Original Article

The Efficacy of Thymbra Spicata and Ferulago Angulata on Second Degree Burn Wound: A Triple-Blinded Randomized Controlled Trial

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Abstract

Background: Burns are categorized as a kind of trauma, as they may be a strong, life-threatening stressor that causes major problems, delaying the healing process. So, this study was aimed at investigating the effect of the combined ointment of *Thymbra spicata* and *Ferulago angulata* extracts on second-degree burn wounds. **Materials and Methods:** In 2024, a triple-blind, randomized controlled trial was conducted on 72 second-degree burn patients randomly allocated into two groups with randomized allocation software. The 36 participants in the intervention group received a combination ointment of *Thymbra spicata* and *Ferulago angulata* extracts thrice a day for 6 days with routine treatment. The Bates-Jensen Wound Assessment Tool (BWAT) was completed before and after the intervention. Statistical tests were included: paired *t*, independent *t*, Chi-square, and difference-in-difference regression, which were conducted at the level of significance considered less than 0.05. **Results:** Before the intervention, there was no meaningful variation in the mean scores of wound healing between the control and intervention groups by independent *t* ($p = 0.600$). However, after the intervention, its variation was meaningful in the intervention group by paired *t* (26.59 (1.55)) versus the control group (35.10 (1.69)) ($p < 0.001$). Also, assessing the group effect and time effect and adjusting the model showed that in the intervention group, second-degree burn wound healing has statistically significant variation by difference in difference regression ($p < 0.001$). **Conclusion:** Using a combination of *Ferulago angulata* and *Thymbra spicata* extracts thrice a day may significantly improve burn wound healing after second-degree burns.

Keywords: *Burns, herbal medicine, randomized controlled trial, wound healing*

Introduction

Burns are one of the most common and costly tissue injuries in the world, imposing a significant burden on healthcare systems and the quality of life of individuals.[1] The World Health Organization (WHO) says that burns are a major public health issue all over the world, but especially in countries with low and middle incomes. Every year, fires kill more than 195,000 people. More than 95% of these deaths happen in countries with low or middle income, where children under 5 and those over 70 are more at risk and have the greatest death rates. In addition to deaths, millions more suffer from non-fatal burn injuries that often require extended hospital stays, leave them disfigured, and make them disabled, sometimes with stigma and rejection.[2] According to official data from the Iranian Ministry of Health, approximately 28,000 people are hospitalized annually due to burns, and nearly 3,000 people die, a statistic that only partially reflects the extent of this problem.[2] Second-degree burns, which affect the epidermis and part of the dermis, present particular treatment challenges due to their depth of penetration and the possibility of infectious complications, chronic inflammation, and delayed healing.[3] These injuries start a complex process that needs effective treatment because they cause inflammation, reduce blood flow to tissues, and weaken the body's cell protection systems.[4] Conventional methods such as advanced dressings, debridement, systemic antibiotics, and skin grafting, although contributing to reducing mortality and improving outcomes, face challenges such as high costs, drug resistance, and side effects.[5]

Medicinal plants have gained increasing attention as potential alternatives in

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disease treatment due to their bioactive compounds and historical use in traditional medicine.[6] According to the World Health Organization, 11% of essential medicines are derived from plant sources, underscoring their value in modern pharmacology.[7] Iran's diverse flora and long-standing tradition of herbal remedies offer fertile ground for such research.[8] In the meantime, *Thymbra spicata* has shown significant antimicrobial, antifungal, and antioxidant properties due to its phenolic compounds.[9] *Thymbra spicata* plant has been confirmed as an effective therapeutic agent, playing a role in inhibiting pathogens and reducing tissue oxidative stress.[10] Similarly, *Ferulago angulata*, a species native to the Zagros regions of Iran, contains monoterpenes and sesquiterpenes that have been shown to have antimicrobial and antioxidant activity in preliminary studies.[11] The *Ferulago angulata* plant has long been known in traditional Iranian medicine as a natural antiseptic and preservative agent and has great potential for clinical applications.[12] However, the available data on the use of these two plants, especially in combination, in the treatment of burn wounds is limited.[13]

