



www.bioinformation.net
Volume 22(9)



Research Article

Received September 1, 2026; Revised September 30, 2026; Accepted September 30, 2026, Published September 30, 2026

DOI: 10.6026/973206300225594

SJIF 2026 (Scientific Journal Impact Factor for 2026) = 8.478

2022 Impact Factor (2023 Clarivate Inc. release) is 1.9

Declaration on Publication Ethics:

The author's state that they adhere with COPE guidelines on publishing ethics as described elsewhere at <https://publicationethics.org/>. The authors also undertake that they are not associated with any other third party (governmental or non-governmental agencies) linking with any form of unethical issues connecting to this publication. The authors also declare that they are not withholding any information that is misleading to the publisher in regard to this article.

Declaration on official E-mail:

The corresponding author declares that lifetime official e-mail from their institution is not available for all authors

License statement:

This is an Open Access article which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly credited. This is distributed under the terms of the Creative Commons Attribution License

Comments from readers:

Articles published in BIOINFORMATION are open for relevant post publication comments and criticisms, which will be published immediately linking to the original article without open access charges. Comments should be concise, coherent and critical in less than 1000 words.

Disclaimer:

Bioinformation provides a platform for scholarly communication of data and information to create knowledge in the Biological/Biomedical domain after adequate peer/editorial reviews and editing entertaining revisions where required. The views and opinions expressed are those of the author(s) and do not reflect the views or opinions of Bioinformation and (or) its publisher Biomedical Informatics. Biomedical Informatics remains neutral and allows authors to specify their address and affiliation details including territory where required.

Edited by Vini Mehta

E-mail: vmehta@statsense.in

Citation: Samraj *et al.* Bioinformation 22(9): 5594-5598 (2026)

Comparative evaluation of nitrous oxide, melatonin and their combination for managing pediatric dental anxiety – An *in vivo* study

A. Infant Samraj*, Vikas Verma, Vanika Arora, Ravindra Pratap Singh, Prerana Bhatt & H. Hareesh

Department of Pediatric and Preventive Dentistry, Divya Jyoti College of Dental Science and Research, Modinagar, Ghaziabad, Uttar Pradesh, India; *Corresponding author

Affiliation URL:

<https://djdentalcollege.com/>

Author contact:

A. Infant Samraj - E-mail: infantians222001@gmail.com; Phone: +91 6383723406

Vikas Verma - E-mail: vvikas5074@gmail.com; Phone: +91 7838392722

Vanika Arora - E-mail: vanikaarora1@gmail.com; Phone: +91 9045666440

Ravindra Pratap Singh - E-mail: ravindra.singh.rs22@gmail.com; Phone: +91 9837022113

Prerana Bhatt - E-mail: drpreranabhatt@gmail.com; Phone: +91 9897288344

H. Hareesh - E-mail: hareeshsharma214@gmail.com; Phone: +91 8248975436

Abstract:

Dental anxiety in children remains a major challenge in paediatric dentistry and safer adjuncts such as nitrous oxide and melatonin are being explored to improve behavior management. Therefore, it is of interest to compare tell-show-do, nitrous oxide inhalation sedation, oral melatonin and their combination in children aged 5–12 years with negative behavior who required treatment under local anesthesia. Anxiety was assessed using a Visual Analogue Scale and pulse rate before and after intervention and the combination of nitrous oxide with melatonin produced the greatest reduction in anxiety and physiologic stress, followed by nitrous oxide alone and melatonin alone. The control group showed the least improvement and greater physiologic reactions. Thus, we show that combining nitrous oxide and melatonin may offer a promising and safe approach for managing dental anxiety in children.

Keywords: anxiety, nitrous oxide, melatonin, pediatric dentistry, inhalation sedation, dental fear.

