IJPSR (2019), Volume 10, Issue 11 **(Research Article)**

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 19 February 2019; received in revised form, 06 June 2019; accepted, 14 June 2019; published 01 November 2019

NEUROLOGICAL, HAEMATOLOGICAL AND HISTOLOGICAL EFFECTS OF CHRONIC EXPOSURE TO NITROUS OXIDE IN WISTAR ALBINO RATS

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Keywords:

Nitrous oxide, Neurological effects of nitrous oxide, Methemoglobinemia, Nitrous oxide analgesia

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ABSTRACT: Objectives: The objectives of the study were to investigate the neurological, hematological, and histological effects of chronic exposure to nitrous oxide (N_2O) in male Wistar albino rats. **Methodology:** 12 adults, male, Wistar albino rats (150-200g) were divided into 2 groups of 6 animals each. Group 1 served as control. Group 2 received $N_2O + O_2$ (70:30 mixture) for 1 h every day for 60 days. Neurological assessments were done by forced swim test, tail suspension test, actophotometer, rotarod, and elevated plus maze models at baseline and every 2 weeks, after that for 60 days. Complete blood count, liver function tests, renal function tests, and serum methemoglobin levels were assessed at baseline and on day 60. Rats were sacrificed on the 60th day, and histopathological examination of liver, kidney, spleen, and brain was done. **Results:** On comparing the results with the control group, the following changes were observed in nitrous oxide exposed rats. A: The duration of immobility was significantly reduced in forced swim test and tail suspension test, implying antidepressant-like activity. B: The activity of the animals was significantly reduced in actophotometer model, implying a reduction in the locomotor activity. C: No significant changes were observed in rotarod and elevated plus maze. D: Serum methemoglobin was significantly elevated. E: Histopathological examination showed hepatitis and interstitial nephritis. **Conclusion:** Chronic nitrous oxide exposure resulted in significant neurological changes, methemoglobinemia, hepatitis and interstitial nephritis. Hence healthcare workers such as dentists, doctors and paramedical staff need to take necessary precautions to prevent chronic occupational exposure of nitrous oxide.

INTRODUCTION: Nitrous oxide is one of the nitrogen-containing oxides, commonly known as laughing gas or Nitrous. It is a sweet-smelling, colorless and non-inflammable gas. Though it is non-inflammable, it supports combustion.

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DOI: 10.13040/IJPSR.0975-8232.10(11).4959-67

This article can be accessed online on www.ijpsr.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.10(11).4959-67

Nitrous oxide is also a powerful oxidizing agent, like oxygen [1]. Its chemical name is Di Nitrogen monoxide, and the molecular formula is N_2O. N_2O is one of the oldest, inhalational anesthetic and analgesic agents. In the initial days, ether and chloroform were also used as anesthetics, but only N_2O is in clinical use today [1].

The mechanism of action of nitrous oxide is not clearly understood. The neurological effects of nitrous oxide are attributed for its ability to bind proteins present in the neuronal membrane and subsequent changes in the ion fluxes and synaptic

transmission. Nitrous oxide can produce analgesia even at minimal concentration [2].

The possible mechanisms [2] of its analgesic effects could be,

i. Its interaction with the endogenous opioid system.
ii. Modulation of various ligand-gated ion channels and
iii. Nitrous oxide imitating Nitric oxide in the Central Nervous system.

The Mean Alveolar Concentration (MAC) of nitrous oxide is more than 100%, and hence, it is difficult to induce anesthesia in a concentration less than 100%. The blood gas partition coefficient of nitrous oxide is 0.47 as its solubility is very minimal in blood. Due to these properties, the induction and recovery are fast for nitrous oxide anesthesia. During the administration of Nitrous oxide, initially, there is a rapid transfer of nitrous oxide from alveoli to pulmonary circulation, creating a vacuum in the alveoli, which helps to pull more gas into alveoli.

When the administration is terminated, nitrous oxide diffuses rapidly to lung alveoli and eliminates via exhaled air. This reduces the oxygen exchange in the pulmonary circulation and causes diffusion hypoxia. To avoid diffusion hypoxia, sometimes, 100% oxygen is administered after terminating nitrous oxide administration [2].

