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Original Article

Comparison between oral melatonin and 24% sucrose for pain management during retinopathy of prematurity screening: a randomized controlled trial

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ABSTRACT

Background. Preterm neonates perceive multiple painful procedures during Neonatal Intensive Care Unit (NICU) stay, having long term neurobehavioral effects. This study aims to compare the analgesic efficacy of oral melatonin with 24% sucrose in neonates during retinopathy of prematurity (ROP) screening.

Methods. A prospective, non-blinded, randomized controlled trial was conducted in a tertiary care NICU. All preterm neonates with gestational age (GA) <34 weeks or birth weight (BW) < 2000 grams eligible for ROP screening were randomized into oral melatonin (4 mg/kg) and oral 24% sucrose (0.5 ml) groups. Both groups received standard non-pharmacological measures and topical proparacaine. The intensity of pain was measured by Premature Infant Pain Profile (PIPP) score during the procedure, at 1st and 5th minutes following the procedure and compared between the two groups by Mann-Whitney U test with p value <0.05 considered as significant.

Results. A total of 60 preterm neonates were randomized with 30 neonates in the melatonin (median [interquartile range] GA: 30.86 [3.78] weeks, BW: 1160 [430] grams) and 30 neonates in the 24% sucrose (median [IQR] GA: 29.29 [4.68] weeks, BW: 1070 [315] grams) group. The median PIPP score during the procedure in the melatonin and sucrose groups were 17 and 16, respectively (p=0.64). The median (Q1-Q3) PIPP score at the 1st minute was significantly lower among the melatonin group (7 [5.25-10]) vs 24% sucrose group (9.5 [7.25-11]) (p=0.02); and at the 5th minute, the median (Q1-Q3) PIPP scores in the melatonin group (5 [4-6]) was comparable to the 24% sucrose group (5.5 [3.25-7]) (p= 0.52).

Conclusions. Oral melatonin is not inferior to oral 24% sucrose for pain management during ROP screening.

Key words: retinopathy of prematurity, melatonin, analgesic, neonate.

Neonates are anatomically and physiologically capable of feeling pain; and inadequate pain management evokes long-term consequences.[1,2] A number of validated tools are used for pain assessment and various non-pharmacological (nesting, swaddling, non-nutritive sucking, facilitated tucking, kangaroo mother care etc.) as well as pharmacological (sucrose, dextrose, opioids, acetaminophen etc.) interventions are used for pain management in neonates.[3,4] Retinopathy of prematurity (ROP) screening is an essential procedure for the prevention of visual morbidity of preterm neonates. It inflicts severe pain during an eye examination with the persistence of residual pain up to 30 min after the procedure.[5] The recent recommendation includes various non-pharmacological measures, local anesthetics and oral 24% sucrose before ROP screening.[6]

Melatonin is a hormone synthesized and secreted by the pineal gland. In a meta-analysis,

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melatonin was effective in reducing chronic pain in the adult population.[7] There is only a single study evaluating the analgesic activity of intravenous melatonin in mechanically ventilated preterm neonates.[8] The present study aimed to explore the analgesic effect of oral melatonin and to compare its efficacy with 24% sucrose solution in neonates undergoing ROP screening.

Material and Methods

This prospective, non-blinded, parallel, randomized controlled trial was conducted in the neonatal intensive care unit of Kalinga Institute of Medical Sciences, Bhubaneswar from February 2021 to July 2021 after approval from the institutional ethics committee and drug trial (CTRI) registration (Institutional Ethics Committee, Kalinga Institute of Medical Sciences, CTRI/2021/02/031458).

