

Effectiveness of Peppermint (*Mentha piperita* L.) Leaf Extract Gel on Dorsal Incised Wound Healing in Male Wistar Rats (*Rattus norvegicus*)

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ABSTRACT

Peppermint (*Mentha piperita* L.) contains constituents with potential anti-inflammatory, antioxidant, and antimicrobial activity. This study evaluated peppermint leaf extract gels at 5%, 10%, and 15% for dorsal incised wound healing in male Wistar rats (*Rattus norvegicus*). A laboratory pretest–posttest control group design used 30 rats, with five animals in each of six groups. K1 was the normal control with an induced wound and no topical treatment; K2 received the gel base, K3 received Betadine Gel, and K4–K6 received the extract gels. The Federer calculation indicated a minimum of 24 animals. All groups had an initial incision length of 20 mm, and macroscopic healing was recorded on days 3, 7, 10, 13, and 14. Omnibus tests showed overall group differences on days 3, 7, 10, and 13 ($p < 0.001$). The 15% gel had the highest mean healing among the extract groups before day 14, reaching 64.20% on day 10 and 77.90% on day 13; Betadine Gel reached 71.60% and 78.30%, respectively. The untreated control reached 26.10% and 46.90%. None of the nonparametric pairwise comparisons met the Bonferroni threshold of 0.00333; the day-13 Betadine Gel versus 15% peppermint comparison had an exact p value of 0.095. All groups reached 100% macroscopic closure on day 14. The 15% preparation warrants further preclinical investigation, but equivalence to Betadine Gel and an optimal concentration were not established.

Keywords: peppermint, *Mentha piperita*, leaf extract gel, incised wound, wound healing, wistar rats

INTRODUCTION

Skin injury disrupts the anatomical continuity of tissue and compromises the barrier that protects the body against physical trauma, microorganisms, and environmental exposure. Incised wounds are produced by sharp instruments and generally have relatively regular margins, making them useful for controlled experimental evaluation. Their apparent simplicity does not eliminate the possibility of delayed repair: local contamination, tissue damage, poor perfusion, and systemic conditions can alter the course of healing. The distinction between acute and chronic wounds is therefore relevant when interpreting an experimental model, because a standardized acute incision does not reproduce all the biological constraints associated with a chronic wound [1], [2], [3].

Wound repair proceeds through overlapping phases of hemostasis, inflammation, proliferation, and remodeling. Hemostasis limits blood loss and establishes a temporary matrix, whereas inflammatory cells remove damaged tissue and participate in microbial defense. Fibroblast activity, angiogenesis, extracellular matrix deposition, and epithelial migration subsequently restore tissue continuity. Remodeling

continues after surface closure and determines the organization and mechanical quality of the repaired tissue. Excessive inflammation, oxidative stress, and infection can interfere with these processes. This biological framework has encouraged the investigation of plant constituents that may influence several aspects of the wound environment [4].

Peppermint, *Mentha piperita* L., is an aromatic member of the Lamiaceae family. Its leaves contain volatile constituents, including menthol and menthone, as well as nonvolatile compounds such as flavonoids, phenolic compounds, and rosmarinic acid. The European Medicines Agency describes peppermint leaf and essential oil preparations as distinct herbal materials, a distinction that matters when comparing experimental products. The presence of potentially active constituents provides a rationale for investigation, but the botanical name alone does not establish the chemical composition, potency, or safety of a particular extract [5].

Experimental evidence supports further examination of peppermint in wound repair. Amirzade-Iranaq et al. investigated topical peppermint essence in male Wistar rats and reported favorable macroscopic

and histological findings together with increased transforming growth factor beta expression. Their formulation and wound model differed from those evaluated here, so the findings support biological plausibility rather than direct interchangeability of products [6]. Rozza et al. evaluated menthol-containing cream and described effects on inflammatory responses, antioxidant defense, and epithelialization. An isolated menthol preparation is likewise not equivalent to a whole-leaf extract, although the study helps explain why peppermint constituents are of interest [7].

The delivery vehicle is another determinant of topical treatment performance. Gels are semisolid preparations in which a liquid phase is retained within a structured matrix. Their consistency facilitates application, distribution over the treatment site, and continued contact between the formulation and the skin. A hydrated vehicle may also help maintain a suitable local environment. Formulation principles require attention to the compatibility of the active material with the base, as changes in concentration can alter the behavior of the complete product. Consequently, a vehicle-only group is needed to distinguish the contribution of the extract from that of the gel base [8], [9].