Currently, nitrous oxide is used for general anesthesia and obstetric analgesia. It is also used for pain management in fracture reduction, dental procedures, burn dressing, sickle cell crisis, positioning for radiological procedures, and ureteric colic [3].

Adverse Events of Nitrous Oxide Anesthesia: The major drawback of nitrous oxide anesthesia is that it causes significant post-operative nausea and vomiting. Long term administration of nitrous oxide interferes with the DNA synthesis in white blood cells and red blood cells and reduces the leucocyte function and also causes mild megaloblastic changes in the bone marrow.

It may sometimes lead to agranulocytosis, distension of the bowel, damage to the middle ear, and tympanic membrane rupture. If nitrous oxide is used frequently, it can cause myeloneuropathy [4]. Nitrous oxide can make the people experience the feeling of 'high', 'flying', 'diving', 'floating' at high concentrations. Hence, people start abusing nitrous oxide for recreational purposes. Chronic abuse of nitrous oxide can lead to sub-acute degeneration of the spinal cord, vocal cord injury and vitamin B 12 deficiencies [4].

Nitrous oxide is a pregnancy C category drug according to the US FDA classification of drugs based on drug exposure and foetal risk. Pregnancy "Category C" means foetal adverse events are reported in animal reproductive studies with no significant data of foetal adverse events in well-controlled human studies [5].

Administration of Nitrous Oxide: Nitrous oxide is administered *via* an inhalational route through a face mask or tracheal tube using an anesthetic apparatus. It is mixed with oxygen in various ratios (50:50 or 70:30) and used. Nitrous oxide has both analgesic and anesthetic properties. It is a good analgesic, but a poor anesthetic and it fails to create an adequate anesthetic effect on its own; hence, it is combined with appropriate doses of other anesthetics when used for general anesthesia. There are sophisticated equipment available in operation theatres for providing general anesthesia with nitrous oxide [6].

In dental practice [7], nitrous oxide is one of the most commonly used inhalational anesthetics. Anesthesia produced by N_2O is called 'conscious sedation' as it reduces pain, anxiety, and self-awareness without really causing loss of consciousness. It is used in both children and adults but requires cooperation from patients. It may be useful in patients who have 'needle phobia' for local analgesic injections in the mouth. Sometimes, the dose is titrated for the inspired nitrous oxide to produce a light level of sedation and mild analgesia. This allows procedures to take place under the subsequent local anesthetic block.

In anesthesia practice, open and closed systems are used for the administration of inhalational agents. In open system, the inhalational drugs administered *via* Boyle's apparatus will be exhaled into the environment of the operation theatres. Unless there

is an air scavenging system placed in the operation theatres, the health care professionals in the theatres will be significantly exposed to the anesthetic agents. In a closed system, the exhaled air will be routed to a soda lime canister which will take the drugs in the exhaled air and hence the people in the operation theatre will get only a minimal exposure compared to open system.

Nitrous oxide is not metabolized in the body and the amount of nitrous oxide ingested is almost completely eliminated through exhaled air. Health care professionals in dental clinics who perform dental extractions and other dental procedures, operation theatres without proper air scavenging units and theatres that use open anesthetic systems will get constant exposure to nitrous oxide. On the long term, nitrous oxide exposure may lead to adverse events such as head ache, addiction, increased suicidal tendency, euphoria, and methemoglobinemia.

In females, there are chances for increased miscarriages and teratogenicity [1]. Due to the sophisticated air scavenging systems in the operation theatres, the possibility of chronic nitrous oxide exposure has come down, however, it can still happen in dental clinics that have only a split air conditioner and operation theatres that use open anesthetic systems and have no proper air circulation.