Background:

Children's dental anxiety is one of the most frequent behavioral problems that is seen in pediatric dentistry and has a significant impact on treatment acceptance, cooperation and oral health outcomes [1]. Fear of injections, unfamiliar clinical environments, painful previous experiences and separation from parents are all significant factors in dental anxiety among children undergoing dental procedures. Higher anxiety can result in avoidance of dental care, behavioral management issues and sub-optimal treatment [2]. A number of pharmacological and non-pharmacological methods have been developed to reduce dental anxiety in children. Nitrous oxide inhalation sedation is one of the most accepted and safe sedation techniques in dentistry because of its anxiolytic, analgesic, titratable and rapid recovery properties [3]. Horace Wells introduced nitrous oxide sedation into dentistry [4]. Melatonin has recently been studied as an anxiolytic in children. Melatonin is a naturally occurring neurohormone produced by the pineal gland which controls sleep and circadian rhythm. Melatonin has been shown to have sedative, anxiolytic, analgesic and antioxidant effects with little to no side effects and does not cause significant respiratory depression or cognitive impairment, unlike benzodiazepines, making it a potentially safer option in children [5, 6]. Therefore, it is of interest to evaluate the efficacy of Nitrous oxide sedation, oral melatonin and Nitrous oxide sedation combined with oral melatonin in pediatric dental anxiety were comparatively assessed.

Materials and Methods:**Study design:**

The study was an *in vivo*, randomized clinical trial carried out in the Department of Pediatric and Preventive Dentistry with 60 children aged 5–12 years.

Inclusion criteria:

- [1] Children aged between 5–12 years
- [2] Children who need dental treatment using local anaesthetic.

- [3] Children showing Frankl behavior ratings 2 and 3
- [4] Children who are healthy but fall into ASA I category.

Exclusion criteria:

- [1] Medically compromised children
- [2] Children with respiratory disorders or nasal obstruction.
- [3] Children who are allergic to melatonin or nitrous oxide.
- [4] Children for whom parents did not consent
- [5] Children taking sedative and/or anxiolytic drugs

Group allocation:

Children were randomly divided into four groups of 15 each:

Group 1: Control group (tell show do)

Group 2: Nitrous oxide inhalation sedation

Group 3: Oral melatonin

The combination of nitrous oxide and melatonin was used in Group 4. In Group 4, nitrous oxide and melatonin were combined.

Parameters assessed:

The following parameters were assessed:

- [1] The Visual Analogue Scale (VAS) for anxiety assessment is used to assess anxiety. Anxiety is assessed using the Visual Analogue Scale (VAS).
- [2] Pulse rate (pulse oximeter): Measurements were taken prior to intervention and following the completion of treatment

Intervention protocol:**[1] Group 1: Control group:**

Children were provided with regular behavioral guidance (tell show do) without the use of drugs to manage anxiety.

[2] Group 2: Nitrous oxide sedation:

Dietary restrictions and preoperative evaluation and nasal patency assessment were performed in patients. Sedation was achieved by incremental administration of nitrous oxide after 100 percent O₂ for 5 minutes and then gradually titrated to an optimum level of sedation, as assessed by

Ramsay Sedation Score, SpO₂ and pulse rate during the procedure. Local anesthesia was followed by optimal sedation. Post procedure, nitrous oxide was withdrawn and oxygen administered; recovery was assessed via walking and a puzzle game.

[3] Group 3: Oral melatonin:

The Oral Melatonin group also noted dietary restrictions and preoperative evaluation; dosage (0.5 mg/kg) was calculated based on body weight and given in water. Vitals and anxiety were recorded and all sedations were recorded digitally. A stopwatch was used to measure the time to onset of sedation. Patients were kept in a sitting position for 30-45 minutes and sedation level was evaluated by the Ramsay Sedation Score, which includes items such as drowsiness response before local anesthesia is given. Patients were monitored in the post-operative phase until they reached Ramsay Sedation Score 2. Follow-up was planned for 1 day and telephonic monitoring 3 hours until full recovery, water intake encouraged. Vital signs, anxiety levels and sedation time were documented.