Related formulation research illustrates the importance of evaluating both the active constituent and its delivery system. Alsareii et al. investigated a piperine-containing bioactive hydrogel in a rat excision wound model [10]. Serbezeanu et al. evaluated polyvinyl alcohol and xanthan gum hydrogels loaded with neomycin sulfate [11], whereas Popescu et al. studied silver-containing nanocomposite hydrogels as antibacterial materials [12]. These products differ from peppermint leaf extract gel in composition and intended mechanism. They provide formulation context and identify useful avenues for characterization, but their results cannot establish the effectiveness of the present peppermint preparation.

Further context comes from investigations of hydrophilic matrices and membrane interactions. Ward et al. examined hydration-related behavior using focus variation microscopy and dissolution imaging [13]. Moreno et al. investigated the interactions of amphiphilic compounds with P-glycoprotein in a membrane environment [14]. Neither study directly evaluated peppermint-mediated wound healing. Their relevance lies in the broader observation that physical properties and molecular interactions can influence drug delivery. A higher nominal extract concentration should therefore be evaluated experimentally rather than assumed to produce a proportionate increase in tissue exposure or therapeutic activity.

An established topical comparator is useful alongside the vehicle control. Betadine Gel contains povidone iodine, an antiseptic used in wound care. Its antimicrobial action provides a basis for its use as a positive comparator when evaluating a candidate topical preparation. However, comparing macroscopic healing with an antiseptic does not determine whether the candidate product acts through antimicrobial

activity, modulation of inflammation, or another mechanism [15]. The scoping review by Araújo et al. also emphasizes variation in peppermint-derived formulations, concentrations, experimental models, and outcome measures. This heterogeneity limits direct comparisons and prevents the designation of a universally optimal peppermint formulation [16].

Male Wistar rats provide a practical model for controlled observations of cutaneous repair, although animal characteristics, husbandry, allocation, and outcome definitions must be reported consistently [17], [18], [19]. Standardization is a broader concern in preclinical research; for example, Sun et al. addressed reproducibility and comparability in rat femoral defect models. Their work concerns bone rather than skin, but illustrates why the experimental injury and its measurement require explicit definition [20]. The present study evaluated peppermint leaf extract gels at concentrations of 5%, 10%, and 15% in a dorsal incised wound model, compared their observed healing trajectories with an untreated wounded control, the gel base, and Betadine Gel, and examined differences between groups at the recorded observation times.

RESEARCH METHODS

Study design and setting

The study used a laboratory experimental pretest-posttest control group design. Baseline wound measurements were obtained before topical treatment, and subsequent measurements were used to describe the course of healing. Thirty male Wistar rats were allocated to six groups of five animals: an untreated wounded control, a vehicle control, a positive control, and three peppermint extract groups. All 30 animals had an induced dorsal incision, and wound-healing outcomes were evaluated in groups K1-K6. Clear reporting of group allocation, experimental units, and outcome definitions is consistent with the ARRIVE 2.0 recommendations for animal research [21]. NM Laboratory was used for animal maintenance, acclimatization, wound induction, treatment, observation, and gel preparation. The study was conducted in 2026, and the experimental observation period extended to day 14 after wound induction.

Animals and allocation

The experimental animals were male Wistar rats, *Rattus norvegicus*. The eligibility criteria specified an age of 8–12 weeks, a body weight of 150–250 g, healthy and active behavior during acclimatization, and the absence of anatomical or skin abnormalities affecting the dorsal treatment area. Exclusion criteria included illness before treatment, local skin infection or abnormality, and injuries unrelated to the experimental procedure. Death, severe infection, complications requiring treatment cessation, or loss of observation data were specified as reasons for withdrawal after treatment began.