Apart from the usefulness of nitrous oxide in general anesthesia, it has definite advantages in small outpatient procedures in dentistry and surgical practice. Nitrous oxide with oxygen gives good analgesia without significantly affecting the level of consciousness and vital parameters. It is especially preferred in pediatric dental procedures as it does not involve painful injections. With all its advantages, there is a limitation for its use in clinics and operation theatres that do not have proper air scavenging systems. The doctors, dentists, nurses, technicians, and other support staff working in such an environment may get chronic exposure of nitrous oxide, which may adversely affect their health. Hence, this study was planned to systematically study the effects of long term exposure of nitrous oxide in animal models.

The study aimed to assess the toxicity of chronic exposure to nitrous oxide in Wistar albino rats.

The objectives were to evaluate them,

Neurological Effects: By Tail suspension test, Forced swim test, Actophotometer, Rotarod, and Elevated plus maze.

Hematological Effects: By complete blood count, renal function tests, liver function tests, and serum methemoglobin.

Histological Effects: On liver, kidney, brain, and spleen.

MATERIALS AND METHODS: The study was initiated after obtaining approval from the institutional animal ethics committee. The approval number was IAEC 3/Desp.No.12 dated 04.09.17. Twelve (12) adult, male, Wistar albino rats weighing 150-200 gms were used in the study. The animals were housed at 24±2°C with 12:12 hour "light and dark" cycle with free access to food and water. The animals were acclimatized for seven days before the start of the experiment and were divided into two groups of 6 animals each. Group 1 animals were control animals that did not receive any intervention. Group 2 animals were tested with animals that were exposed to Nitrous oxide. The test animals received nitrous oxide + oxygen (70:30) at the flow rate of 0.7 liters/minute of nitrous oxide and 0.3 liters/minute of oxygen for one hour every day for 60 days. Every day, the test animals were placed in the exposure chamber and exposed to the gases. Food and water were not provided during the exposure period of one hour.

The exposure chamber was custom made by 1/8 inch thick Lucite transparent plastic material measuring 20 cm × 20 cm × 20 cm. It was a leak-proof chamber with two connecting ports for the inflow of gases. Nitrous oxide and oxygen cylinders were connected through flow meters via the gas connecting tubes and the animals inside the chamber were exposed to nitrous oxide and oxygen at 70:30 ratio for one hour every day for 60 days.

Before the start of the study, the following baseline neurological assessments were carried out.

- Tail Suspension Test (TST)
- Forced Swim Test (FST)
- Actophotometer model
- Rotarod model

- Elevated Plus Maze (EPM)

Among them, TST and FST are considered as models for depression, EPM for anxiety, actophotometer for locomotor activity, and rotarod for motor coordination. These tests were further carried out every 2 weeks until the end of the study (day 60). Body weight of the animals was measured at baseline and every 2 weeks, after that.

The following hematological parameters were assessed at baseline and the end of the study. 0.5 ml of blood was collected by retro-orbital vein puncture and used for the assessment of the following blood parameters.

- Complete Blood Count (CBC) - Hemoglobin, Packed Cell Volume (PCV), total RBC count, differential leucocyte count, total WBC count, and platelet count
- Liver function tests – Bilirubin (direct, indirect and total), Serum glutamic oxaloacetic transaminase (SGOT), Serum glutamic pyruvic transaminase (SGPT), Alkaline Phosphatase (ALP), total protein
- Renal Function tests – Urea and Creatinine
- Serum Methemoglobin (ELISA method)

The animals (both control and test) were sacrificed on day 60 using a high dose of halothane (5 ml). After sacrifice, the animals were affixed to the dissecting board and abdomen was opened through a midline abdominal incision starting at xiphoid cartilage and extending caudally down for approximately 4 cm. The incision was deepened, and the peritoneal cavity was opened. Liver, kidneys, and spleen were dissected and removed in total and stored in 10% formalin. Similarly, the brain was removed by doing craniotomy and stored in 10% formalin solution. The specimens were processed for paraffin embedding. They were sectioned at 5 µm thickness and subsequently stained with hematoxylin - eosin. The light microscopic examination was done to record the histopathological changes.

RESULTS: In this study that investigated the long term effects of nitrous oxide exposure in male Wistar albino rats, 12 animals were included in test and control groups, with 6 in each group and all the animals completed the study.