Neonates with a gestational age <34 weeks or birth weight < 2000 grams undergoing ROP screening during Neonatal Intensive Care Unit (NICU) stay, and receiving partial paladai feeds (at least 7 ml/kg of breastmilk per feeding) were included in the study. Neonates on mechanical ventilation, ionotropic support, opioid analgesics, sedatives or anticonvulsants during ROP screening were excluded. As per unit protocol neonates with gestational age ≤ 28 weeks or birth weight ≤ 1250 grams were subjected to the first ROP screening in the second or third week of age whereas neonates of higher gestational age underwent the first screen by the fourth week of age. Some of them were subjected to repeated follow-up screens till full vascularization of the retina. The eligible neonates after randomization were included only once, out of several ROP examinations during their NICU stay, for study purposes. Parental consent was taken prior to case recruitment. Patients were assigned into the intervention and control groups, in a ratio of 1:1, by computer generated random list and allocation concealment was done in an opaque sealed envelope. Sample size calculation: In a previous study, 43% of neonates had no painful reaction during ROP examination with oral 24% sucrose vs 22% neonates with placebo.[9] Assuming 10% difference points in analgesic effect between 24% sucrose and oral melatonin, alpha error 5%, power 80% and 1:1 allocation ratio, the required sample size was 54 (27 in each arm). Considering a 10% attrition rate, calculated sample size was 60 (30 in each arm).

The baseline demographic neonatal characteristics, heart rate (HR) and oxygen saturation (SpO_2) denoted by Multipara monitors were recorded in a pre-structured proforma. Indirect ophthalmoscopy for ROP screening was done by a trained ophthalmologist. Prior to the procedure eye drop containing 0.8% tropicamide with 5.0% phenylephrine (Auromide Plus Drop by "Aurolab") was used four times at 10 minute intervals to dilate the pupils, and the infants were fed at least an hour before screening. Neonates in the intervention group were given melatonin (Syrup Trunap, 3mg/5ml, "Brio Bliss Life Science Pvt. Ltd") at a dose of 4mg/kg (~ 6.6ml/kg) orally 20 minutes prior to the procedure. The syrup Trunap contains melatonin as an active ingredient with the presence of minor ingredients as vehicle, preservative, flavoring agent similar to any other oral pediatric formulation. The control group received 0.5 ml oral 24% sucrose (Arbineo sachet by "Raptakos") 2 minutes prior to the procedure. Eye drop 0.5% proparacaine (Aurocaine drop by "Aurolab") was used for neonates in both arms just before the procedure. All the neonates were provided with non-pharmacological interventions such as nesting, swaddling and facilitated tucking by nursing staff in a dim light environment throughout the procedure. The chronological age (days), post menstrual age (gestational age plus chronological age in weeks) and weight of the baby at the time of the ROP examination, were duly noted in the proforma. Premature Infant Pain Profile (PIPP) score was used for the assessment of the severity of pain - during the procedure, at 1 minute and 5 minutes after the procedure. The parameters of the PIPP Scale are

gestational age, behavioral state, highest heart rate, lowest SpO_2, brow bulge, eye squeeze and nasolabial furrow wherein each parameter is scored from 0 to 3 with the maximum score being 21. The pain scoring was done by the primary investigator whereas timekeeping was done by the nurse educator with a stopwatch. The severity was categorized as mild/no pain (<6), moderate pain (6-12) and severe pain (>12). Complete pain relief was denoted by a PIPP score of less than 6 at any time during the study. Neonates recorded to have moderate to severe pain 5 minutes after the procedure (PIPP score ≥ 10) received oral paracetamol, 10 mg/kg.

Any adverse effects such as apnea, respiratory distress, arrhythmias, vomiting or feeding difficulties were monitored for the next 24 hours in both arms. Stoppage of the trial was planned in case of any major adverse effect such as any acute life threatening event noted in either of the groups.

Statistical analysis

All data was recorded in Microsoft Excel format. Statistical analysis was performed using SPSS version 20.0 (SPSS Inc., Chicago, IL, USA). Quantitative variables are expressed as mean with standard deviation (SD) or median with quartile range (Q1-Q3). The qualitative variables are described in terms of frequencies & proportion. The significance of the differences between the study groups was tested using the Mann-Whitney U test. Differences in categorical variables were tested using a chi-square test/ Fisher exact test. A p value < 0.05 was considered statistically significant in all statistical tests.

