Original Research

A STUDY PROTOCOL FOR A DOUBLE-ARM RANDOMISED CONTROLLED TRIAL TO EVALUATE THE ANAESTHETIC EFFICACY OF A NOVEL TOPICAL AYURVEDIC EMULGEL DURING NEONATAL VENIPUNCTURE

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ABSTRACT

Background: Effective pain management in neonates during procedures such as venipuncture is critical to minimise potential adverse neurodevelopmental and psychological consequences. Conventional synthetic topical anaesthetics, while effective, may pose safety concerns in this vulnerable population. This study protocol proposes to evaluate a novel topical ayurvedic emulgel (NTAE) containing Anacyclus pyrethrum (Akarkarabha) and Syzygium aromaticum (Lavanga) as a potentially safer alternative to standard lidocaine–prilocaine cream (LPC) for neonatal venipuncture.

Objectives: The primary objective is to evaluate the anaesthetic efficacy of NTAE in reducing pain during venipuncture in neonates. Secondary objectives include comparison with LPC and assessment of the incidence of local adverse effects following topical anaesthetic application.

Methods: This will be a single-centre, prospective, randomised-controlled, parallel-group, single-blind clinical trial. A maximum proposed sample size of one hundred neonates will be randomly assigned to either the intervention group receiving NTAE or the control group receiving LPC. The trial will be conducted in two phases based on application duration: Phase I (30-minute application) and Phase II (60-minute application), in accordance with the revised IEC-approved protocol. The study drug will comprise Anacyclus pyrethrum ethanol extract and Syzygium aromaticum oil formulated as an emulgel using standardised procedures. Pain will be assessed using the Neonatal Infant Pain Scale (NIPS) and the Neonatal Facial Coding System (NFCS), along with monitoring of physiological parameters such as heart rate and oxygen saturation.

Conclusion: This study protocol aims to generate evidence regarding the feasibility, efficacy, and safety of an ayurvedic topical anaesthetic formulation for neonatal venipuncture and may inform the design of future integrative and multicentric clinical trials in neonatal pain management.

Keywords: Neonatal Pain, Procedural Pain Management, Ayurvedic Topical Anaesthetic, Neonatal Pain Assessment.

INTRODUCTION

Neonates are a vulnerable population with altered pain perception and a limited ability to verbally express pain, making effective pain management crucial to prevent adverse neurodevelopmental and psychological consequences.[1] Painful procedures such as venipuncture are routine in neonatal intensive care units (NICUs), which makes the need for safe and effective pain relief strategies even more critical.

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Current synthetic anaesthetics, while commonly used, present safety concerns, including the risk of toxicity and allergic reactions.[2] This highlights a significant gap in neonatal care and the need to explore safer, efficacious alternatives.

Traditional Ayurvedic literature describes several botanicals with analgesic and anaesthetic properties. Anacyclus pyrethrum (Akarkarabha) and Syzygium aromaticum (Lavanga) have long been used in traditional medicine for their analgesic and anaesthetic properties, with studies supporting their pharmacological efficacy and safety. Anacyclus pyrethrum exhibits a wide range of biological activities, including antibacterial, antiviral, analgesic, and local anaesthetic effects.[3] Notably, ethanol extract of Anacyclus pyrethrum has shown local anaesthetic potency comparable to that of

lidocaine in experimental studies. Preclinical trials have confirmed its local anaesthetic activity in guinea pigs.[4] Likewise, Syzygium aromaticum contains eugenol, a compound known to reduce pain perception during dental procedures and minor surgeries.[5] However, the clinical application of these phytogenic anaesthetics in neonatal care remains underexplored, with most evidence limited to paediatric dentistry.[6]

Although Anacyclus pyrethrum and Syzygium aromaticum demonstrate substantial potential as anaesthetic agents, their effectiveness and safety in neonatal pain management, specifically during venipuncture, require clinical investigation. Lidocaine-Prilocaine cream (LPC) is widely used for pain mitigation in neonates but concerns about systemic absorption and the risk of methaemoglobinaemia, especially with prolonged use, have raised safety issues.[7]

Given this context, the present study protocol aims to evaluate the anaesthetic efficacy and safety of a novel topical ayurvedic emulgel (NTAE) formulation combining Anacyclus pyrethrum and Syzygium aromaticum for pain management during neonatal venipuncture.