The following parameters were assessed in control and test groups to assess the toxicity.

- ❖ Body weight of the animals
- ❖ Duration of immobility in tail suspension test
- ❖ Duration of immobility in the forced swim test
- ❖ Score count indicating motor activity in actophotometer
- ❖ Time is taken by the animals to fall off from the rotating rod
- ❖ Elevated plus maze
  - ▪ Number of entries into open and closed arms
  - ▪ Time spent in open and closed arms

Body Weight: The mean weight of the animals in the control group was 186.33 grams (± 26.58) at baseline and 189.33 grams (± 46.35) at the end of the study (60 days). In the nitrous oxide exposed animals, the mean body weight was 190.67 grams (± 25.07) at baseline and 195.00 grams (± 23.28) at the end of the study. The mean difference in the body weight of the animals between end of the study and baseline was 3.00 grams in control group and 4.33 grams in N_2O exposed group. The changes observed in body weight within the groups and between the groups were not statistically significant.

Tail Suspension Test: (**Table 1** and **Fig. 1**) In the control group, the mean duration of immobility (in seconds) was 185.00 (± 42.58) at baseline and 184.83 (± 32.00) on day 60 and change observed was not statistically significant (paired t-test). In N_2O exposed animals, the duration of immobility was significantly reduced from 189.50 (± 63.81) at baseline to 106.50 (± 33.36) at day 60 with the p-value 0.0041 (paired t-test). The difference observed between the groups for the duration of immobility was also statistically significant (p-value 0.01, unpaired t-test). The results indicate that N_2O produced antidepressant-like activity in the exposed animals.

TABLE 1: NEURO PHARMACOLOGICAL ASSESSMENTS

Animal	Tail suspension test (Duration of Immobility in seconds)				Forced Swim test (Duration of Immobility in seconds)				Actophotometer (Score count indicating motor activity)			
	Control		Test (N_2O)		Control		Test (N_2O)		Control		Test (N_2O)	
	Baseline	Day 60	Baseline	Day 60	Baseline	Day 60	Baseline	Day 60	Baseline	Day 60	Baseline	Day 60
1	219	221	263	161	174	169	166	84	825	758	825	470
2	131	153	141	63	179	148	188	114	619	654	619	500
3	213	174	173	94	164	183	181	102	375	381	375	251
4	233	150	98	90	190	174	184	92	785	845	785	599
5	144	188	215	108	182	185	179	80	675	588	675	288
6	170	223	247	123	179	188	188	71	657	698	657	561
Mean	185	184.83	189.51	106.51	178	174.5	181	90.51	656	654	656	444.83
SD	42.58	31.99	63.80	33.36	8.64	14.81	8.11	15.61	158.67	160.07	158.67	143.60

Statistics: Control group: Baseline *vs.* day 60, paired t test, $p > 0.5$, Not significant, Test Group: Baseline *vs.* day 60, paired t test, $p < 0.5$, Significant. Test *vs.* Control: unpaired t test, $p < 0.05$, Significant

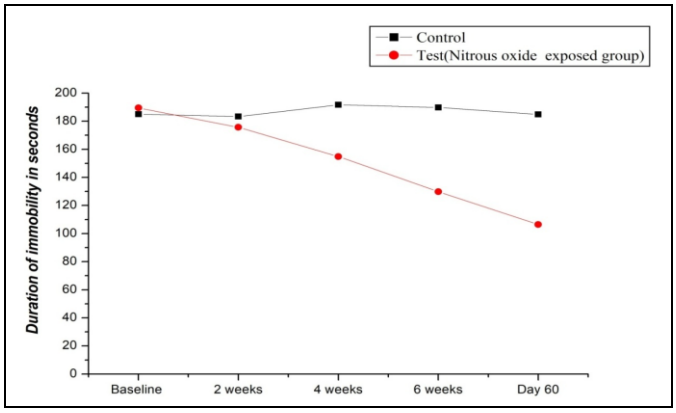

FIG. 1: TAIL SUSPENSION TEST

Forced Swim Test: (**Table 1** and **Fig. 2**) The mean duration of immobility (in seconds) was 181.00 (± 8.20) before nitrous oxide exposure in the test group, and it was reduced to 90.50 (± 15.62) at the end of the study. The reduction was statistically significant (p-value less than 0.0001, paired t-test). In the control group, the changes observed were only minimal and not statistically significant. The difference in the changes observed between test and control groups was statistically significant (p-value 0.0001, unpaired t-test). The results are similar to TST with N_2O producing antidepressant-like activity in the exposed animals.

