

Original Article

Cannabidiol in wound healing: validation of an experimental protocol

Cannabidiol en la cicatrización de heridas: validación de un protocolo experimental

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Abstract

Validating well-structured experimental protocols is essential to ensure reproducibility and data quality in preclinical studies. This work presents a rigorous methodological framework for assessing wound healing in a murine model using cannabidiol (CBD) ointments at 1.5% and 3% concentrations. A completely randomized block design (CRBD) was applied, using sex as a blocking factor. Thirty CD1 murin biomodels were considered and selected based on strict inclusion and exclusion criteria and housed individually under controlled conditions with environmental enrichment. The approval of the Bioethics Committee of the Agrarian University of Ecuador was proposed, with technical support from INSPI and CBD formulations provided by ALLYMA CORP. The protocol included an adaptation phase, standardized wound induction, daily treatment application, and 21-day clinical evaluation. Photographic documentation was standardized using a fixed metallic base. The results demonstrated successful execution across all phases, highlighting the structured statistical organization, control of external variables, and consistency in data collection. This preclinical study provides a valuable contribution to research involving cannabidiol and other underexplored compounds in alternative medicine, offering a solid, ethical, and reproducible methodological foundation for future investigations.

Key words: Cannabidiol, preclinical model, experimental protocol, alternative medicine

Resumen

La validación de protocolos experimentales bien estructurados es clave para garantizar la reproducibilidad y calidad en estudios preclínicos. En este trabajo se presenta un diseño metodológico riguroso para evaluar el proceso de cicatrización en un modelo murino, utilizando pomadas a base de cannabidiol (CBD) en concentraciones de 1,5% y 3%. Se aplicó un diseño de bloques completamente al azar (DBCA), considerando el sexo como factor de bloqueo. Se consideró la utilización de 30 biomodelos murinos de cepa CD1, con criterios de inclusión y exclusión definidos, alojados individualmente bajo condiciones controladas con enriquecimiento ambiental. Se planteó la aprobación del Comité de Bioética de la Universidad Agraria del Ecuador, con apoyo técnico del INSPI y colaboración del laboratorio ALLYMA CORP. El protocolo incluyó una fase de adaptación, inducción estandarizada de heridas, aplicación diaria de tratamientos y evaluación clínica durante 21 días. La documentación fotográfica fue estandarizada mediante una base metálica fija. Los resultados evidencian la correcta ejecución del protocolo en todas sus fases, destacando la organización del análisis estadístico, el control de variables y la consistencia en la recolección de datos. Esta investigación preclínica representa una contribución relevante para estudios con compuestos como el cannabidiol y otros derivados poco explorados en el campo de la medicina alternativa, ofreciendo una base metodológica sólida, ética y reproducible para futuras investigaciones.

Palabras Claves: Cannabidiol, modelo preclínico, protocolo experimental, medicina alternativa

Introduction

Cannabis is a plant widely recognized for its therapeutic properties, primarily due to its active compounds, cannabidiol (CBD) and tetrahydrocannabinol (THC) (1). Historically, it has been used for medicinal purposes in various cultures to treat conditions such as chronic pain and anxiety, owing to its interaction with the endocannabinoid system (ECS). This system, present in mammals, plays a crucial role in regulating physiological functions such as immune response, skin homeostasis, and inflammatory

processes (2). Evidence has shown that cannabis modulates inflammatory pathways, immune cell activity, and the behavior of skin cells such as keratinocytes and fibroblasts, in addition to influencing Toll-like receptors and apoptosis (3,4).

The growing interest in its therapeutic applications has been facilitated by more flexible regulations governing its study, allowing for greater exploration of its benefits in various pathologies. However, to properly understand its mechanisms of action and ensure reproducibility of results, it is essential to implement rigorous experimental protocols (5).

Animal models remain fundamental tools in biomedical research, providing valuable data on the safety, efficacy, and mechanisms of action of new therapies prior to clinical application. Nonetheless, for these models to be reliable and representative, it is necessary to optimize their design, standardize procedures, and enhance their translational relevance (6). When studying emerging compounds such as cannabis derivatives, the development of precise experimental models continues to be a critical challenge.

The National Centre for the Replacement, Refinement and Reduction of Animals in Research (NC3Rs) has reported that inadequate methodological descriptions and poor reporting of results in animal studies lead to scientific, ethical, and economic consequences. Many studies fail to clearly define their hypothesis, animal characteristics, or strategies to reduce bias. Furthermore, those employing statistical analysis often lack detailed descriptions of their methods (7). Therefore, scientific publications must prioritize the inclusion of sufficient methodological detail to support proper evaluation and avoid unnecessary duplication of studies (7).

In this context, the ARRIVE guidelines (Animals in Research: Reporting In Vivo Experiments) aim to improve quality and transparency in animal research. These guidelines define key elements that must be reported in scientific publications, including animal housing, handling conditions, and experimental methodologies (7). Thus, the development of experimental models for preclinical studies using innovative compounds such as cannabis gains particular relevance, especially in dermatological conditions involving disruption of skin integrity.