[4] Group 4: Combination group:

The group that received oral Melatonin nitrous oxide also received dietary restrictions and preoperative evaluation, dosage (0.5 mg/kg) oral Melatonin was calculated according to body weight and given with water. Vitals and anxiety levels were taken. The time of sedation was all recorded digitally. Time to effect of sedation was timed with a stopwatch. Patients were placed in a chair for 30-45 minutes and sedation level was evaluated using the Ramsay Sedation Score, which includes criteria such as drowsiness and then nitrous oxide was used prior to local anesthetic. It was observed that the desired level of sedation was reached at less concentration of nitrous oxide i.e. 30 percentage. Patients were monitored in the post-operative phase until they reached Ramsay Sedation Score 2. Drinking water was promoted. Follow up was to be conducted at 1 day and telephonic monitoring 3 hours until full recovery. Vital signs, anxiety and sedation time were noted.

Statistical analysis:

Data obtained were tabulated and statistically analyzed using one-way ANOVA and paired t-test. A p-value less than 0.05 was considered statistically significant.

Results:

Combination therapy resulted in the highest reduction in anxiety scores (62.1%), followed by Nitrous Oxide (53.8%) and Melatonin (50.0%), which were equally effective and the control/TSD group had a 26.2% reduction. Intragroup comparison showed that the combination group ($p = 0.01$), the Nitrous Oxide group ($p = 0.01$) and the Melatonin group ($p = 0.01$) had statistically significant decreases in anxiety scores compared to the placebo group ($p = 0.07$) (**Table 1 and Figure 1**). The Combination group had a significantly greater reduction in anxiety than the Nitrous Oxide group (mean difference: 8.3%, $p = 0.02$) and the Melatonin group (mean difference: 12.1%, $p = 0.01$); the Nitrous Oxide group and the Melatonin group had similar reductions in anxiety, with no statistically significant difference between them (mean difference: 3.8%, $p = 0.07$). The placebo/TSD group had the smallest decrease in anxiety scores. Likewise, the Combination group had the greatest reduction in pulse rate (7.49%), followed by the Nitrous Oxide (5.52%) and Melatonin (5.26%) groups, which had similar reductions, while the placebo group had only a 2.67% reduction (**Table 2, Figure 2**). The Combination, Nitrous Oxide and Melatonin groups had significant decreases in pulse rate ($p \leq 0.03$), whereas the placebo group did not ($p = 0.08$). The behavior score also showed a maximum improvement in the Combination group (58.6%), followed by Nitrous Oxide (50.0%) and Melatonin (44.4%), with the placebo/TSD group showing the least improvement (17.9%). The Combination group had statistically significant differences when compared to the other groups, with the Combination group having better behavioral improvement than the Nitrous Oxide group and the Melatonin group (**Table 3, Table 4 and Figure 3**).

Table 1: VAS Anxiety Scores (Pre Op, Post Op)

Group	Before(Mean $\pm$ SD)	After ((Mean $\pm$ SD)	Mean Reduction $\pm$ SD	% Reduction $\pm$ SD	P-value	Significance
Placebo (TSD)	6.5 $\pm$ 1.2	4.8 $\pm$ 1.1	1.7 $\pm$ 1.3	26.2%	0.07	Not significant
Nitrous Oxide	6.5 $\pm$ 1.2	3.0 $\pm$ 0.9	3.5 $\pm$ 1.5	53.8%	0.01	Significant
Melatonin	6.4 $\pm$ 1.3	3.2 $\pm$ 1.0	3.2 $\pm$ 1.6	50.0%	0.01	Significant

Table 2: Pulse Rate (Pre Op, Post Op)

Group	Before(Mean $\pm$ SD)	After ((Mean $\pm$ SD)	Mean Reduction $\pm$ SD	% Reduction $\pm$ SD	P-value	Significance
Placebo	97.2 $\pm$ 6.3	94.6 $\pm$ 5.7	2.6 $\pm$ 3.2	2.67%	0.08	Not significant
Nitrous Oxide	97.8 $\pm$ 6.2	92.4 $\pm$ 5.0	5.4 $\pm$ 3.9	5.52%	0.03	Significant
Melatonin	96.9 $\pm$ 6.5	91.8 $\pm$ 5.3	5.1 $\pm$ 4.1	5.26%	0.03	Significant
Combination	97.5 $\pm$ 6.0	90.2 $\pm$ 4.8	7.3 $\pm$ 3.6	7.49%	0.01	significant
Combination	6.6 $\pm$ 1.1	2.5 $\pm$ 0.8	4.1 $\pm$ 1.4	62.10%	0.01	Highly significant