The minimum sample size was calculated using the Federer criterion, $(t - 1)(n - 1) \geq 15$, where t denotes the number of groups and n the number of

animals per group. With six groups, the calculation yielded $n \geq 4$, corresponding to a minimum of 24 animals. The actual study used five animals in each of the six groups, giving a total of 30 rats. Thus, 24 represents the calculated minimum, whereas 30 represents the number used in the experiment. Simple random selection and allocation were performed after eligibility assessment. Each rat constituted an experimental unit, and the five-per-group allocation was maintained when describing the treatment groups. Distinguishing the calculated minimum from the actual group size provides the sample-size information needed to assess the experimental design [22].

Acclimatization and ethical approval

Animals underwent a seven-day acclimatization period before the experimental procedures. They were maintained in prepared cages with bedding, standard feed, and drinking water available *ad libitum*. Health and activity were observed during this period. Body weight was measured with a digital balance and recorded by animal identification code. These procedures were intended to provide a consistent starting environment and to identify conditions that could interfere with subsequent wound observations [17], [18].

The study protocol was approved by the Health Research Ethics Committee (Komisi Etik Penelitian Kesehatan, KEPK) of Universitas Prima Indonesia under approval no. 047/KEPK/UNPRI/VIII/2026, dated 27 August 2026. The ethics approval was valid from 27 August 2026 through 27 August 2027. Anesthesia preceded wound induction in the experimental sequence. Animal welfare considerations include appropriate perioperative care, monitoring, and criteria for discontinuing procedures, consistent with general laboratory animal guidance [17], [22].

Gel preparation and treatment groups

Peppermint leaf extract was incorporated into a gel base to produce nominal concentrations of 5%, 10%, and 15%. The preparations were described as homogeneous and ready for topical administration.

Each group contained five rats. K1, designated as the normal control, had an induced dorsal incision and received no topical treatment. K2 received the gel base after wound induction, K3 received Betadine Gel, and K4, K5, and K6 received peppermint extract gels at 5%, 10%, and 15%, respectively. K1 served as the untreated wounded control for observing spontaneous repair. The vehicle and positive comparator groups allowed the extract preparations to be assessed alongside the gel base and an active topical product.

Topical products were applied directly to the wound, with the same quantity specified across the treated groups during the observation period. The vehicle group was used to account for the contribution of the base, whereas the povidone iodine group provided an active topical comparator [8], [9], [15]. The extract concentrations were the treatment factor, while the vehicle was held constant across the three extract groups.

Wound induction and baseline measurement

The dorsal hair was shaved in all six groups, and the exposed skin was cleaned with 70% alcohol before incision. A sterile scalpel was used to create the experimental wound, followed by baseline measurement before topical treatment in K2–K6. The baseline wound records documented an initial incision length of 20 mm in all groups. K1 underwent wound induction but received no subsequent topical treatment. A common initial incision length was used to reduce variation in the starting wound dimension between groups.

Wound documentation used photography and measurement equipment, including a ruler or digital caliper; ImageJ was also listed among the possible assessment tools. Figure 1 illustrates baseline measurement and topical formulation application. The documented 20-mm value refers to incision length and should not be interpreted as wound area. The distinction between a linear wound dimension and a two-dimensional wound area is important when interpreting the healing endpoint.



Figure 1. Experimental wound measurement and topical treatment

From left to right: baseline measurement of the dorsal incision with a digital caliper displaying 20.0 mm; a prepared gel formulation beside the incision; and gel application over the incision. These photographs document the experimental procedures and do not constitute a serial assessment of healing.

Outcomes and observation schedule

The outcome presented in the results was the percentage of macroscopic wound healing. Baseline measurements preceded treatment, and the results recorded follow-up observations on days 3, 7, 10, 13, and 14. This schedule defined the recorded time-specific outcomes. The earlier protocol specified days 0, 4, 7, 10, and 14, whereas the recorded results used the schedule stated above.

At each observation time, wound appearance was documented and measurements were entered into an observation sheet. The data were subsequently checked, coded, entered, cleaned, and tabulated in IBM SPSS Statistics. The evaluated endpoint was macroscopic closure; histopathology, collagen content, growth-factor expression, microbial burden, and biomechanical strength were not measured. Accordingly, statements about cellular or molecular mechanisms are interpretations based on the cited literature rather than outcomes of this experiment.