Results

A total of 108 preterm neonates were eligible for ROP screening and 48 neonates were excluded (25 discharged prior to ROP screening, 6 left against medical advice and 8 neonatal deaths before the screen, no parental consent for 9 neonates). The remaining 60 neonates were randomized into 30 in the melatonin and 30 in the 24% sucrose groups. Fig. 1 depicts the flow diagram of the study participants. The baseline characteristics of neonates were similar in both groups (Table I).

During the procedure, the median (Q1-Q3) PIPP score in the melatonin and 24% sucrose groups were 16 (14-17) and 15.50 (13.25-17) respectively and the neonates of both groups perceived severe pain. The median PIPP score was significantly lower in the melatonin vs 24% sucrose group at 1 minute after the procedure (p=0.02) but was not significantly different between the groups (p=0.52) at 5 minutes after the procedure (Table II). Only one neonate in the melatonin group had moderate pain at the 10th minute after the procedure (PIPP score-10) and needed an add on analgesic i.e. oral paracetamol. None of the neonates in the oral 24% sucrose group needed additional analgesics.

One neonate in the sucrose group had two episodes of apnea one minute after the procedure and was revived with tactile stimulation. One baby in the melatonin group had respiratory distress after a few minutes of the procedure requiring low flow oxygen at 1 L/min and it subsided within the next 30 minutes. Two neonates in the melatonin group developed one episode of non-bilious vomiting within one hour of the procedure. In neither of the arms, any of the neonates had difficulty in oral feeding after the procedure. There was no significant difference in adverse events between both groups.

Discussion

We have shown that the analgesic effect of oral melatonin is not inferior to oral 24% sucrose during the post-procedural period of ROP screening. The pain inflicted during ROP screening was not well relieved with either of the analgesic agents, in spite of the use of additional non-pharmacological measures and topical anesthetic agents. At 1 minute after the procedure, the median PIPP score in the melatonin group (7) was significantly lower (p=0.02) compared to the 24% sucrose