Table 3: Descriptive Statistics – VPT Anxiety Scores (Pre Op, Post Op)

Group	Before	After
Nitrous Oxide	3.5	1.5
Melatonin	3.6	1.6
Combination	3.6	1.2

Placebo	3.5	2.8
---------	-----	-----

Table 4: Comparison of change Venham’s Picture Test score within each group

Group	Before (Mean ± SD)	After (Mean ± SD)	Mean Reduction ± SD	% Reduction± SD	P-value	Significance
Placebo (TSD)	2.8 ± 0.7	2.3 ± 0.6	0.5 ± 1.6	17.9%	0.09	Not significant
Nitrous Oxide	2.8 ± 0.7	1.4 ± 0.6	1.4 ± 0.8	50.0%	0.01	Significant
Melatonin	2.7 ± 0.8	1.5 ± 0.5	1.2 ± 0.7	44.4%	0.01	Significant
Combination	2.9 ± 0.6	1.2 ± 0.5	1.7 ± 0.7	58.6%	0.01	Highly significant

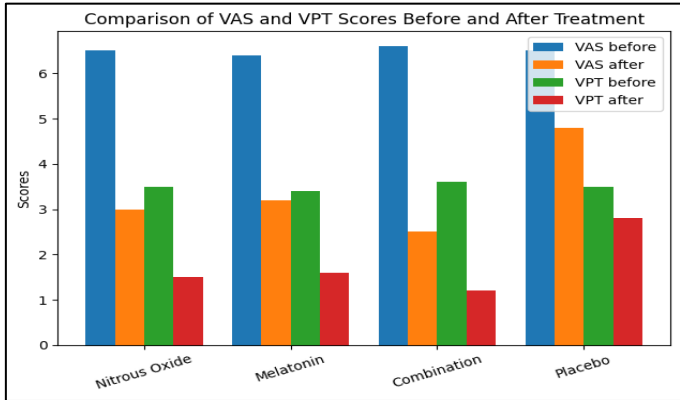


Figure 1: Comparison of change Visual Analog Scale and Venham’s Picture Test score within each group