Statistical analysis

Healing percentages were summarized as means and standard deviations for all six groups, with five rats per group. The Shapiro-Wilk test assessed distributional assumptions within groups, and Levene's test assessed variance homogeneity. Kruskal-Wallis tests were used on days 3, 7, and 13, followed by Mann-Whitney U comparisons. The six-group design yielded 15 pairwise comparisons, giving a Bonferroni threshold of 0.05/15, or approximately 0.00333. The exact pairwise p values are distinguished from this adjusted

decision threshold. The untreated wounded control K1 was included in both the descriptive summaries and the comparisons of healing outcomes.

Day 10 was analyzed using one-way analysis of variance across the six groups, followed by Tukey's honestly significant difference procedure. Although Levene's test was not significant on that day, the 10% peppermint group failed the Shapiro-Wilk normality test; this qualification is retained when interpreting the reported ANOVA. Day-14 values in all six groups were 100% with zero reported variance and were described without inferential testing. The analyses compared groups separately at each time point and did not provide a joint longitudinal estimate of treatment effects [18].

RESULT AND DISCUSSION

Baseline characteristics and experimental groups

The experiment included 30 male Wistar rats, with five animals assigned to each of the six groups. The available baseline weight listing contained four measurements per group, or 24 recorded measurements. Among these entries, ages ranged from 9 to 12 weeks, animals were recorded as healthy at the initial assessment, and body weights ranged from 200 to 276 g, with an overall recorded mean of 225.04 g. Table 1 presents the actual group allocation alongside these available baseline weight summaries. The weight summaries therefore describe the recorded subset and should not be interpreted as summaries of all 30 animals.

Table 1. Experimental groups and available baseline weight measurements

Group	Treatment	n	Mean weight (g)	Weight range (g)	Initial incision (mm)
K1	Normal control, wounded and untreated	5	217.00	200–240	20
K2	Gel base	5	219.50	204–230	20
K3	Betadine Gel	5	216.25	214–222	20
K4	Peppermint extract gel 5%	5	235.50	202–276	20
K5	Peppermint extract gel 10%	5	228.00	222–240	20
K6	Peppermint extract gel 15%	5	234.00	226–244	20

Note: $n = 5$ rats per group; total allocation = 30 rats. Mean weights and ranges summarize the four available baseline measurements per group. Recorded ages were 9–12 weeks. K1 was the normal control with an induced wound and no topical treatment. One recorded weight in K4 was 276 g.

Among the available baseline measurements, the highest mean weight was recorded in the 5% peppermint group, at 235.50 g, and the lowest in the Betadine Gel group, at 216.25 g. The 5% group also had the widest range, including one rat weighing 276 g. This value exceeded the upper limit of the stated eligibility range and represents a departure from the weight criterion. The baseline wound records documented a common incision length of 20 mm in all six groups, including the untreated wounded control K1.

Macroscopic healing over time

Table 2 presents the reported healing percentages for all six wounded groups at the recorded observation times. Each group contained five rats. Betadine Gel had the highest mean percentage from day 3 through day 13, whereas the untreated wounded control K1 had the lowest. The peppermint groups showed a consistent descriptive ordering over that period: 15% exceeded 10%, and 10% exceeded 5%. The vehicle group had lower mean values than each of the peppermint groups and higher values than K1 at the corresponding observations. These comparisons describe the tabulated values and do not by themselves establish statistically significant treatment effects.

Table 2. Macroscopic healing percentages in all six groups over time

Group and treatment	Day 3	Day 7	Day 10	Day 13	Day 14
K1 untreated control	12.50 ± 0.00	22.80 ± 5.90	26.10 ± 5.02	46.90 ± 5.24	100.00 ± 0.00
K2 gel base	19.20 ± 3.72	32.00 ± 4.57	41.00 ± 2.83	57.20 ± 3.38	100.00 ± 0.00
K3 Betadine Gel	40.50 ± 2.26	55.70 ± 1.26	71.60 ± 1.64	78.30 ± 0.27	100.00 ± 0.00
K4 peppermint 5%	26.70 ± 2.08	42.10 ± 1.98	48.90 ± 1.43	62.80 ± 2.66	100.00 ± 0.00
K5 peppermint 10%	30.00 ± 1.06	46.00 ± 0.35	54.20 ± 1.99	75.10 ± 1.85	100.00 ± 0.00
K6 peppermint 15%	34.90 ± 1.60	50.30 ± 3.01	64.20 ± 1.92	77.90 ± 0.22	100.00 ± 0.00

Values are means ± standard deviations (%), with $n = 5$ rats per group. K1 was the normal control with an induced wound and no topical treatment; K2 received the gel base, K3 received Betadine Gel, and K4–K6 received peppermint extract gels at 5%, 10%, and 15%, respectively.