Despite advances in burn management, challenges such as resistant infections, the high cost of chemical treatments, and long-term complications remain unresolved.[14] Previous studies have shown that *Thymbra spicata* extract can inhibit pathogens like Pseudomonas aeruginosa and promote tissue regeneration in animal models, though clinical evidence in humans remains limited and inconsistent.[15] Likewise, *Ferulago angulata* has demonstrated antimicrobial and regenerative effects in preclinical studies, but the absence of human trials has hindered its clinical application.[16] While both plants have shown promise individually, there is a striking lack of studies investigating their combined therapeutic use, particularly in human subjects. This represents a critical and unmet need in the field of burn care.[17] Specifically, no experimental research has yet evaluated the effects of combining *Thymbra spicata* and *Ferulago angulata* extracts in the treatment of second-degree burns in humans, despite encouraging results in animal models.[18] Addressing this gap is essential for advancing alternative, plant-based approaches to burn wound healing and forms the rationale for the present study.[19] Although various approaches have been used to manage second- and third-degree burn wounds, few studies have employed randomized controlled trials—particularly those using triple-blinded designs—to rigorously evaluate treatment effectiveness. Moreover, there is a lack of clinical research on plant-based therapies for burn healing. Therefore, this study aimed to evaluate the effect of a combined ointment containing *Thymbra spicata* and *Ferulago angulata* extracts on the healing of second-degree burn wounds in humans. The primary objective was to determine whether this herbal formulation could improve wound-healing outcomes compared to standard treatment.

Materials and Methods

This was a triple-blinded randomized controlled experiment done in 2024, authorized by the Ethics Committee of Ilam University of Medical Sciences. The participants in the study were burn victims who went to the burn ward of Emam Khomeini Hospital in Ilam province in 2024. They were chosen using the convenience sampling method based on inclusion criteria like being at least 18 years old, having thermal second-degree burns, scoring 24 or higher on the Mini-Mental Status Examination (MMSE), having at least 3% burns on their hands and feet (non-sensitive areas),[20] being able to read and write, and having been hospitalized for between 3 and 6 days. Exclusion criteria were contained participants, who were unwilling to take part in the study because they were pregnant, had diabetes, had tattoos,[21] had burns on their face, had used corticosteroid drugs in the past year,[22] had eczema in the burned areas, had an autoimmune disease, had used intravenous, oral, or topical corticosteroids, while they were in the hospital, had laser treatment in the burned areas, or had electrical burns were also not allowed. A type 1 error of $\alpha = 0.05$ and a test power of 80% are considered, and if the new treatment is expected to be 30% more effective than the standard treatment, the sample size is calculated according to the formula for calculating the sample size in a clinical trial study. (Fixed score (C = 7.9), (P1 = 0.50), (P2 = 0.8)).

Firstly, using the convenience sampling for recruitment, 72 samples were distributed randomly to the control group (36 participants) and the intervention group (36 participants) by Randomized Allocation Software (RAS). The final list was exported by RAS and just dedicated to the research secretary (constant outcome assessor) for supervising the correct selection, which led to the main researcher no longer having any ability to change the allocation. In this study, the researchers, outcome assessor, participants, and data analyzer were blinded, which strengthened the study protocol.

The first tool was a tool that included age, gender, marital status, burning percentage, and education. The second one was the MMSE, which was developed by Folstein and associates to assess cognitive dysfunction. Each question has a total score ranging from 1 to 30. A score of 24 or above signifies no cognitive impairment, a score between 18 and 23 denotes mild cognitive impairment, a score ranging from 10 to 17 reflects moderate cognitive impairment, and a score below 10 implies Alzheimer's disease.[23] The study reported this tool had a suitable and acceptable validity (0.79), and its reliability was measured to be 0.85 using Cronbach's alpha internal consistency.[24] The third tool was the Bates-Jensen Wound Assessment Tool (BWAT), which was used to evaluate wound status and monitor healing progress with subjective assessment. It measures 13 different aspects of a wound, including its size, depth, edges, undermining, necrotic tissue type

and amount, exudate type and amount, surrounding skin color, peripheral tissue edema and induration, granulation tissue, and epithelialization. The scoring system uses a five-point Likert format, with scores ranging from 13 to 65. Higher scores indicate more severe burn damage and a delay in the healing process. For assessing the validity and reliability of the English version, its validity was reported as acceptable (0.93), and its reliability was measured to be 0.87 using Cronbach's alpha internal consistency.[25] For the Persian version's validity and reliability, its validity was reported to be good (0.89), and its reliability was measured to be 0.86 using Cronbach's alpha internal consistency.[26] In this research, its reliability was checked on 20 burn patients, and its result showed 0.93 using Cronbach's alpha internal consistency.