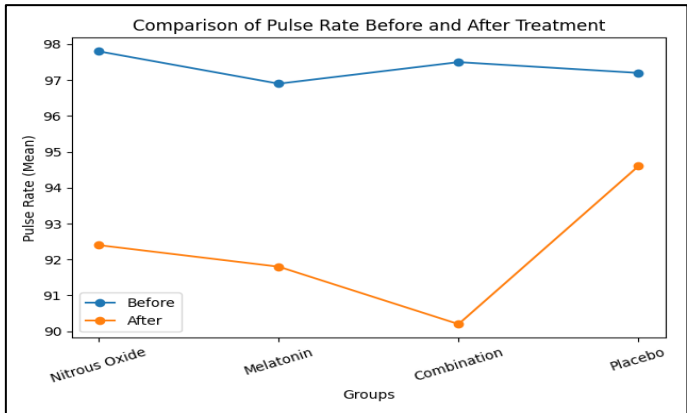


Figure 2: Comparison of change in pulse rate within each group

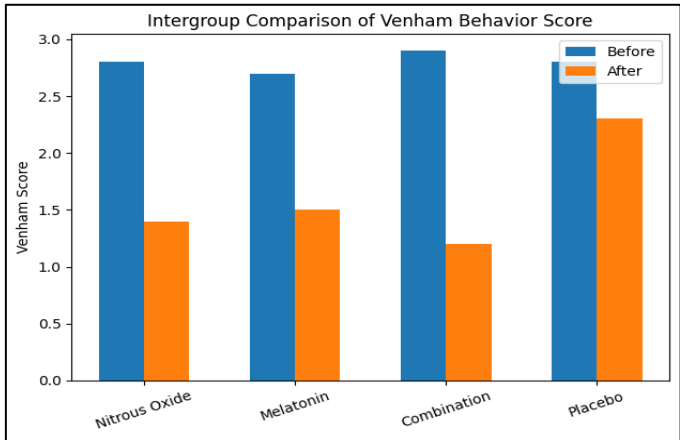


Figure 3: Comparison of change Venham’s Picture Test score within each group

Discussion:

Dental anxiety is a common psychological disorder that affects many people, especially children and can have a negative impact on their oral health. Dental anxiety is a common problem among children and they tend to avoid dental visits, leading to delayed treatment and poor oral hygiene [7]. Approximately 20% of children suffer from this anxiety due to fear of pain, unfamiliar clinical environments and past negative dental experiences. Non-pharmacological interventions are typically recommended as first-line treatment, as reported by Shehani *et al.* [8]. Conventional techniques such as Tell Show-Do (TSD), modeling and distraction therapy have shown a significant effect. TSD is a process of explaining and demonstrating procedures in a child friendly way and increases cooperation to decrease fear, according to Browder *et al.* (2012) [9]. Likewise, Nada Farhat-McHayleh *et al.* (2009) [10] showed that children could be helped to normalize dental procedures by watching peers being treated in a model. Khandelwal *et al.* (2019) [11] mentioned that distraction techniques such as audiovisual therapy are effective in diverting the child's attention. Areef *et al.* (2024) [12] introduced alpha wave music for the purpose of soothing anxious children. Positive reinforcement has also been found to be beneficial in line with Shehani *et al.* (2024) [8] who found that it can help to increase compliance and decrease dental-related fear. In some cases, psychological techniques are not sufficient and pharmacological techniques are used, including oral sedation, nitrous oxide inhalation and general anesthesia. Nitrous oxide (N₂O) is popular for its quick recovery time. The 60 paediatric patients aged 5-12 years were selected in this study as the age of 5-12 years is the age at which dental anxiety is the highest. The selection of extractions as the standard procedure for assessing anxiety was based on their higher stress induction than restorative treatments. Assessment was done using the Visual Analogue Scale (VAS) which was reported as reliable and simple by Shehani *et al.* (2024) [8] and physiological monitoring was carried out using digital pulse oximetry to ensure precision as reported by Sukumaran *et al.* (2025) [13]. The statistical analysis showed that nitrous oxide sedation was the most effective method and it significantly reduced anxiety and had a quicker onset. According to Wu *et al.* (2025), N₂O exerts its main effects by modulation of GABAergic pathways and NMDA receptor inhibition, leading to relaxation and analgesia without loss of consciousness [14]. Physiological responses were also observed, such as decreased pulse and blood pressure, which were signs of decreased sympathetic activity Kanagasundaram *et al.*, (2001) [15]. Children showed higher levels of stress when there was no anxiety control, as evidenced by higher VAS scores and physiological parameters, which is consistent with the