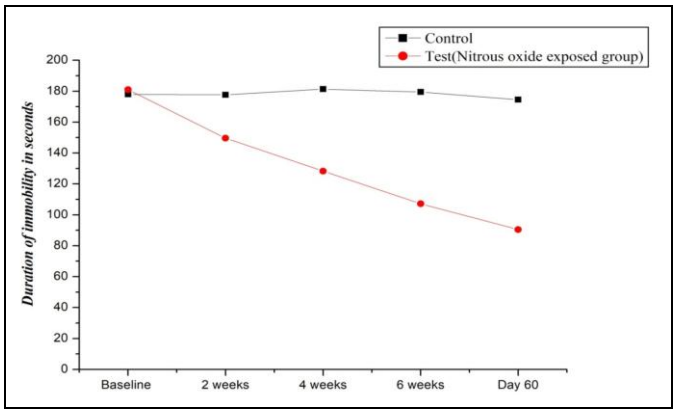

FIG. 2: FORCED SWIM TEST

Motor Activity in Actophotometer: (**Table 1** and **Fig. 3**) In actophotometer model, the movements of animals were significantly reduced in the N_2O exposed animals as indicated by the score counts (p-value 0.0098, paired t-test). The mean score was initially 656 (± 158.68), which was reduced to 444.83 (± 143.60). There were no significant changes observed in movements of the control animals. The difference between test and the control groups was statistically significant (p-value 0.0047, unpaired t-test).

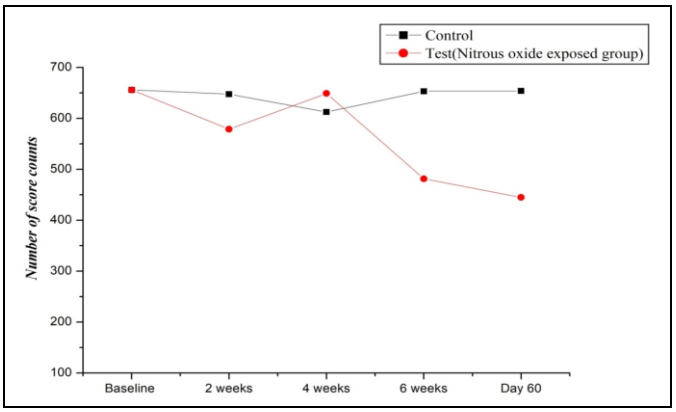

FIG. 3: ACTOPHOTOMETER

Rotarod and Elevated Plus Maze: There were no significant changes observed in these assessments in control and test and animals.

Serum Methemoglobin: (**Table 2** and **Fig. 4**) Serum methemoglobin levels were measured at the baseline and the end of the study. There were no significant changes in the methemoglobin levels in the control group. In the N_2O exposed group, the mean methemoglobin levels were significantly increased at the end of the study compared to the levels at the baseline (p-value less than 0.0001, paired t-test). It was 162.133 µg/ml (± 5.39) at baseline and 297.00 µg/ml (±7.56) at the end of the

study. The differences observed in methemoglobin levels between test and control groups were also statistically significant (p-value less than 0.0001, unpaired t-test).