METHODS

Trial Design

This study will be a single-centre, prospective, randomised-controlled, parallel-group, two-phased, single-blind (participants blinded) clinical trial. Eligible neonates will be randomly assigned in a 1:1 ratio to one of two groups: the intervention group, which will receive the NTAE, or the control group, which will receive the LPC. The trial will be conducted in two phases: Phase I (30-minute drug application) and Phase II (60-minute drug application), in accordance with the revised IEC-approved protocol.

The study will follow the Standard Protocol Items: Recommendations for Interventional Trials (SPIRIT) 2013 guidelines to ensure comprehensive and transparent trial methodology. It has been prospectively registered with the Clinical Trials Registry - India (CTRI) under registration number CTRI/2024/03/063807.

Trial Amendment

During the early implementation phase of the study, it was recognised that the 30-minute application duration may not fully align with pharmacodynamic evidence available for lidocaine–prilocaine formulations, which typically require longer application periods (60–120 minutes) for optimal anaesthetic efficacy.

In order to ensure a methodologically robust and clinically fair comparative evaluation, a protocol amendment was undertaken. The amendment was approved following verbal communication and was formally ratified by the Institutional Ethics Committee in its meeting held on 7 August 2025 (Approval No.: BVDUCOA/EC/2062/2025-26).

The approved amendment increased the planned sample size to 50 neonates per group and incorporated a 60-minute application subgroup (Phase II). Recruitment under the amended protocol is ongoing. A corresponding update has been submitted to the Clinical Trials Registry – India (CTRI), and confirmation of registry modification is awaited.

Study Setting

The trial will be conducted in the Neonatal Intensive Care Unit (NICU) of Bharati Ayurved Hospital, Bharati Vidyapeeth (Deemmed to be University), Pune, Maharashtra. The target population will comprise neonates aged between 48 hours and 28 days.

Eligibility Criteria

Neonates eligible for inclusion will be required to be ≥48 hours old, will weigh ≥2500 grams at the time of procedure, will have a gestational age of at least 37 weeks, and will be scheduled for venipuncture. Neonates with major congenital abnormalities, dermatological issues at the application site, or requiring life support will be excluded. Additional exclusions will include neonates on systemic medications (except nutritional supplements), those born to immunocompromised mothers, and those currently enrolled in other clinical trials.

Intervention

Eligible neonates will be randomized into two parallel groups: a trial group that will receive the NTAE and a control group that will receive LPC. The trial formulation will consist of Anacyclus pyrethrum and Syzygium aromaticum, compounded into an emulgel using standardized pharmaceutical procedures. The control formulation, LPC, will be a commercially available eutectic mixture cream containing 2.5% Lidocaine and 2.5% Prilocaine.

In accordance with the IEC-approved protocol amendment, participants will receive the assigned formulation for either 30 minutes (Phase I) or 60 minutes (Phase II), depending on the phase of recruitment. Approximately one gram of the assigned anaesthetic will be applied under an occlusive dressing to the intended venipuncture site. After the designated application duration, the dressing will be removed, and the site will be cleansed with isopropyl alcohol. Venipuncture will then be performed under aseptic conditions.

Pain and physiological parameters will be assessed at baseline, during the procedure, and 30 minutes post-procedure using the Neonatal Infant Pain Scale (NIPS),[8] the Neonatal Facial Coding System (NFCS),[9] and physiological monitors.

Standardised protocols will be followed across both groups and phases to ensure uniformity of application and monitoring.

Discontinuation Criteria

The intervention will be discontinued if adverse reactions such as skin lesions, severe irritation, systemic allergic responses, or new medical conditions are observed. Parental withdrawal of consent will also result in immediate discontinuation. Modifications in dosage or formulation may be considered based on emerging safety data.