results of Achmad *et al.* (2019) [16]. The results highlight the importance of using effective, child-friendly anxiety management techniques in dental care. Managing dental anxiety is crucial for effective pediatric dental care. Nitrous oxide inhalation sedation is the standard for minimal to moderate conscious sedation due to its rapid onset, titratability, anxiolytic effects and rapid recovery [17]. Melatonin is a promising anxiolytic and sedative agent because of its favorable safety profile and physiologic mechanism of action, which includes binding to melatonin receptors in the central nervous system and modulating gamma-aminobutyric acid (GABA) transmission, which helps to decrease anxiety and increase calmness [18]. Recent evidence further supports the efficacy of both agents in paediatric dental anxiety management. Sinha *et al.* (2025) reported that oral melatonin and nitrous oxide inhalation significantly reduced anxiety, pain, salivary cortisol levels and pulse rate in children, with no significant difference between the two groups in sedation efficacy or post-operative recovery, suggesting that melatonin may represent a viable alternative to nitrous oxide for paediatric dental procedures [19]. The current study showed that melatonin combined with nitrous oxide had greater anxiety reducing effect than either alone. The synergistic effect could be explained by the anxiolytic effect of melatonin before surgery followed by sedation during surgery from nitrous oxide. The children who were treated with combination therapy showed improved cooperation, reduced pulse rate and reduced VAS scores than the control group. The results indicate that combination therapy is effective in reducing the psychological and physiological stress reactions of dental procedures. Oral administration and lack of significant adverse effects were also favorable attributes of melatonin that contributed to its favorable acceptance. Melatonin does not cause any significant respiratory depression or paradoxical reactions as compared to benzodiazepines like Midazolam. Based on the results of this study, combined nitrous oxide and melatonin sedation is a safe and effective approach to managing dental anxiety in children.

Conclusion:

Combination therapy with N₂O and melatonin was most effective in reducing anxiety and pulse rate and improving cooperative behavior in pediatric dental patients. N₂O alone and

melatonin alone showed comparable effects, while the placebo/TSD group demonstrated minimal and non-significant improvements. The N₂O-melatonin combination appears to be a safe and promising approach for improving anxiolytic and behavioral outcomes in anxious children undergoing dental treatment.

References:

- [1] Al-Beltagi M *et al.* *World J Clin Pediatr.* 2025 **14**:106778. [PMID: 40881091]
- [2] Shim YS *et al.* *J Dent Anesth Pain Med.* 2015 **15**:53. [PMID: 28879259]
- [3] Dahlander A *et al.* *Dent J (Basel).* 2019 **7**:68. [PMID: 31266156]
- [4] <https://www.jaypeedigital.com/book/9788184489163>
- [5] Sharma D. *J Indian Soc Pedod Prev Dent.* 2024 **42**:S118. [DOI: 10.4103/jisppd.jisppd_59_24]
- [6] Cavallo A. *J Pediatr.* 1993 **123**:843. [PMID: 8229515]
- [7] Appukuttan DP. *Clin Cosmet Investig Dent.* 2016 **8**:35. [PMID: 27022303]
- [8] Shehani F *et al.* *Eur Arch Paediatr Dent.* 2024 **25**:577. [PMID: 38982009]
- [9] Browder DM *et al.* *Teach Educ Spec Educ.* 2012 **35**:212. [DOI: 10.1177/0888406411432650]
- [10] Farhat-McHayleh N *et al.* *J Can Dent Assoc.* 2009 **75**:283. [PMID: 19422751]
- [11] Khandelwal M *et al.* *Int J Clin Pediatr Dent.* 2019 **12**:18. [PMID: 31496566]
- [12] Areef FS *et al.* *Cureus.* 2024 **16**:e66526. [PMID: 39246978]
- [13] Sukumaran A & Joseph B. *IntechOpen.* 2025. [DOI: 10.5772/intechopen.1009439]
- [14] Wu H *et al.* *Med Gas Res.* 2025 **15**:85. [PMID: 39436171]
- [15] Kanagasundaram SA *et al.* *Arch Dis Child.* 2001 **84**:492. [PMID: 11369566]
- [16] Achmad MH *et al.* *Pesqui Bras Odontopediatria Clin Integr.* 2019 **19**:e4655. [DOI: 10.4034/PBOCI.2019.191.97]
- [17] <https://pmc.ncbi.nlm.nih.gov/articles/PMC2526224/>
- [18] Subhadarshini S *et al.* *Cureus.* 2024 **16**:e60575. [PMID: 38894785]
- [19] Sinha S *et al.* *Eur Arch Paediatr Dent.* 2026 **27**:777. [PMID: 41201777]

Caveat Emptor is applicable among the literate community where required and possible. The publisher, its journal, editors and the internal/external reviewers take adequate steps to check, evaluate, correct, edit, revise and improve content where possible and required.