TABLE 2: SERUM METHEMOGLOBIN LEVELS

Animal	Control group (µg/ml)			Test group (N_2O exposed rats) (µg/ml)		
	Baseline	Day 60	Difference	Baseline	Day 60	Difference
1	190.00	202.00	12	160.00	304.00	144
2	185.00	200.00	15	162.00	300.00	138
3	185.00	195.00	10	155.00	285.00	130
4	200.00	210.00	10	170.00	293.00	123
5	140.00	190.00	50	167.00	295.00	128
6	125.00	220.00	95	160.00	305.00	145
Mean	170.83	202.83	32	162.33	297.00	134.67
SD	30.56	10.78	34.5	5.39	7.56	9.03

Statistics: Control group: Baseline *vs.* day 60, paired t test, p = 0.0722, Not significant, Test Group: Baseline *vs.* day 60, paired t test, p = 0.0001, Significant, Test *vs.* Control: unpaired t test, p = 0.0001, Significant

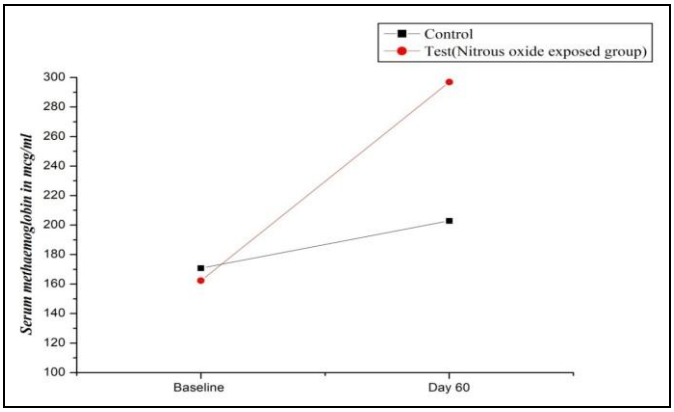

FIG. 4: SERUM METHEMOGLOBIN LEVELS

Hematological, Biochemical Investigations: Among the various parameters measured in the blood samples during baseline and at the end of the study, there were no significant changes observed within the groups and between the groups for Hemoglobin, PCV, Total RBC count, Differential leucocyte count (Neutrophil, Lymphocyte, Monocyte), SGOT, SGPT, Urea, and Creatinine. There were significant changes noted in total WBC count, total bilirubin, direct bilirubin, indirect bilirubin, ALP, and total protein in nitrous oxide treated group. Platelets levels were significantly increased in both control and test groups.

Histopathological Examination: The following changes were observed in the histopathological examination conducted in the vital organs of nitrous oxide treated animals.

Liver: The examination showed sinusoidal dilatation with congestion, minimal peri-portal inflammation, piecemeal necrosis, focal peri-portal infiltrates, and lymphoid aggregates. These changes were suggestive of drug-induced hepatitis.

Kidneys: Mild focal necrosis with minimal lymphoid cells was noted in the interstitium, suggestive of interstitial nephritis. The adrenal glands appeared normal.

Brain: Mild edema was noted in the cerebral cortex and cerebellum. There were no other significant changes.

Spleen: There was marked congestion and edema with the sinus histiocytes loaded with pigment.

DISCUSSION: This study that investigated the toxicological effects of chronic nitrous oxide exposure in Wistar albino rats can detect significant changes in the following neurological assessments.

- Tail Suspension Test (TST)
- Forced Swim Test (FST)
- Actophotometer

Tail suspension and forced swim tests are behavioral tests used to screen the pharmacological agents for their antidepressant activity. These tests are usually applied when depression-related behaviors are investigated in rodents. The drugs having antidepressant activity will reduce the duration of immobility in the animals. Classical antidepressants like imipramine, desipramine, and amitryptiline reduce the duration of immobility. Psychostimulants and atropine also reduce the duration of immobility, while, sedatives and hypnotics like diazepam increase the duration of immobility [8].

Nitrous oxide, in this study, reduced the duration of immobility from 184.83 seconds to 106.50 seconds in tail suspension test and 181 seconds to 90.50

seconds in the forced swim test. These effects produced by nitrous oxide are similar to the antidepressants. Nitrous oxide is not a complete anesthetic agent, though it produces analgesia and amnesia. It does not cause hypnosis but produces a hilarious feeling in the patients exposed to Nitrous oxide. It depresses the CNS areas with inhibitory functions, thereby releasing other areas with stimulatory functions [9]. This could be the reason for the stimulant activities noticed after administration of nitrous oxide in TST and FST.