The intervention commenced after capturing the ethics code, randomization, and criteria assessments. Before the study, a constant outcome assessor filled out the BWTA in the online questionnaire platform (Porsline), and then the intervention commenced. In the intervention group, a constant nurse who did not know about the aim of the research did six times per day (every 4 hours for targeted intervention, then the next 4 hours, routine treatment implemented) a combination ointment with a total concentration of 1% in 100 g tubes along with routine treatment (A + D ointment consisting of 85,000 international units of vitamin A and 8,500 international units of vitamin D3 in 30 g tubes) for six days. In more detail, first, A + D ointment was applied topically to the patient three times a day (every 4 hours) on the burn area with a thickness of 3 mm. After 4 hours passed from that time, the burn area was washed with normal saline and dried sterilely. Then, the combination ointment was applied topically on the burn area with a thickness of 3 mm, and a dressing was applied three times. This process continued for up to 6 days for both ointments, one after the other (with a 4-hour interval).[27] In the control group, that constant nurse only applied the dressing with A + D ointment to the patient six times a day (every 4 hours) locally on the burn area with a thickness of 3 mm. After 4 hours, the burn area was washed with normal saline and dried in a sterile manner. Then, the dressing was applied again with A + D ointment. This process continued for 6 days. During these times, the main researchers were never allowed to enter the burn ward except in cases of allergic reactions or patient non-consent. At the start and finish of the research, BWTA was filled with the constant outcome assessor on the online questionnaire platform (Porsline) into SPSS V.16 directly to prevent data leakage to the research members. Additionally, the data analyzer was unaware of the research aim to prevent any biases and analyzed the received SPSS data package. The demographic data were examined through descriptive statistics, presenting variables as mean, standard deviation, frequency, and percentage. The statistical techniques employed included the Kolmogorov-Smirnov (K-S) test for normality assessment, the independent *t*-test and Chi-square test for demographic comparisons between two groups, the paired *t*-test for pre- and post-score comparisons, and difference-in-difference regression (DID) for evaluating time effects, group effects, and adjusting for additional relationships. Analyses were performed using SPSS V.16, with a significance threshold set at less than 0.05 [Figure 1].

Ethical consideration

The research adhered to ethical guidelines, which encompassed obtaining ethical approval from Ilam University of Medical Sciences (IR.MEDILAM.REC.1403.089), registering the study in the Iranian Registry of Clinical Trials (IRCT20211110053030N6), acquiring informed consent from participants, ensuring data confidentiality, and complying with the principles established in the Declaration of Helsinki.

Results

Table 1 presents the distribution of participants based on age and burn percent across the intervention and control groups. The Kolmogorov–Smirnov test was employed to assess the normality of these continuous variables, and the results confirmed a normal distribution in both groups ($p > 0.05$). Therefore, the independent *t*-test was used to compare the mean values between the two groups. The mean age of participants was 35.88 ± 11.99 years in the intervention group and 33.70 ± 11.10 years in the control group, with no statistically significant difference between them ($p = 0.428$). Similarly, the mean burn percent was 26.63 ± 8.08% in the intervention group and 30.23 ± 9.52% in the control group; this difference was also not statistically significant ($p = 0.128$). These findings indicate that the two groups were comparable at baseline with respect to these key clinical variables [Table 1].

Most of the samples were female, married, and had diplomas. Performing the Chi-square test, there was not any meaningful differentiation between the gender ($p = 0.199$), marital situation ($p = 0.544$), and education ($p = 0.431$) [Table 2].