Outcome Measures

Primary Outcomes

The primary outcomes will include pain perception measured using the Neonatal Infant Pain Scale (NIPS) and the Neonatal Facial Coding System (NFCS), changes in physiological parameters (heart rate, respiratory rate, and oxygen saturation), time taken to return to a calm state post-procedure, and the success rate of venipuncture on the first attempt. These outcomes are planned to be analysed using appropriate statistical methods and will be summarised as means, medians, or proportions depending on the nature of the variable.

Secondary Outcomes

Secondary outcomes will focus on the incidence of adverse events within 24 hours of the procedure, such as inflammation, erythema, skin integrity changes, or local infections.

Participant Timeline

Following informed consent, enrolled neonates will undergo baseline assessments. The assigned intervention will be applied 30 to 60 minutes prior to venipuncture. Pain scores and physiological signs will be monitored during and after the procedure. Adverse events will be evaluated 24 hours post-intervention. The detailed schedule is illustrated in **Table 1**.

Table 1: Participant Timeline for Enrolment, Interventions, Assessments, and Monitoring

Study Activity	Day 0 (enrolment)	T–X min* (intervention application)	T0 (venipuncture)	T+30 min (post-procedure)	T+24 hrs (follow-up)
ENROLMENT					
Screening for eligibility	✓				
Informed consent process	✓				
Randomization and allocation	✓				
Case record form initiation	✓				
INTERVENTION APPLICATION					
Trial emulgel or control cream application		✓			
ASSESSMENTS					
Pain score assessment (NIPS, NFCS)		✓	✓	✓	
Vital signs (HR, RR, SpO_2)		✓	✓	✓	
CRF documentation	✓	✓	✓	✓	✓
APPLICATION SITE MONITORING					
Application site inspection			✓	✓	✓
ADVERSE EVENT MONITORING		✓	✓	✓	✓
Observation for reactions/discomfort		✓	✓	✓	✓
Management of adverse events (if applicable)			As needed	As needed	As needed
WITHDRAWAL/DISCONTINUATION CRITERIA					
Monitoring for withdrawal (e.g., skin lesion, illness, parental request)	✓	✓	✓	✓	✓

*T–X refers to application timepoint:
- T–30 min for Phase I (n = 50 neonates)
- T–60 min for Phase II (n = 50 neonates)

Sample Size

The initial sample size was determined based on feasibility considerations and estimated availability of eligible neonates, with IEC approval for a minimum of 36 participants per group.

Following review of emerging methodological considerations and pharmacological evidence regarding application duration, an IEC-approved protocol amendment expanded the planned sample size to 50 neonates per group (total n = 100) and incorporated a second phase with a 60-minute application duration.

The revised sample size is expected to enhance statistical robustness and allow subgroup analyses based on duration of application. Recruitment remains ongoing under the amended protocol.

Recruitment Strategy

Neonates meeting the inclusion criteria will be screened for participation in the study. Prior to screening, parents will be provided with detailed information about the study, including the research objectives, the study drugs, their administration, and anticipated outcomes. Parents expressing willingness based on the information provided will be approached, and neonates of willing parents will then be screened for eligibility.

Specific criteria will be established for neonatal dropout during the study. A neonate will be withdrawn if the parent chooses to discontinue participation at any point, or if the neonate develops skin lesions at the site of anaesthetic application or falls ill before the venipuncture procedure.

A case record form will be used to document relevant information collected from parents. Written proxy consent will be obtained from parents whose neonates meet the inclusion criteria. Before agreeing to participate, the informed consent process will ensure that parents are fully aware of the study's objectives, procedures, potential risks, and benefits. This consent process will adhere to the recommendations of the American Academy of Paediatrics (AAP) and the Indian Academy of Paediatrics (IAP).[10,11]

Participant Allocation

Participants will be randomly assigned in a 1:1 ratio to either the trial group or the control group. Randomization will be performed using a chit-based selection method. Sequentially numbered, opaque envelopes containing pre-assigned group allocation chits will be prepared in advance to ensure allocation concealment. Upon enrolment, an envelope will be drawn at the bedside to determine group assignment.