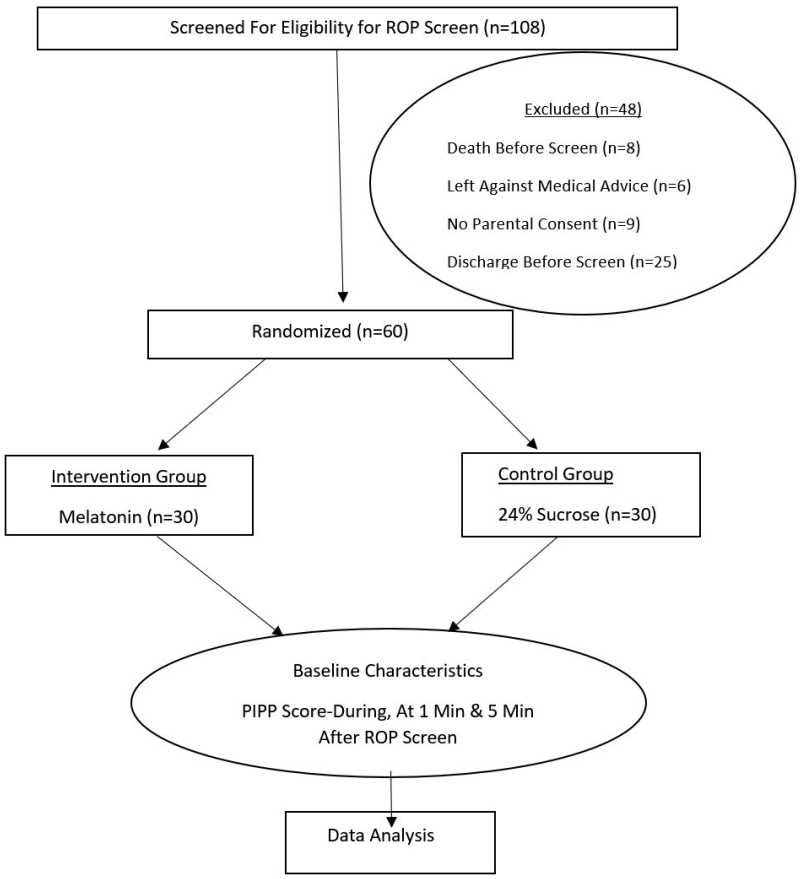

Fig. 1. Study participants flow diagram.
PIPP: Premature Infant Pain Profile, ROP: Retinopathy of prematurity

Table I. Comparison of baseline characteristics of neonates between Melatonin and Sucrose groups.

Variables	Median (Q_1-Q_3)		*p*-value
	Melatonin Group (N=30)	Sucrose Group (N=30)	
Gestational age (weeks)	30.86 (29.33-33.11)	29.29 (28.07-32.75)	0.15
Birth weight (grams)	1160.00 (1002.50-1432.50)	1070.00 (980.00- 1295.00)	0.23
Day of examination	26.50 (21.25- 41.50)	27.50 (20.25-40.00)	0.93
PMA at ROP screen (weeks)	35.64 (33.78-37.00)	35.86 (33.11-36.82)	0.59
Weight at ROP screen (grams)	1600.00 (1388.75-1787.50)	1485.00 (1240.00-1702.50)	0.13
Baseline heart rate (BPM)	149.00 (141.25-157.75)	146.50 (136.25-157.00)	0.49
Baseline SpO_2(%)	97.50 (96.00-99.00)	98.00 (95.00-99.00)	0.80

PMA: Post-menstrual age, BPM: Beats per minute, ROP: Retinopathy of prematurity

Table II. Comparison of pain scoring (PIPP score) between the two groups during and after examination.

Parameters	Median (Q_1-Q_3)			*p*-value
Pain Score	Melatonin Group (N=30)	Sucrose Group (N=30)	Total (N=60)	
PIPP score during examination	16.00 (14.00-17.00)	15.50 (13.25-17.00)	16.00 (14.00-17.00)	0.64
PIPP score at 1st minute	7.00 (5.25-10.00)	9.50 (7.25-11.00)	8.00 (7.00- 10.00)	0.02
PIPP score at 5th minute	5.00 (4.00-6.00)	5.50 (3.25-7.00)	5.00 (4.00- 6.00)	0.52

PIPP: Premature Infant Pain Profile

group (9.5) and around one third of neonates in the melatonin vs one tenth of neonates in the sucrose group had no pain (*p*=0.05). At the 5th minute after the procedure a majority of neonates in both the melatonin (median PIPP score-5, 86.66% with PIPP score <6) and sucrose (median PIPP score-5.5, 70% with PIPP score < 6) groups had no or minimal pain. To the best of our knowledge, this is the first study to explore the analgesic effect of oral melatonin and compares its efficacy with standard analgesic agent 24% sucrose in a randomized control trial.

The inconclusive effectiveness of either melatonin or sucrose during the ROP examination could be related to the severity of pain during the procedure. In a Cochrane systematic review, 24% sucrose was found to be a safe and effective analgesic agent in mild to moderate pain in neonates.[10] Grabska et al.[11] and Rush et al.[12] were unable to demonstrate a significant analgesic effect between a placebo and 24% sucrose during ROP screening in neonates. The analgesic effect of sucrose in combination with a pacifier was found to be greater than a placebo with a pacifier in two studies.[13,14]

The evidence of the analgesic effect of melatonin is very limited in neonates. The exact anti-nociceptive action of melatonin is not known and possible pathways are mostly explored from animal studies. Melatonin may regulate pain via various receptors i.e. MT1/MT2 –melatonin receptors, opioid l-receptors, GABA receptors present in both the central and peripheral nervous system, release of β-endorphins and the nitric oxide-arginine pathway.[15] In adult human studies, melatonin reduces acute pain during the post-operative period.[16] The anti-inflammatory cytokine pathway was found in a neonatal study for its late onset nociceptive effect.[8] However, the early analgesic effect of melatonin noticed in the present trial needs further studies to explore its mechanism.