On day 3, mean healing was 40.50% in the Betadine Gel group, 34.90% with 15% peppermint, 30.00% with 10% peppermint, 26.70% with 5% peppermint, 19.20% with the vehicle, and 12.50% in the untreated wounded control. By day 7, these values had increased to 55.70%, 50.30%, 46.00%, 42.10%, 32.00%, and 22.80%, respectively. The 15% preparation thus showed the greatest early macroscopic healing among the extract concentrations, while the positive comparator remained numerically ahead. Healing also progressed in K1, demonstrating spontaneous repair without topical treatment.

The separation between the extract groups was more evident on day 10. Mean healing reached 64.20% with 15% peppermint, compared with 54.20% at 10% and 48.90% at 5%; the vehicle group reached 41.00%, Betadine Gel reached 71.60%, and the untreated wounded control reached 26.10%. On day 13, the corresponding peppermint values were 77.90%, 75.10%, and 62.80%. The 15% group was then 0.40 percentage points below Betadine Gel, which reached 78.30%, while the vehicle and untreated wounded control reached 57.20% and 46.90%, respectively.

All reported day-14 percentages in K1–K6 were 100.00%, with standard deviations of 0.00. This common endpoint provided no between-group variation for statistical comparison. Complete macroscopic closure was therefore observed in the untreated wounded control, the vehicle group, and the active-treatment groups. Interpretation of treatment performance consequently emphasizes the trajectories before day 14 rather than the final shared closure percentage.

Distributional assumptions and variance homogeneity

Table 3 summarizes the assumption checks relevant to the chosen tests. Normality was not satisfied by the Betadine Gel group on day 3, the vehicle group on day 7, the 10% peppermint group on day 10, or the Betadine Gel and 15% peppermint groups on day 13. Levene's test indicated unequal variances on days 3, 7, and 13. These results explain the use of nonparametric comparisons at those observations and qualify the interpretation of the parametric analysis on day 10.

Table 3. Reported assumption checks for time-specific analyses

Day	Groups failing Shapiro-Wilk	Levene statistic	Levene p	Variance assessment
3	K3: $p = 0.009$	6.133	0.001	Unequal
7	K2: $p = 0.012$	12.846	< 0.001	Unequal
10	K5: $p = 0.040$	2.494	0.059	No evidence against equality
13	K3: $p = 0.006$; K6: $p < 0.001$	4.783	0.004	Unequal
14	Not estimable: constant values	Not tested	Not tested	Zero variance

The Levene tests included all six groups, with degrees of freedom of 5 and 24. The day-3 Shapiro-Wilk statistic for K1 was not estimable because its recorded values were constant. Values printed as $p = 0.000$ are expressed as $p < 0.001$.

Levene's test on day 10 gave a statistic of 2.494 and $p = 0.059$. This result did not provide evidence against equal variances at the 0.05 threshold, but it did not resolve the normality violation in K5, where Shapiro-Wilk $p = 0.040$. The reported ANOVA is consequently presented with that limitation. The absence of a significant assumption test also needs cautious interpretation in small groups, because limited sample sizes restrict the information available about the underlying distribution.

Overall differences between groups

The six-group Kruskal-Wallis tests gave $H = 28.272$ on day 3, $H = 28.114$ on day 7, and $H = 27.678$ on day 13, each with five degrees of freedom and $p < 0.001$. The day-10 ANOVA yielded $F(5, 24) = 175.622$ with $p < 0.001$. Table 4 summarizes these omnibus results and their follow-up analyses. These tests compared healing outcomes across all six wounded groups, including the untreated control K1, and indicated overall differences at each observation before day 14.