Blinding

The study will be conducted as a single-blind trial, wherein only the participants (neonates and their parents or legal guardians) will be blinded to group allocation. Due to distinguishable differences in the colour, consistency, and aroma of the two formulations, blinding of the clinical staff and outcome assessors may not be feasible. Consequently, outcome assessments, including behavioural pain scores and physiological parameters, will be conducted using standardised protocols to minimise observer bias. Investigators will be aware of group assignments, and outcome assessors may remain unblinded due to practical constraints.

Data Collection, Management, and Analysis

Outcome data, including pain scores and physiological parameters, will be collected using validated tools and direct observation. Data will be managed in a secure database with restricted access and regular audits to ensure integrity and confidentiality.

Statistical analysis is planned to include both descriptive and inferential techniques using SPSS software. Continuous variables will be analysed using parametric or non-parametric tests, depending on data distribution. Categorical variables will be assessed using Chi-square or Fisher's exact tests. All analyses are planned to be conducted on an intention-to-treat basis, with a significance threshold set at $p < 0.05$.

Monitoring

While the study lacks an independent Data Monitoring Committee, the co-investigator will oversee the trial conduct. The Institutional Ethics Committee will regularly review the study's ethical compliance and adverse events. Any adverse events will be reported and treated promptly, and periodic audits will ensure adherence to the protocol. Study progress reports will be shared with the funding agency in line with their requirements.

Harms

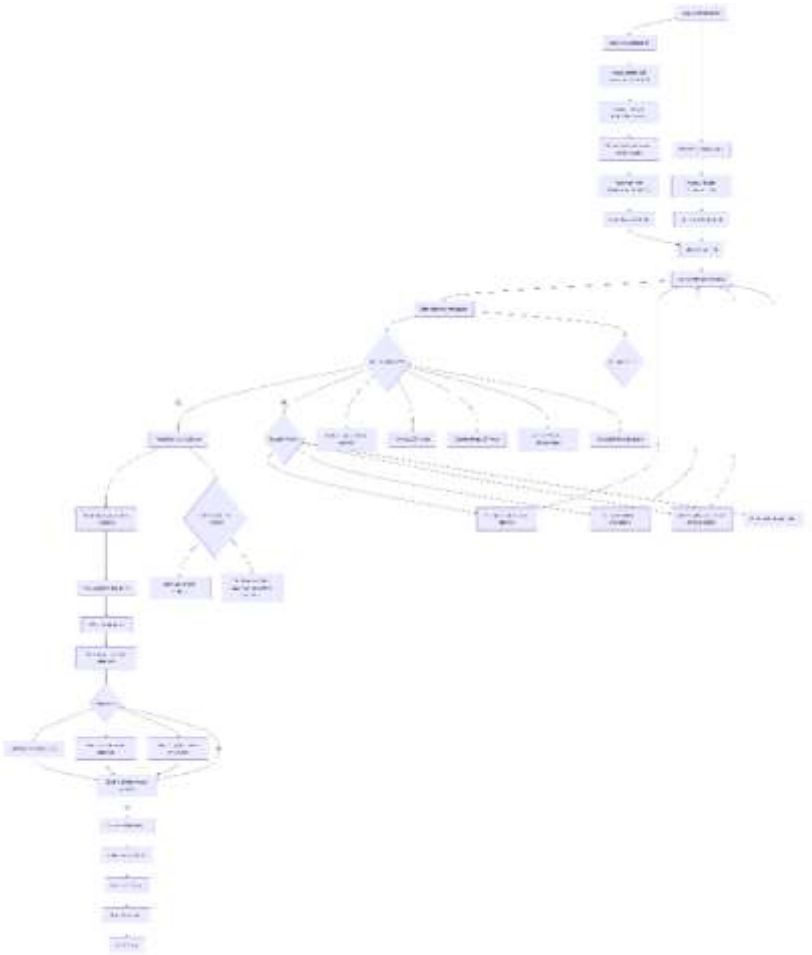
While every effort will be made to ensure the safety and well-being of participating neonates, potential risks associated with the study will include skin irritation, redness, or other local reactions at the application site, as well as the possibility of allergic responses to the topical agents. To minimise these risks, preliminary skin sensitivity testing will be conducted, with close observation for any adverse reactions. All adverse events will be documented and will be reported to the Institutional Ethics Committee in accordance with regulatory guidelines. If a neonate develops a reaction or discomfort, venipuncture will be performed at an unaffected site, and the area will be gently cleansed with a mild cleanser and water, followed by application of a soothing, hypoallergenic moisturiser if needed. Necessary medical care will be provided without delay.

