning/memory activities after a long-term daily thinner inhalation regime treatment for 12 weeks, this suggests a gradual worsening of these chemicals with respect to the duration of exposure.

Malondialdehyde (MDA) marks the level of oxidative stress and the antioxidant state in cancer patients. Too much production of MDA is sequel to an increase in free radicals. Level of MDA increased in mice exposed to nitrocellulose, this agrees with Halifeoglu, Canatan, Ustundag, Ilhan and Inanc (2000) who observed a statistically significant difference in workers whose MDA levels of thinner was increased as against the control group. A product of lipid peroxidation which MDA is was observed to be significantly higher ($p<0.001$) in workers who had thinner exposure as against the control group. The response of polyunsaturated fatty acids with oxidative free radicals is termed lipid peroxidation.

Interleukin-6 (IL-6) is increased in circulation with lingering stress and this leads to neurobehavioral problems (Niraula, Witcher, Sheridan and Godbout, 2019). Its implication in stress-induced neuropsychiatric deficits cannot be overemphasized. In clinical and experimental stress, it is used as a cytokine biomarker. Treatment of resistant mood disorders is associated with IL-6. The level of IL-6 increased significantly in the nitrocellulose group indicating the presence of oxidative stress markers elicited by the paint thinner.

In this study, there was a decline in the number of neurons in layer 5 and 6 of nitrocellulose group in the prefrontal cortex indicating that stress caused by nitrocellulose inhalation is detrimental to the pyramidal neurons as corroborated by Gelazonia, Japaridze and Svanidz (2006) reported a 26% decrease in the quantity of pyramidal neurons in rats who inhaled toluene in contrast to the control in CA3 field of the subgroup II of the juvenile animals only. This loss of pyramidal neurons in CA3 field will lead to destruction of the neural circuits in the hippocampus and impair memory and other activities in the rats. However, the cellular composition in the hippocampus has been reported to be affected by several factors including the age and neurodegenerative process. Although as previously studied by Kalanjati, Hendrata and Ardana (2019), constitution of neurons and glia (neuron-glia ratio) with no major nonconformities was differentiated from the type seen in juvenile adult human cortices who had no serious pathology seen as against the brain of subjects who are in the initial stages of normal aging (with no obvious symptoms of neurodegenerative diseases).

We also reported an increase in the number of glia in nitrocellulose group. This might be a response to inflammation caused by active components of the paint thinner especially toluene. Astrocytes and oligodendroglia are indispensable to unraveling toluene's toxic effect on the neurons because of their important function in attending to damage done to neurons and sustaining the extracellular milieu through neurotransmitter and neurohormone control (Eisenberg, 2003). Fukui, Utsumi, Tamada, Nakajima, Niraula and Ibada (1996) investigated hippocampal astrocytes by immunocytological methods after 2000ppm of toluene was exposed to rats for four weeks, four hours daily. In the dentate gyrus, exposure to toluene caused multiplication of glial processes (not numbers) in cells that were marked with glial fibrillary acidic protein (GFAP).

Everyday use of toluene includes its use as solvents in paints, inks, synthetic fragrances, adhesives, coatings, cleaning agents and primers. From a public health perspective, nitrocellulose, should be used with caution either orally or by ingestion considering the toxic chemicals that it contains like toluene. One can get exposed to toluene from breathing ambient or indoor air affected by such sources. Primarily, the central nervous system becomes the main target organ for toxicity in both humans and animals from acute and chronic exposures to toluene and similar chemicals.

CONCLUSION

Dexamethasone may enhance learning and memory by dampening the inflammatory effect related with oxidative stress in the prefrontal cortex but not necessarily the density of cells in the hippocampus.

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