Prior to the intervention, the mean wound healing score, as measured by the BWAT, was 34.00 ± 1.53 in the control group and 33.75 ± 1.68 in the intervention group. The difference between groups was not statistically significant ($p = 0.600$), indicating baseline comparability [Table 3]. Following the intervention, the

Table 1: Distribution of age and burn percent based on two groups

Variables	Mean (SD)		*p***
	Intervention	Control	
Age	35.88 (11.99)	33.70 (11.10)	0.428**
*p**	0.509*	0.847*	
Burn percent	26.63 (8.08)	30.23 (9.52)	0.128**
*p**	0.920*	0.054*	

*Kolmogorov–Smirnov. **Independent-*t*

control group demonstrated a slight increase in the mean BWAT score to 35.10 ± 1.69, whereas the intervention group exhibited a substantial decrease to 26.59 ± 1.55, reflecting a marked improvement in wound healing. This between-group difference was statistically significant ($p < 0.001$). The mean change in BWAT scores (post-pre) was + 1.10 in the control group and –7.16 in the intervention group. An independent *t*-test confirmed that this difference in mean change was statistically significant ($p < 0.001$) [Table 3]. Within-group comparisons using paired t-tests revealed no statistically significant difference in the control group before and after the intervention ($p = 0.154$), while the intervention group showed a highly significant improvement ($p < 0.001$) [Table 3].

Table 2: Manifesting the samples pile by demographic variables in both groups

Variables	Sub categories	Groups		*p**
		Intervention *n* (%)	Control *n* (%)	
Gender	Male	14 (39%)	12 (33%)	0.199*
	Female	22 (61%)	24 (67%)	
Marital situation	Single	6 (17%)	10 (28%)	0.544*
	Married	30 (83%)	26 (72%)	
Education	Elementary	2 (5%)	6 (17%)	0.431*
	High school	4 (11%)	4 (11%)	
	Diploma	16 (45%)	18 (50%)	
	Bachelor and higher	14 (39%)	8 (22%)	

*Chi-square

A DID regression model was performed to evaluate the effect of the intervention while controlling for time, group, and demographic characteristics. The interaction term between group and time (Group × Time) was statistically significant (B = –7.16, $p < 0.001$), indicating that the intervention group experienced significantly greater improvement in wound healing compared to the control group after adjusting for age, burn percentage, gender, marital status, and education level [Table 4].

Discussion

This study investigated the effect of a combined ointment containing *Thymbra spicata* and *Ferulago angulata* extracts on the healing of second-degree burn wounds. The results demonstrated a statistically significant improvement in wound healing scores in the intervention group compared to the control group after the intervention. This finding suggests that the combination of *Ferulago angulata* and *Thymbra spicata* may have synergistic therapeutic properties that promote wound healing in second-degree burn patients.

In line with these results, previous studies have also reported the positive effects of medicinal plants on wound healing. For example, Qashqai *et al*. demonstrated in a study on laboratory mice that the essential oil of *Thymbra spicata* significantly inhibited the growth of *Pseudomonas aeruginosa* and accelerated the healing of burn wounds. In that study, 50 mice were divided into intervention and control groups, and after 14 days, the intervention group showed significant improvement in tissue regeneration and reduced infection. The similarity of that study with the

Table 3: Comparison of the mean scores of Bates-Jensen wound assessment Tool (BWAT) in samples before and after the intervention in both groups

Variables		Group				*p**
		Control		Intervention		
		Mean (SD)	95% CI (lower-upper)	Mean (SD)	95% CI (lower-upper)	
Wound healing	Before	34.00 (1.53)	(34.50 to 35.50)	33.75 (1.68)	(33.20 to 34.20)	p=0.600*
	After	35.10 (1.69)	(34.55 to 35.65)	26.59 (1.55)	(26.08 to 27.10)	p<0.001*
Mean difference		1.10		–7.16		p<0.001*
p**		p=0.154**		p<0.001**		-

*Independent *t*. **Paired *t*

Table 4: Effect of intervention on second-degree burn wound healing by considering demographic variables using difference in difference regression (DID)

Variables	Coefficient (B)	T	*p**	95% CI (lower–upper)
Group (intervention=1)	–0.25	–0.60	0.552*	–1.09 to 0.59
Time (after=1)	–0.15	–0.38	0.705*	–0.93 to 0.63
Group × time (interaction)	–7.16	–7.53	<0.001*	–9.10 to –5.22
Age (years)	0.01	0.50	0.619*	–0.03 to 0.05
Burn percent (%)	–0.03	–1.00	0.320*	–0.08 to 0.03
Gender (male=1)	–0.18	–0.44	0.661*	–0.99 to 0.63
Marital status (married=1)	0.11	0.28	0.778*	–0.66 to 0.89
Education (higher=1)	0.27	0.75	0.457*	–0.44 to 0.98