Pharmacologically, nitrous oxide has inhibitory action at N-methyl-D-aspartate (NMDA) glutamate receptors, and stimulatory activity at dopaminergic, alpha 1− and alpha 2− adrenergic and opioid receptors. It is further reported that drugs having NMDA antagonism can reduce the duration of immobility in tail suspension test and forced swim test [10].

Nitrous oxide exposure in actophotometer model reduced the movements from 656 counts at baseline to 444.83 counts at the end of the study. Actophotometer model is meant to investigate the locomotor activity of animals. Muscle relaxants and sedatives will reduce the movements of the animals and reduce the score in actophotometer model. It is also reported that drugs having antidepressant activity reduce locomotor activity [11]. In this study, N_2O demonstrated antidepressant-like behavior in TST & FST and at the same time reduced locomotor activity similar to antidepressants.

Chronic N_2O exposure did not significantly change the outcome in rotarod model, which is used to test the motor coordination and balance. There was also no significant change observed in the elevated plus maze model, which tests the anxiety levels of the animals. Hence, it can be noted that N_2O did not affect the motor coordination and balance in spite of reducing locomotor activity in actophotometer and the duration of immobility in TST and FST.

About methemoglobin levels, chronic N_2O exposure led to an increase in methemoglobin levels from162.133µg/ml at baseline to 297.00 µg/ml at the end of the study. It is due to oxidation of iron in hemoglobin from ferrous heme (Fe++) to ferric heme (Fe+++) [12].

Short-term exposure does not result in significant methemoglobinemia as the protective enzyme, methemoglobin reductase, reconverts it to hemoglobin again, but long-term exposure could lead to significant methemoglobinemia as observed in this study. People with abnormal methemoglobin reductase enzyme can easily go for significant methemoglobinemia, and they have to be careful if there is repeated nitrous oxide exposure [13].

Methemoglobin is unable to carry and deliver oxygen like hemoglobin, and people with methemoglobinemia initially develop breathlessness, fatigue, lethargy, and headache. High methemoglobin levels are associated with severe features like cyanosis, altered mental state, seizures, and sometimes arrhythmias and rarely death. Patients with other comorbidities like anemia, cardiovascular and respiratory disorders and sepsis are prone to develop severe features of methemoglobinemia. One of the commonest causes for acquired methemoglobinemia is exposure to agents such as Nitrogen containing oxides that can oxidize transmetal complexes like iron in hemoglobin. Methemoglobinemia needs medical management in asymptomatic patients when its level is more than 30% and in symptomatic patients when it is more than 20%. Intravenous infusion of methylene blue is the treatment of choice in the management of methemoglobinemia. Patients not responding to methylene blue have to undergo exchange transfusion to remove methemoglobin in the circulation [12].

In this study, the hematological parameters such as hemoglobin, PCV, total RBC, WBC counts, and differential count were assessed during baseline investigations and at the end of the study. There were no significant changes in hemoglobin, PCV, total RBC count, and differential count. Platelet counts were significantly increased in both control, and nitrous oxide exposed animals. The total WBC count was reduced in nitrous oxide exposed animals from 13,600 to 6,616, and the change was statistically significant. Though these changes were observed in hematological indices, their functional significance seems to be minimal as they lie within normal limits.

The histopathological examination of the liver has shown features suggestive of hepatitis. The changes

observed in the examination were sinusoidal dilatation with congestion, minimal peri-portal inflammation, piecemeal necrosis, focal peri-portal infiltrates, and lymphoid aggregates. The liver function tests showed significant increases in bilirubin (total, direct and indirect) and total protein. Alkaline Phosphatase was significantly reduced, and there were no significant changes in liver enzymes SGOT and SGPT. The statistically significant changes in the liver function tests of bilirubin, protein, and alkaline phosphatase, do not seem to be functionally significant.