We used oral melatonin suspension due to non-availability of intravenous formulations in this part of the country. To date, the safety and efficacy of melatonin have been established in the intravenous route at a dose of 3-10 mg/kg in neonates.[8,17-22] Based on an allometric evaluation, the oral melatonin dosage was estimated between 0.5-5 mg/kg for preterm neonates in a pharmacokinetic study.[23,24] The analgesic effect of melatonin was noticed with its oral administration 30 minutes prior to venipuncture in pediatric study participants aged between 1-14 years.[25] The paucity of pharmacokinetics data is the major limitation in establishing appropriate analgesic dosage of melatonin in neonates. The dose of melatonin used in the current study was extrapolated from available relevant literature.

The anti-inflammatory, antioxidant and neuroprotective behavior of melatonin have been studied in various neonatal diseases i.e. hypoxic ischemic encephalopathy, sepsis, respiratory distress syndrome, bronchopulmonary dysplasia and neonatal surgery.[18-22,26] The myelination in white matter of the preterm brain could be protected by melatonin and its metabolites.[27,28] Preterm neonates

usually require multiple painful procedures during their hospital stay. Considering its neuroprotective effect, melatonin could be explored as an analgesic drug that might be used multiple times. However, repeated use of sucrose analgesia has worse neurobehavioral development and physiologic outcomes in a preterm neonatal study by Johnston et al.[29] Again sucrose has no effect on pain related brain activity in an EEG based neonatal study by Slater et al.[30] In a systematic review, authors were concerned regarding the neurodevelopmental outcome with multiple times use of oral sucrose as analgesia.[31] Further research is needed for a head-to-head comparison of multiple doses of sucrose versus melatonin for the long-term neurodevelopmental outcomes.

In the 24% sucrose group, one neonate had apnea within one minute of the procedure. In a study by Dilli et al.[13], around one-third of total neonates had apnea and bradycardia following ROP screening both in the sucrose and placebo groups. Most of the neonates well tolerated the oral melatonin at 4 mg/kg dose apart from one episode of vomiting noted in two neonates and one neonate had transient respiratory distress. The vomiting episodes could be due to the adverse effect of melatonin or post-procedural pain and there was no persisting difficulty in paladai feeding. As 10% of neonates in the melatonin group faced some kind of adverse events, the safety of the drug needs to be evaluated in future studies.

This study has many limitations, one of them being a monocentric study with a relatively small sample size. The analgesic action of melatonin is evaluated only in a single procedure in hemodynamically stable neonates after completion of intensive care management. Hence its safety and effectiveness may not be generalized to critically ill neonates and also for different types of procedures. The sample size is relatively small to address the adverse effect of a novel drug like melatonin. Additionally, in this study, the analgesic effect of melatonin was measured along with standard non-pharmacological measures and topical anesthesia, thus the isolated effect of melatonin was not evaluated.

Oral melatonin may be an alternative medication to oral 24% sucrose for moderate to severe pain management in neonates. Hence further studies are needed to explore the analgesic effect of melatonin in neonatal practice with long term neurodevelopmental effects.

Ethical approval

Institutional Ethics Committee (KIMS/KIIT/IEC/535/2020) dt. 29/12/2020 CTRI Number – CTRI/2021/02/031458.

Author contribution

Study conception and design: SKP; data collection: SSB, AD; analysis and interpretation of results: BN, SKP; draft manuscript preparation: SKP, SSB, BN, AD. All authors reviewed the results and approved the final version of the manuscript.

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Conflict of interest

The authors declare that there is no conflict of interest.