Table 4. Overall statistical comparisons and follow-up findings

Day	Omnibus test	Statistic	df	p	Follow-up result
3	Kruskal-Wallis	$H = 28.272$	5	< 0.001	No pair met Bonferroni threshold
7	Kruskal-Wallis	$H = 28.114$	5	< 0.001	No pair met Bonferroni threshold
10	One-way ANOVA	$F = 175.622$	5, 24	< 0.001	K4 and K5 shared a Tukey subset
13	Kruskal-Wallis	$H = 27.678$	5	< 0.001	No pair met Bonferroni threshold
14	Not tested	Not applicable	—	—	All six group means were 100%

All six wounded groups, K1–K6, were included. ANOVA = analysis of variance; df = degrees of freedom. The Bonferroni threshold was approximately 0.00333 for 15 comparisons. The day-10 ANOVA should be interpreted with the normality limitation described in Table 3.

The untreated wounded control provides a reference for spontaneous repair, while the vehicle control helps separate the contribution of the base from that of the extract. Significant omnibus tests indicate that the six groups did not share the same outcome distribution or mean at the relevant observations, depending on the test used. They do not identify the specific pairs responsible for the differences. Treatment-specific conclusions therefore require examination of the post hoc analyses together with the observed healing trajectories.

Post hoc comparisons

Across the reported pairwise comparisons on days 3, 7, and 13, the smallest exact Mann-Whitney p value was 0.008, exceeding the Bonferroni threshold of 0.00333. None of the 15 pairwise comparisons at these

observations therefore met the adjusted significance criterion. This finding should be distinguished from the significant omnibus results. Failure to detect a pairwise difference does not establish that treatments have identical effects [23].

For day 10, the Tukey homogeneous-subset output placed K1 in subset 1, K2 in subset 2, K4 and K5 together in subset 3, K6 in subset 4, and K3 in subset 5 (Table 5). The K4–K5 subset significance value was 0.056. The available summary describes the subset pattern but does not provide the complete matrix of adjusted pairwise differences and confidence intervals. The 0.056 value is therefore identified as a subset significance value rather than a separately verified pairwise p value. Interpretation of the Tukey analysis remains subject to the day-10 normality limitation.

Table 5. Day-10 Tukey homogeneous subsets for all six groups

Group and treatment	Mean healing (%)	Homogeneous subset
K1 untreated control	26.10	1
K2 gel base	41.00	2
K4 peppermint 5%	48.90	3
K5 peppermint 10%	54.20	3
K6 peppermint 15%	64.20	4
K3 Betadine Gel	71.60	5

All six groups are shown. The K4–K5 subset significance value was 0.056. This value is not a separately verified pairwise p value. The full adjusted pairwise matrix and confidence intervals were not presented in the main results.

The day-13 pairwise results are presented in Table 6. The Betadine Gel versus 15% peppermint comparison gave $U = 4.000$, $Z = -2.032$, and an exact p value of 0.095. The vehicle versus 5% peppermint comparison gave an exact p value of 0.032. Neither

comparison satisfied the Bonferroni threshold, and the same decision applied to the remaining pairs. The small difference between the 15% preparation and Betadine Gel therefore remains a descriptive observation rather than proof of equivalence.

Table 6. Mann–Whitney pairwise comparisons on day 13

Comparison	U	Z	Exact p	Bonferroni decision
K1 vs K2	0.500	-2.514	0.008	Not significant
K1 vs K3	0.000	-2.652	0.008	Not significant
K1 vs K4	0.000	-2.627	0.008	Not significant
K1 vs K5	0.000	-2.611	0.008	Not significant
K1 vs K6	0.000	-2.694	0.008	Not significant
K2 vs K3	0.000	-2.652	0.008	Not significant
K2 vs K4	2.000	-2.207	0.032	Not significant
K2 vs K5	0.000	-2.611	0.008	Not significant
K2 vs K6	0.000	-2.694	0.008	Not significant
K3 vs K4	0.000	-2.668	0.008	Not significant
K3 vs K5	0.000	-2.652	0.008	Not significant
K3 vs K6	4.000	-2.032	0.095	Not significant
K4 vs K5	0.000	-2.627	0.008	Not significant
K4 vs K6	0.000	-2.712	0.008	Not significant
K5 vs K6	0.500	-2.595	0.008	Not significant

All 15 comparisons among K1–K6 are shown. Exact p values are unadjusted; decisions use the Bonferroni threshold $\alpha = 0.05/15 \approx 0.00333$. Every pair exceeded that threshold, including K3 versus K6 (exact p = 0.095).