Material and methods

To ensure clarity, consistency, and reproducibility of the study, the experimental design and procedures used throughout the research are detailed below.

Hypothesis

Topical treatment with CBD-based ointment enhances the reepithelialization process and promotes more effective healing in skin wounds.

Experimental Design and Sample Size

An experimental study was conducted using a completely randomized block design (CRBD), with sex considered as a blocking factor to reduce variability. A total of 30 CD1 mice were used, distributed into three experimental groups (n = 10 per group), with 5 males and 5 females in each group:

- Control group: Daily wound care with chlorhexidine antiseptics, without ointment application.
- Treatment group 1 (TT1): Daily topical application of CBD ointment at 1.5%.
- Treatment group 2 (TT2): Daily topical application of CBD ointment at 3%.

Inclusion Criteria

- CD1 strain mice
- Body weight between 40–60 g
- Sex: male and female
- Optimal health condition, without visible diseases or wounds

Exclusion Criteria

- Mice outside the 40–60 g weight range
- Poor health condition or abnormal anatomical or physiological characteristics

Animal Housing

Each mouse was housed individually to prevent injury from interactions and to minimize healing-related bias. Environmental and nutritional enrichment strategies were implemented to reduce stress and ensure animal welfare throughout the study.

Randomization

An open-access software was used to randomly assign animals to each experimental group, thereby avoiding selection bias. During randomization, the even distribution of males and females in each group was taken into consideration.

Ethical Approval and Collaborators

The methodology and experimental protocol were approved by the Bioethics Committee of the Agrarian University of Ecuador, in accordance with ethical and scientific guidelines for animal research with medical purposes. The CBD ointment used in the study was provided by ALLYMA CORP, which supplied the 1.5% and 3% formulations for evaluation of their healing effects.

Experimental Phases

Adaptation Phase

Prior to the start of the study, the mice underwent a three-week acclimatization period. During this time, they were handled daily by the experimenter to promote a calm and docile behavior during the procedures.

Wound Induction and Treatment Application

At the end of the adaptation period, standardized 2 cm × 2 cm skin incisions were performed using a template to ensure uniformity. A species-appropriate anesthesia protocol was followed using ketamine and xylazine administered intraperitoneally, and 5% lidocaine subcutaneously for local analgesia. Treatments were applied daily according to group assignment under controlled conditions to minimize external variables.

Wound Healing Evaluation

Healing progression was monitored daily for 21 consecutive days through:

- Macroscopic evaluation: Daily photographs and wound area measurements were taken using a fixed metallic base that served as a tripod, ensuring a consistent 30 cm distance from the wound site to maintain uniform image quality throughout the study.
- Skin assessment: Use of the modified Vancouver scale, assessing vascularization, pigmentation, elasticity, exudate, and tissue thickness.

- Adverse effect monitoring: Evaluation of inflammatory signs and local reactions using the CADESI-4 scale as a reference.

Statistical Analysis

Data were analyzed using JASP software. Normality (Shapiro-Wilk), homogeneity of variance (Levene), and Pearson correlation tests were applied. Based on data distribution, either ANOVA or Kruskal-Wallis tests were used, followed by post hoc comparisons using Tukey or Dunn's test.

Animal Ethics and Welfare

The study was conducted in accordance with international animal welfare guidelines, ensuring appropriate housing, handling, and analgesia. Humane euthanasia was performed using a CO₂ chamber, following the protocols of the National Institute of Public Health Research (INSPI) and the ethical standards set by the Bioethics Committee of the Agrarian University of Ecuador.

Results

The implementation of a rigorous and structured experimental protocol yielded highly satisfactory outcomes across all phases of the study. The use of a completely randomized block design (CRBD), incorporating sex as a blocking factor, proved effective in reducing statistical variability and potential bias in the interpretation of wound healing data. Including two treatment groups with different CBD ointment concentrations (1.5% and 3%) allowed for a broader comparative analysis of the compound's effect on skin regeneration.

The application of clearly defined inclusion and exclusion criteria enabled the precise selection of individuals, minimizing biological variability among the subjects. The housing strategy—individual enclosures for each mouse—was particularly beneficial in maintaining optimal behavior and minimizing stress. No signs of distress or stereotypic behaviors were observed throughout the experiment. Environmental and nutritional enrichment contributed to the animals' well-being, and individual identification by color-coded labels facilitated consistent tracking and data recording.

Ethical oversight by the Bioethics Committee of the Agrarian University of Ecuador and technical guidance from the National Institute of Public Health Research (INSPI) were key to refining

animal care procedures and ensuring compliance with international ethical standards. The institutional infrastructure and hygiene protocols also contributed significantly to reducing experimental bias, including the prevention of contamination by external agents such as insects.