REFERENCES

1. Brummelte S, Grunau RE, Chau V, et al. Procedural pain and brain development in premature newborns. Ann Neurol 2012; 71: 385-396. https://doi.org/10.1002/ana.22267
2. Valeri BO, Holsti L, Linhares MBM. Neonatal pain and developmental outcomes in children born preterm: a systematic review. Clin J Pain 2015; 31: 355-362. https://doi.org/10.1097/AJP.0000000000000114

3. Witt N, Coynor S, Edwards C, Bradshaw H. A Guide to pain assessment and management in the neonate. Curr Emerg Hosp Med Rep 2016; 4: 1-10. https://doi.org/10.1007/s40138-016-0089-y

4. Hall RW, Anand KJS. Pain management in newborns. Clin Perinatol 2014; 41: 895-924. https://doi.org/10.1016/j.clp.2014.08.010

5. Mitchell AJ, Green A, Jeffs DA, Roberson PK. Physiologic effects of retinopathy of prematurity screening examinations. Adv Neonatal Care 2011; 11: 291-297. https://doi.org/10.1097/ANC.0b013e318225a332

6. Samra HA, McGrath JM. Pain management during retinopathy of prematurity eye examinations: a systematic review. Adv Neonatal Care 2009; 9: 99-110. https://doi.org/10.1097/ANC.0b013e3181a68b48

7. Zhu C, Xu Y, Duan Y, et al. Exogenous melatonin in the treatment of pain: a systematic review and meta-analysis. Oncotarget 2017; 8: 100582-100592. https://doi.org/10.18632/oncotarget.21504

8. Gitto E, Aversa S, Salpietro CD, et al. Pain in neonatal intensive care: role of melatonin as an analgesic antioxidant. J Pineal Res 2012; 52: 291-295. https://doi.org/10.1111/j.1600-079X.2011.00941.x

9. Gal P, Kissling GE, Young WO, et al. Efficacy of sucrose to reduce pain in premature infants during eye examinations for retinopathy of prematurity. Ann Pharmacother 2005; 39: 1029-1033. https://doi.org/10.1345/aph.1E477

10. Stevens B, Yamada J, Ohlsson A, Haliburton S, Shorkey A. Sucrose for analgesia in newborn infants undergoing painful procedures. Cochrane Database Syst Rev 2016; 7: CD001069. https://doi.org/10.1002/14651858.CD001069.pub5

11. Grabska J, Walden P, Lerer T, et al. Can oral sucrose reduce the pain and distress associated with screening for retinopathy of prematurity? J Perinatol 2005; 25: 33-35. https://doi.org/10.1038/sj.jp.7211199

12. Rush R, Rush S, Ighani F, Anderson B, Irwin M, Naqvi M. The effects of comfort care on the pain response in preterm infants undergoing screening for retinopathy of prematurity. Retina 2005; 25: 59-62. https://doi.org/10.1097/00006982-200501000-00008

13. Dilli D, İlarslan NEÇ, Kabataş EU, Zenciroğlu A, Şimşek Y, Okumuş N. Oral sucrose and non-nutritive sucking goes some way to reducing pain during retinopathy of prematurity eye examinations. Acta Paediatr 2014; 103: e76-9. https://doi.org/10.1111/apa.12454

14. Mitchell A, Stevens B, Mungan N, Johnson W, Lobert S, Boss B. Analgesic effects of oral sucrose and pacifier during eye examinations for retinopathy of prematurity. Pain Manag Nurs 2004; 5: 160-168. https://doi.org/10.1016/j.pmn.2004.06.001

15. Chen WW, Zhang X, Huang WJ. Pain control by melatonin: Physiological and pharmacological effects. Exp Ther Med 2016; 12: 1963-1968. https://doi.org/10.3892/etm.2016.3565

16. Andersen LPH, Werner MU, Rosenberg J, Gögenur I. A systematic review of peri-operative melatonin. Anaesthesia 2014; 69: 1163-1171. https://doi.org/10.1111/anae.12717