Ethics and Dissemination

The study received university approval from Institutional Ethics Committee of College of Ayurved, Bharati Vidyapeeth (Deemed to be University), Pune, Maharashtra [Approval No.: BVDUCOA/EC/1560/2023-24, dated 28/12/2023]. The IEC approved the protocol to ensure compliance with ethical standards and the protection of participant rights. A subsequent protocol amendment modifying application duration and planned sample size was approved by the Institutional Ethics Committee (Approval No.: BVDUCOA/EC/2062/2025-26; dated 7 August 2025). Any major protocol amendments, including changes to eligibility criteria, outcomes, or analyses, will be submitted to the IEC for approval and communicated to all relevant stakeholders, including participants, registries, journals, and regulators. Informed proxy consent will be obtained from parents or guardians of neonates, following AAP and IAP guidelines. The Principal Investigator ensures that consent is documented after explaining the study's objectives, procedures, risks, and benefits. No additional consent is required as no ancillary data or biospecimens are being collected. Participant confidentiality will be maintained through anonymised, coded data accessible only to authorised personnel. Access to the final dataset will be limited to the Principal Investigator and Co-Investigator, with data sharing governed by ethical and institutional guidelines. Ancillary care will be provided for any trial-related adverse effects, but no post-trial care or long-term follow-up is planned. Results will be disseminated through peer-reviewed publications, conferences, and public databases without restriction. Authorship will reflect substantial contributions to the study; while professional writers may assist, all authors will meet eligibility criteria and approve the final manuscript. The full protocol, de-identified participant data, and statistical code will be made publicly available in accordance with institutional and funder requirements. At the time of submission of this protocol, participant recruitment is ongoing.

Figure 1 provides a schematic overview of the study protocol, outlining key phases from drug preparation and participant recruitment to pain assessment, follow-up, and data analysis.

Figure 1: Detailed flowchart of the study protocol, including drug preparation, participant recruitment, data collection, observations, and analysis phases.



Strengths and Anticipated Limitations

This study protocol outlines a randomised controlled trial designed to evaluate the anaesthetic efficacy and safety of a novel topical ayurvedic emulgel for pain management during neonatal venipuncture. A key strength of the proposed study is the use of validated neonatal pain assessment tools, including the Neonatal Infant Pain Scale (NIPS) and the Neonatal Facial Coding System (NFCS), in conjunction with objective physiological parameters, enabling a comprehensive assessment of procedural pain.

The parallel-group randomised design and the inclusion of a standard comparator, lidocaine–prilocaine cream, are expected to enhance internal validity and clinical relevance. Additionally, the evaluation of two planned application durations may help optimise the practical applicability of the intervention in neonatal settings.

Several anticipated limitations are acknowledged. As a single-centre study, the findings may have limited generalisability across different clinical settings. Blinding of clinical staff and outcome assessors may not be feasible due to distinguishable sensory characteristics of the formulations, which could introduce observer bias despite the use of standardised assessment protocols.

Pain assessment in neonates relies on observer-based scales, which may be subject to inter-observer variability. Furthermore, the study focuses on short-term outcomes related to procedural pain and immediate adverse events; longer-term safety outcomes or effects of repeated exposure are not addressed within the scope of this protocol.

Despite these anticipated limitations, the study is intended to generate preliminary data regarding the feasibility, safety, and clinical applicability of integrative topical anaesthetic approaches in neonatal care, and to inform the design of future multicentric and methodologically robust trials.

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Declaration of Competing Interests

The authors have jointly filed a provisional patent application for the study drug NTAE evaluated in this study. The formulation is currently under patent pending status (Indian Patent Application No. 202521069149). The authors declare no other conflicts of interest.

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