*: Difference in difference regression

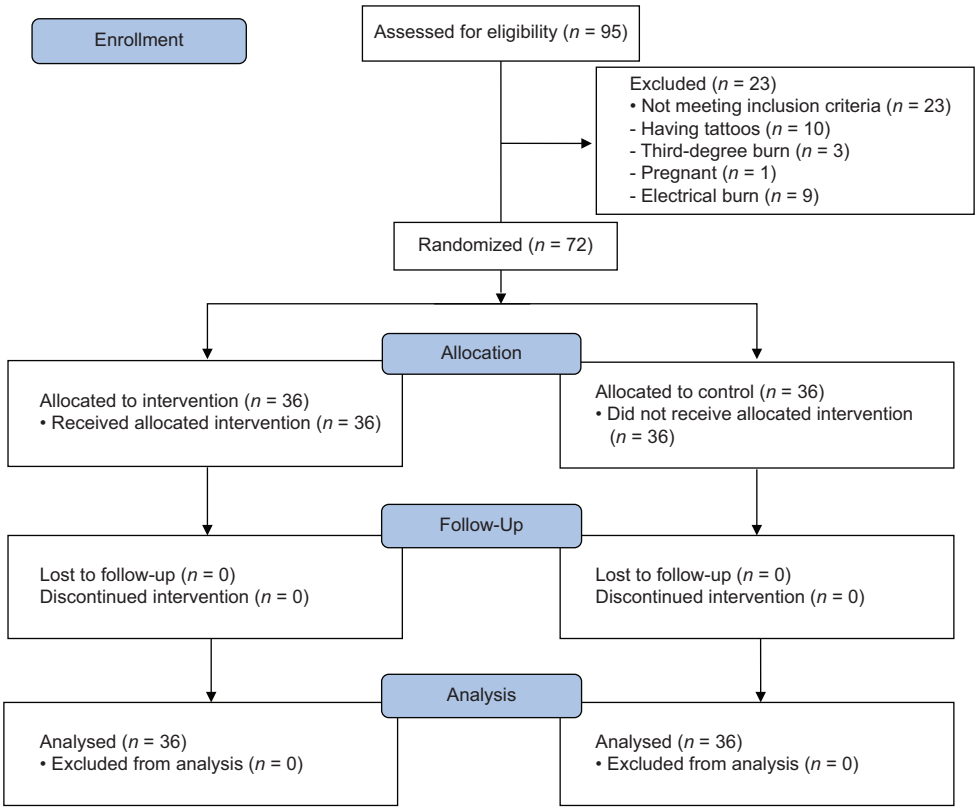

Figure 1: The sequence of selecting and study protocol related on control and intervention groups

present one lies in the antimicrobial and regenerative effects of *Thymbra spicata*. However, the key difference is that our study investigated a combination of *Thymbra spicata* and *Ferulago angulata* in a clinical setting involving 72 human patients. This combination may have been more effective due to a synergistic interaction between *Thymbra spicata* and compounds such as monoterpenes and sesquiterpenes, which have been shown in previous studies to enhance antimicrobial and healing properties.[28] Another relevant study by Mousavinejad *et al*. examined the effects of *Thymbra spicata* combined with violet and pine extracts on second-degree burn wounds infected with Candida glabrata in mice. Their findings demonstrated enhanced wound healing and reduced inflammation in the fungal-infected model. While their study highlighted the antifungal potential of a multi-herbal intervention, the present study differs in two important aspects: it focused on bacterial infection risks in human patients and employed a simpler, dual-herbal formulation (*Thymbra spicata* and *Ferulago angulata*). These differences underscore not only the broader clinical applicability of our results in bacterial contexts but also the practical advantages of our intervention in terms of ease of formulation and potentially lower production costs compared to multi-herbal remedies.[29] Compared to chemical interventions, the patent by Sage used a chemical antibiotic spray (silver sulfadiazine) to treat burns, which, although effective, had side effects such as dark spots remaining at the wound site. In contrast, the combined ointment used in this study, due to the use of natural extracts, did not show such side effects and was superior in terms of safety and patient acceptance. This finding is also comparable to the study by Wang, who introduced a complex herbal ointment with more than 10 components. The complexity of its formulation increased manufacturing and cost challenges compared to the simplicity of the two-component ointment in this study, while the results of the present study showed high efficacy with minimal components.[30,31]