17. Dixon BJ, Reis C, Ho WM, Tang J, Zhang JH. Neuroprotective strategies after neonatal hypoxic ischemic encephalopathy. Int J Mol Sci 2015; 16: 22368-22401. https://doi.org/10.3390/ijms160922368

18. Gitto E, Karbownik M, Reiter RJ, et al. Effects of melatonin treatment in septic newborns. Pediatr Res 2001; 50: 756-760. https://doi.org/10.1203/00006450-200112000-00021

19. Gitto E, Reiter RJ, Amodio A, et al. Early indicators of chronic lung disease in preterm infants with respiratory distress syndrome and their inhibition by melatonin. J Pineal Res 2004; 36: 250-255. https://doi.org/10.1111/j.1600-079X.2004.00124.x

20. Gitto E, Reiter RJ, Cordaro SP, et al. Oxidative and inflammatory parameters in respiratory distress syndrome of preterm newborns: beneficial effects of melatonin. Am J Perinatol 2004; 21: 209-216. https://doi.org/10.1055/s-2004-828610

21. Gitto E, Reiter RJ, Sabatino G, et al. Correlation among cytokines, bronchopulmonary dysplasia and modality of ventilation in preterm newborns: improvement with melatonin treatment. J Pineal Res 2005; 39: 287-293. https://doi.org/10.1111/j.1600-079X.2005.00251.x

22. Gitto E, Romeo C, Reiter RJ, et al. Melatonin reduces oxidative stress in surgical neonates. J Pediatr Surg 2004; 39: 184-189; discussion 184-9. https://doi.org/10.1016/j.jpedsurg.2003.10.003

23. Nair AB, Jacob S. A simple practice guide for dose conversion between animals and human. J Basic Clin Pharm 2016; 7: 27-31. https://doi.org/10.4103/0976-0105.177703

24. Carloni S, Proietti F, Rocchi M, et al. Melatonin pharmacokinetics following oral administration in preterm neonates. Molecules 2017; 22: 2115. https://doi.org/10.3390/molecules22122115

25. Marseglia L, Manti S, D'Angelo G, et al. Potential use of melatonin in procedural anxiety and pain in children undergoing blood withdrawal. J Biol Regul Homeost Agents 2015; 29: 509-514

26. Aly H, Elmahdy H, El-Dib M, et al. Melatonin use for neuroprotection in perinatal asphyxia: a randomized controlled pilot study. J Perinatol 2015; 35: 186-191. https://doi.org/10.1038/jp.2014.186

27. Biran V, Phan Duy A, Decobert F, Bednarek N, Alberti C, Baud O. Is melatonin ready to be used in preterm infants as a neuroprotectant? Dev Med Child Neurol 2014; 56: 717-723. https://doi.org/10.1111/dmcn.12415

28. Villapol S, Fau S, Renolleau S, Biran V, Charriaut-Marlangue C, Baud O. Melatonin promotes myelination by decreasing white matter inflammation after neonatal stroke. Pediatr Res 2011; 69: 51-55. https://doi.org/10.1203/PDR.0b013e3181fcb40b

29. Johnston CC, Filion F, Snider L, et al. Routine sucrose analgesia during the first week of life in neonates younger than 31 weeks' postconceptional age. Pediatrics 2002; 110: 523-528. https://doi.org/10.1542/peds.110.3.523

30. Slater R, Cornelissen L, Fabrizi L, et al. Oral sucrose as an analgesic drug for procedural pain in newborn infants: a randomised controlled trial. Lancet 2010; 376: 1225-1232. https://doi.org/10.1016/S0140-6736(10)61303-7

31. Gao H, Gao H, Xu G, et al. Efficacy and safety of repeated oral sucrose for repeated procedural pain in neonates: a systematic review. Int J Nurs Stud 2016; 62: 118-125. https://doi.org/10.1016/j.ijnurstu.2016.07.015