The experimental phases were executed in a controlled and orderly manner, beginning with a three-week adaptation period. This phase proved essential for habituating the animals to human interaction, resulting in more docile behavior during handling. The anesthetic protocol, applied intraperitoneally with ketamine and xylazine and followed by appropriate reversal agents, was executed successfully without any fatalities. All animals recovered normally, showing healthy feeding, hydration, and mobility behaviors within their enclosures.

Daily monitoring of the wounds, including photographic documentation and clinical evaluations, was carried out without setbacks. Photographic evaluations were standardized using a fixed metallic base positioned at 30 cm from the wound site, ensuring uniform image quality and reducing variability throughout the study. Minor visual changes were accurately recorded, and adverse effects were minimal or absent throughout the study. Continuous observation enabled the detection of subtle differences in wound healing among the treatment groups. Notably, animals treated with CBD ointment exhibited greater ease of handling, possibly associated with reduced discomfort or stress at the wound site.

Regarding data management and statistical analysis, the information was systematically organized in tables, categorized by treatment group and evaluation criteria. Each wound parameter generated 630 individual data points (3 groups × 10 animals × 21 days), allowing for detailed exploration of data distribution and detection of outliers. The dataset structure allowed for a clear visualization of treatment progression over time. While specific statistical outcomes are reserved for further analysis in a forthcoming publication, the consistency and completeness of the data confirm the robustness of the experimental design.

Lastly, the successful collaboration with ALLYMA CORP ensured the precise formulation of the CBD ointments used in the study. The standardized production of the ointments according to the required concentrations was instrumental in maintaining

consistency and reliability in the experimental treatments.

Discussion

As noted by Weller (2021) (8), many experimental studies present significant gaps in their protocols, hindering the comparison of results and limiting the reproducibility of procedures. This lack of detailed methodological information creates uncertainty regarding the true effectiveness of the treatments evaluated. In contrast, the present study highlights the importance of a rigorous and well-structured experimental protocol for preclinical wound healing research using cannabidiol (CBD). While the therapeutic outcomes of the treatments are not discussed in this manuscript, the consistent application and successful execution of each procedural phase validate the reliability and reproducibility of the experimental design implemented, supported by the detailed and specific data collected throughout the study.

The adoption of a completely randomized block design (CRBD), with sex as a blocking factor, was a key methodological choice that helped reduce biological variability and increased the statistical clarity of the results. This approach aligns with recommendations for minimizing confounding factors in animal studies and contributes to the strength of the experimental conclusions, even when therapeutic outcomes are not yet reported.

Animal welfare and environmental control were prioritized throughout the study, with individual housing, environmental enrichment, and a structured adaptation phase contributing to reduced stress and behavioral consistency among the subjects. These practices reflect current ethical standards in animal research and are consistent with the principles outlined in the ARRIVE guidelines. Since the publication of these guidelines, there has been a marked improvement in the quality of methodological reporting in animal-based experimental studies (9), reinforcing the value of transparency and detail in preclinical research protocols.

Moreover, the use of standardized tools such as a fixed metallic base for photographic documentation ensured consistency in image capture, reducing variability in macroscopic wound evaluations. The individualized identification and daily monitoring of each subject allowed for meticulous data collection and tracking, further enhancing the protocol's reproducibility.

Although the statistical outcomes of the treatments will be addressed in a future publication, the volume and organization of the dataset generated in this study demonstrate the success of the applied protocol. Each evaluation criterion was applied consistently, generating robust and analyzable data. The structure of the dataset allowed for the identification of outliers and trends without compromising the integrity of the results, ensuring a reliable basis for subsequent therapeutic analysis.

Institutional and collaborative support also played a crucial role in the success of this protocol. Ethical oversight by the Bioethics Committee of the Agrarian University of Ecuador and technical support from the National Institute of Public Health Research (INSPI) contributed to the methodological refinement and compliance with ethical standards. Additionally, the collaboration with ALLYMA CORP ensured the accurate preparation of the CBD ointments in the concentrations required, reinforcing the experimental consistency.

Conclusion

The methodological framework presented in this study proved to be effective, ethical, and highly reproducible for preclinical wound healing research involving cannabidiol. From design to execution, every phase of the protocol was carried out under controlled and standardized conditions, ensuring data integrity and consistency. The detailed documentation, adherence to ARRIVE guidelines, and institutional support underscore the value of this protocol as a reference model for future studies. Moreover, this type of preclinical research is essential and highly relevant for the evaluation of cannabidiol and other underexplored compounds in the field of alternative medicine, where methodological rigor is often lacking. This work contributes to the advancement of transparent, high-quality preclinical research and lays a solid foundation for future investigations focused on assessing the therapeutic efficacy of emerging natural treatments.

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Peer review

The manuscript was peer reviewed and approved by the Editorial Team of the INSPILIP journal.

Availability of data and materials

The data supporting this manuscript are available upon request from the corresponding author.

Conflicts of interest of each author

None of the authors has a conflict of interest.

Contribution of the authors

The different phases of the research were carried out by the authors, who contributed equally throughout the process

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