From the perspective of mechanism of action, it seems that the antioxidant and antimicrobial properties of *Thymbra spicata* and *Ferulago angulata* played a key role in reducing oxidative stress and inhibiting pathogens such as Pseudomonas aeruginosa and *Staphylococcus aureus*. This is consistent with the findings of Taran *et al*., who confirmed the antibacterial effect of *Ferulago angulata* extract on wound infectious bacteria. In the present study, the significant improvement in wound healing and acceleration of tissue regeneration in the intervention group was probably the result of these synergistic effects, which were not observed in the control group.[32] The observed enhancement in wound healing can be biologically explained through the well-established roles of antioxidant, antimicrobial, and anti-inflammatory activities in tissue repair. Antioxidants present in *Thymbra spicata* and *Ferulago angulata* help neutralize ROS, which are elevated in burn wounds and contribute to oxidative stress that impairs healing. By reducing oxidative damage, these compounds

protect cellular components and promote a favorable environment for tissue regeneration. Simultaneously, the antimicrobial effects inhibit colonization and proliferation of pathogenic bacteria such as Pseudomonas aeruginosa and *Staphylococcus aureus*, which are known to delay healing through persistent infection and inflammation. Furthermore, the anti-inflammatory properties modulate the local immune response, reducing excessive inflammation that can cause tissue damage and scarring. Together, these mechanisms synergistically accelerate the wound-healing cascade by facilitating cell proliferation, collagen synthesis, and re-epithelialization, ultimately leading to improved clinical outcomes observed in the intervention group.

In another study, Nagarajan *et al*. evaluated the effect of the methanolic extract of Vitex negundo on burn wounds in mice. The study was conducted on 30 mice, and the results showed that flavonoids and tannins present in the extract accelerated wound healing by increasing collagen synthesis and reducing inflammatory cells. This finding is consistent with the present study, as *Thymbra spicata* and *Ferulago angulata* also contain phenolic and antioxidant compounds that reduce inflammation. However, the current study took a simpler and more practical approach by using a two-component formulation and human testing.[33] Additionally, Yen *et al*. investigated the effect of a turmeric-derived *Hibiscus syriacus* L. gel on burn wounds in mice and found that its antioxidant and angiogenic properties significantly accelerated wound healing over 14 days. Like the current study, theirs highlights the value of plant-based compounds with antioxidant effects. However, their intervention used a single active compound (*Hibiscus syriacus* L.), whereas the present study employed a synergistic combination of *Thymbra spicata* and *Ferulago angulata*. This dual combination may offer broader antimicrobial coverage due to the complementary properties of its constituents, although further microbiological studies are needed to confirm this potential.[34] The limitations of the present study consisted of the small sample size, the use of patients with second-degree burns, and the limited study duration, which reduce the generalizability of the results to the broader population. However, the study has several important strengths. Its innovativeness lies in the use of a novel combination of *Thymbra spicata* and *Ferulago angulata* extracts for burn wound healing, which has not been widely explored in clinical settings before. The accessibility of the intervention is notable, as both plants are locally available and easy to obtain, making the ointment a practical option for resource-limited settings. Additionally, the cost-effectiveness of this natural formulation offers a more affordable alternative to conventional chemical treatments, potentially reducing financial burdens on patients and healthcare systems. Finally, the applicability of the findings extends to real-world clinical practice, suggesting that this simple two-component ointment can be effectively integrated into burn care protocols to improve healing outcomes.

Conclusion

Using a combination of *Ferulago angulata* and *Thymbra spicata* extracts thrice a day may significantly improve burn wound healing after second-degree burns. It's suggested to implement these extracts to enhance second-degree burn wound healing. Besides, the findings suggest that these extracts have potential for accelerating wound healing, warranting further investigation and consideration for clinical application. Additionally, future studies should be conducted with larger sample sizes and longer durations and include different burn degrees and electrical burns to confirm and expand upon these results.

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Conflicts of interest

Nothing to declare.

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