Interpretation of the concentration pattern

The observed pattern suggests that the higher peppermint concentrations merit further investigation in this experimental setting. The 15% preparation had the highest mean healing among the extract groups at every recorded observation before complete closure. The difference between the 5% and 15% groups was 8.20 percentage points on day 3, 8.20 on day 7, 15.30 on day 10, and 15.10 on day 13. This progression indicates a concentration-associated descriptive pattern, although it does not demonstrate a linear dose–response relationship.

The 10% and 15% values became close by day 13, at 75.10% and 77.90%. This observation is relevant to subsequent concentration selection because increasing the extract percentage may yield progressively smaller changes near the end of the macroscopic healing period. Only three concentrations were examined, and no concentrations above 15% were included. Moreover, extract composition, topical exposure, and safety were not quantified. The 15% formulation can therefore be described as the highest-performing tested concentration on the recorded macroscopic endpoint, rather than an established optimum.

The heterogeneity summarized by Araújo et al. provides a further reason for caution. Peppermint-derived products have been incorporated into different topical systems and examined under different experimental conditions [16]. A percentage attached to an essential oil, an isolated constituent, or a leaf extract does not imply the same chemical exposure. Comparisons should account for botanical material, extraction, vehicle, wound type, and timing. The present findings identify a formulation and concentration range for further evaluation, without resolving those sources of variation.

Biological plausibility and comparison with earlier research

Earlier peppermint studies support plausible biological explanations for the observed trajectory.

Amirzade-Iranaq et al. associated topical peppermint essence with increased transforming growth factor beta expression and histological changes consistent with repair [6]. Rozza et al. examined menthol-related anti-inflammatory, antioxidant, and epithelial effects [7]. These studies provide complementary evidence at a level not assessed by the present macroscopic endpoint. Differences in the tested substances and vehicles prevent their results from being treated as a mechanistic confirmation of this gel.

Repair depends on a coordinated transition from inflammatory activity to tissue formation and later remodeling. Fibroblasts contribute extracellular matrix, new vessels support developing tissue, and epithelial migration restores the surface barrier. Persistent inflammation or inadequate local conditions can interfere with that sequence [1], [3], [4]. Peppermint-associated antioxidant and anti-inflammatory activity could plausibly influence the wound environment, but the present experiment cannot determine which process accounts for the recorded percentages. It also cannot distinguish epithelial closure from wound contraction or establish the quality of the underlying repaired tissue.

Antimicrobial activity offers another possible explanation, particularly if local microbial exposure delays repair. Peppermint constituents have been investigated for such activity, while povidone iodine provides an established antiseptic comparator [5], [15]. However, wound cultures and bacterial counts were not obtained in this study, and an infected wound model was not specified. Faster macroscopic closure cannot therefore be attributed specifically to a reduction in microbial burden. An antimicrobial mechanism would require direct microbiological assessment alongside the wound endpoint.

Contribution of the topical vehicle

The gel base is a relevant part of the intervention because the preparation delivers the extract and remains in contact with the treatment site. Pharmaceutical formulation principles recognize the

role of the base in the performance of semisolid products [8], [9]. The vehicle group reached 57.20% healing on day 13, compared with 46.90% in the untreated wounded control; both reached 100.00% on day 14. The 10.30-percentage-point difference on day 13 was descriptive, and the corresponding pairwise comparison did not meet the Bonferroni threshold. The progress observed in K1 demonstrates that spontaneous repair contributes to closure, while the vehicle comparison remains necessary to assess whether an extract adds benefit beyond the base.

The hydrogels investigated by Alsareii et al., Serbezeanu et al., and Popescu et al. illustrate different approaches to combining an active constituent with a delivery matrix [10], [11], [12]. Their active materials and experimental endpoints differ from those used here, so the comparison is conceptual. Ward et al. similarly provide formulation context through the behavior of hydrophilic matrices, while Moreno et al. address molecular interactions in a membrane system [13], [14]. Further development of peppermint gel would benefit from physical characterization and release assessment, but neither hydration behavior nor a specific membrane transport mechanism was measured in this experiment.