Cohen *et al*., investigated the possibility of liver, kidney, and neurological disorders associated with chronic nitrous oxide exposure in his clinical study involving more than 60,000 dentists and dental assistants. He reported that the chances of liver diseases are increased by 1.7 times, kidney diseases by 1.2 times and neurological disorders by 1.9 times in males who had repeated exposure. The same was 1.6 times increased risk of developing liver diseases, 1.7 times increased the risk for kidney diseases, and 2.8 times increased the risk for neurological disorders in females [14]. N_2O increases the sympathetic tone and may cause increased splanchnic & hepatic arterial vasoconstriction and reduce portal circulation. Coupled with its ability to inhibit methionine synthase and reduce the function of vitamin B 12, nitrous oxide may cause liver damage. But there is no convincing evidence to associate liver damage with that of nitrous oxide exposure [15].

In the histopathological examination of other organs, brain and spleen showed mild edema, while kidneys showed mild focal necrosis with minimal lymphoid cells in the interstitium, suggestive of interstitial nephritis. Urea and creatinine did not change significantly in the nitrous oxide exposed animals. The literature search does not yield reports of renal injury with nitrous oxide exposure and possible mechanism of nitrous oxide-induced renal damage or interstitial nephritis, and hence, this aspect needs further studies and detailed exploration.

In summary, long-term nitrous oxide exposure produced i) neurological changes related to antidepressant activity and reduced locomotor activity without any significant changes in motor coordination, balance and anxiety, ii) methemoglobinemia and iii) drug-induced hepatitis and interstitial nephritis.

This study was able to demonstrate that nitrous oxide can cause significant functional neurological changes and elevated methemoglobin levels, besides drug-induced hepatitis and interstitial nephritis. Hence the professionals who have the risk of getting nitrous oxide exposure like anesthetists, surgeons, dentists, paramedical staff, and nurses should be aware of these effects and take precautions to reduce the exposure. The following methods can be implemented to check and reduce nitrous oxide exposure.

- ✓ Good air scavenging systems in the working environment should always be provided wherever nitrous oxide and other inhalational agents are used.
- ✓ Nitrous oxide levels can be periodically checked in the atmospheric air to assess air quality.
- ✓ People who are vulnerable to exposure should be surveyed in a scheduled manner to detect the possible changes in their neurological and hematological parameters.

CONCLUSION: This study was conducted in 12 Wistar albino, male rats to investigate the neurological, hematological and histological effects of long-term exposure of Nitrous oxide. Six animals were exposed to N_2O, and the remaining six were controls that were not exposed to any intervention.

Nitrous oxide was administered as a mixture with oxygen in the ratio of 70:30 *via* a custom made, leak-proof, Lucite chamber for one hour every day for 60 days. Neuropharmacological tests, hematological parameters, liver & renal function tests, and serum methemoglobin were assessed during baseline and at the end of the study.

The results show that nitrous oxide exposure caused adverse neurological effects in terms of reduction in the duration of immobility in forced swim test and tail suspension test and reduction in the movements of animals in actophotometer. These changes suggest that chronic N_2O exposure

can produce effects similar to antidepressant drugs and also reduce locomotor activity. It also significantly increased the methemoglobin levels and caused hepatitis and interstitial nephritis.

However, further studies are needed to be conducted in humans to establish these effects. At present based on the results of this study health care workers such as doctors, anesthetists, dentists and paramedical staff and patients who are likely to get repeated exposure to nitrous oxide can be educated to make them aware of such adverse effects and all steps must be undertaken to protect their health.

ACKNOWLEDGEMENT: The authors are grateful to Chettinad Hospital and Research Institute (CHRI), Chettinad Academy of Research and Education (CARE) for supporting the study.

CONFLICT OF INTEREST: The authors declare that there is no conflict of interest.

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How to cite this article:
Neevedha K, Arunkumar R, Ruckmani A, Meti V and Lavanya K: Neurological, haematological and histological effects of chronic exposure to nitrous oxide in wistar albino rats. Int J Pharm Sci & Res 2019; 10(11): 4959-67. doi: 10.13040/IJPSR.0975-8232.10(11). 4959-67.