Comparison with Betadine Gel

Betadine Gel produced higher mean healing percentages than the extract preparations on days 3, 7, and 10. The mean difference between Betadine Gel and 15% peppermint was 5.60 percentage points on day 3, 5.40 on day 7, and 7.40 on day 10, narrowing to 0.40 on day 13. This time-dependent pattern is more informative than the shared day-14 endpoint because it describes how the groups approached closure. The positive comparator's early numerical advantage should remain visible when assessing the candidate preparation.

The day-13 *p* value of 0.095 does not demonstrate that 15% peppermint and Betadine Gel are equivalent. The study was not designed as an equivalence or noninferiority trial, and no acceptable equivalence margin was specified. A failure to detect a difference is therefore insufficient to justify substituting the experimental gel for an established topical product [23]. The two reported means were close on day 13, and the adjusted pairwise analysis did not detect a difference. This result should be interpreted in the context of five animals per wounded group, the multiple-comparison adjustment, and the absence of an equivalence design.

Timing and statistical interpretation

Complete macroscopic closure at day 14 occurred in all six groups, including the untreated wounded control and the vehicle control. This shared endpoint creates a ceiling that prevents further discrimination of the tested interventions. Observations before that ceiling retain information about the healing trajectory, particularly the descriptive ordering of the peppermint groups and the narrowing difference from the positive comparator. Surface closure at day 14 also does not establish completion of remodeling, restoration of

tensile strength, or the absence of later complications [1], [2].

The combination of significant omnibus tests and nonsignificant Bonferroni-adjusted pairwise comparisons should be reported without changing the decision threshold after inspecting the results. The exact *p* values and the adjustment are both needed to understand the findings. Small groups and multiple comparisons limit the ability to identify individual differences. In addition, analyses performed separately at each observation do not account jointly for measurements obtained from the same animals over time. A future study should define the experimental unit and its longitudinal analysis before data collection [18].

Limitations and implications

Several reporting and design limitations constrain interpretation. The actual allocation was 30 rats, with five animals per group, whereas the available baseline weight listing contained four measurements per group. The weight summaries consequently characterize a recorded subset of the animals. One recorded baseline weight exceeded the stated eligibility range, and the planned and recorded observation schedules differed. The small group sizes, multiple pairwise comparisons, and day-10 normality limitation also restrict the strength of treatment-specific inference. Explicit reporting of the experimental unit, group size, outcome definition, and analysis population is particularly important in animal experiments [21], [22].

The extract and gel were not chemically or physically characterized in sufficient detail to establish reproducibility. The extraction method, the basis of the nominal percentages, and the administration regimen also need complete reporting. No histopathological, molecular, microbiological, biomechanical, or dedicated topical safety assessments were performed. The male rat model provides preclinical information but does not establish efficacy in human wounds. General animal-care and experimental-design principles, together with the broader standardization concerns illustrated by Sun et al., support more explicit reporting of future experiments [17], [18], [20].

Subsequent research should use complete animal-level baseline and longitudinal records and groups sized for the planned primary outcome. An untreated wounded control, as used in K1, and a vehicle control should be retained to distinguish spontaneous repair from the contribution of the formulation. Concentrations near 15% could be investigated alongside histological assessment, collagen deposition, epithelialization, inflammatory measures, and safety outcomes. The observation period should capture differences before complete surface closure and, where relevant, continue into tissue maturation. These assessments would help determine whether the descriptive advantage of the 15% formulation represents a reproducible treatment effect [21], [22].

CONCLUSION

Peppermint leaf extract gels at 5%, 10%, and 15% showed increasing macroscopic healing

percentages during follow-up of dorsal incised wounds in male Wistar rats. The 15% preparation had the highest mean values among the tested extract concentrations before day 14, reaching 64.20% on day 10 and 77.90% on day 13. The day-13 mean was close to that of Betadine Gel, at 78.30%, and the reported pairwise comparison did not meet the Bonferroni-adjusted significance criterion. All wounded groups reached a reported 100% macroscopic closure on day 14.

The 30-rat experiment, with five animals per group, supports further preclinical evaluation of the 15% preparation but establishes neither equivalence to Betadine Gel nor an optimal concentration. K1 was the normal control with an induced wound and no topical treatment, and its healing outcomes were included in the six-group comparisons. Complete formulation reporting and assessment of tissue repair, mechanism, and topical safety are needed to establish the reproducibility and translational relevance of the findings.

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