ISSN PRINT 2319 1775 Online 2320 7876

Research paper UGC CARE Listed (Group -I) Journal Volume 11, Iss 12, 2022

Evaluation of the Wound Healing Activity of *Terminalia chebula* Fruit Cream

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Abstract

Background: Many studies have indicated various medicinal activities of *Terminalia chebula* plant. Present study was designed to investigate the folklore use of fruits of *Terminalia chebula* in the wound healing process.
Methods: Incision wound model were used to evaluate the wound healing activity of *Terminalia chebula* fruit cream on Sprague Dawely rats. In this study, 30 animals were divided into 5 groups of 6 animals each. Group I served as control and group V as positive control group. In an incision wound model, group II animals were treated with 20% *Terminalia chebula* fruit cream, group III animals were treated with 40% *Terminalia chebula* fruit cream and group IV animals were treated with 80% *Terminalia chebula* fruit cream. The effect of various concentration of *Terminalia chebula* fruit cream on wound healing assessed by measuring diameter of wound and contraction of wound.
Results: The 80% Terminalia chebula fruit cream promoted wound healing activity significantly in incision wound model when compared to 20% and 40% Terminalia chebula fruit cream treated animals. On day 21, 80% Terminalia chebula fruit cream treated animals showed 98.08% wound healing activity. The wound contraction results of 80% Terminalia chebula fruit cream treated animals were comparable with standard drug treated animals (98.75%)
Conclusion: The data of this study indicated that the 80% Terminalia chebula fruit cream promotes significant wound healing in rats, non-irritant in rabbit and further evaluation of this activity in humans is suggested.

Keywords: *Terminalia chebula*, Cream formulation, Wound Healing Activity, dorsal site of rat, non-irritant in rabbit

1. Introduction

According to FDA (Food and Drug Administration) wound is cutaneous breakdown of skin induced by thermal, chemical or electrical injury.[1, 2] Wound healing is a dynamic and complex process in which myriad of cellular incidences that must be tightly co-ordinate to efficiently repair damaged tissue. Derangement in wound linked cellular behaviors, as occurs with diabetes and aging, can lead to healing impairment and the formation of chronic, non-healing wound.[3] Numerous plants have been widely used in the treatment of variety of disorders

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and diseases but the use of traditional medicinal plants in improving the efficiency of wound has not been much applauded by scientists.[4] Folklore cultures employ a significant number of plants to treat cuts, wounds, and burns.[5] *Terminalia chebula* (Family: Combretaceae) is one of the traditional medicine used in many folk claims and it is called as "King of medicine".[6] It is an middle-sized tree, leaves are ovate, or elliptic, flowers are yellowish white, fruits are yellowish brown in colour distributed throughout India.[7] The plant has extensively used in Ayurveda and Sidda for wound healing, diarrhoea, ulcers, gastroenteritis, asthma, cough, dyspepsia, hemorrhoids, tumors, skin diseases, leprosy, intermittent fever, rheumatism, arthritis, gout, neuropathy, paralysis, memory loss, epilepsy, depression, diabetes, cardiovascular diseases, anorexia.[8] Types of wound causes breakage in the skin which includes pressure injuries, bedsores and diabetes related ulcers.[9] *Terminalia chebula* contains tannin, chebulic acid, glycosides, sugar, triterpenoids, steroids and small quantity of phosphoric acid. The pharmacological activities previously reported are Antibacterial, Antifungal, Antiviral, Anticarcinogenic, Antioxidant, Adaptogenic and Antianaphylactic, Hypolipidemic, Hepatoprotective, Cardio protective, Antidiabetic, Immunomodulatory and Chemo preventive.[10] However there are no reports on the wound healing activity of the fruit cream of the plant available in the literature. Hence, the present study was designed to investigate the folklore use of fruits of T.Chebula in the wound healing process.

2. Methods:

2.1 Collection of plant material

The fresh fruits of *Terminalia chebula* were collected in and around Bijapur, Karnataka after the authentication by Prof. Dr. M. B. Mulimani, department of Botany, KCP Science College Vijayapur. A voucher specimen has been deposited at the museum of college.

Figure 1: *Terminalia chebula*

2.2 Extraction process of fruits of *Terminalia chebula*

Herbal drug product has a special place in the world of pharmaceuticals. *Terminalia chebula* is a deciduous tree, used in traditional medicines. It is reported to contain various bio chemical compounds such as tannins, chebulinic acid, ellagic acid, gallic acid, punicalagin, flavonoids etc. It has been reported as antioxidant, antidiabetic, antibacterial, antiviral, antifungal, anticancerous, antiulcer, antimutagenic, wound healing activities etc.[11] The optimization of physico-chemical parameters like effects of different solvents, soaking time,

ISSN PRINT 2319 1775 Online 2320 7876

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extraction time with hexane, particle size, different solvent percentages, different volumes of hexane with ethanol and methanol as solvents and pH for the extraction of Total Phenolic Content, Chebulinic acid and Quercetin were studied. For the extraction of Total Phenolic Content, the optimum results were observed for the effects of different solvents, soaking time, extraction time with hexane, particle size, different solvent percentages, different volumes of hexane with ethanol as solvent and pH were ethanol, 1 day, 1hrs, 125 microns, 50% (v/v), 1:1 ratio and 7.0 respectively. The highest Total Phenolic Content concentration for optimized conditions was 2.25µg/dl. For the extraction of Chebulinic acid, the optimum results were observed for the effects of different solvents, soaking time, extraction time with hexane, particle size, different solvent percentages, different volumes of hexane with ethanol as solvent and pH were ethanol, 1 day, 1hrs, 125 microns, 50% (v/v), 1:1 ratio and 7.0 respectively. The highest Chebulinic acid concentration for optimized conditions was 3.4mg/ml. For the extraction of Quercetin, the optimum results were observed for the effects of different solvents, soaking time, extraction time with hexane, particle size, different solvent percentages, different volumes of hexane with methanol as solvent and pH were methanol, 2 days, 1hrs, 125 microns, 60% (v/v), 1:1 ratio and 6.0 respectively. The highest Quercetin concentration for optimized conditions was 0.54µg/cl.

The extraction was carried out by employing various organic solvents using Soxhlet extractor method. Ethanol was found to be the best solvent for the extraction of Chebulinic acid from *Terminalia chebula* species. Soxhlet extractor was carried out using ethanol at different extraction times to verify the mathematical model proposed in this work. The final form of the proposed models were Es = 0.1650 (1 - *e* -0.0415t) for Chebulinic acid where *Es* = yield extract (grams of Chebulinic acid per gram of dried sample) and *t* = extraction time (min) and Es = 0.925(1 - *e* -0.0499t) for Total phenolic content where *Es* = yield extract (mg of Total phenolic content per gram of dried sample) and *t* = extraction time (min). The model showed good agreement with the experimental data by generating Average absolute relative deviation (AARD) of about 0.1176 ± 20.80% for Chebulinic acid and 0.7169 ± 16.06% for Total phenolic content. The optimization of physico-chemical parameters required for obtaining the highest production of Chebulinic acid using Baker's yeast (*Saccharomyces cerevisiae*). From the fermentation process, the incubation period, pH, biomass concentration, partition coefficient for the aqueous extract were 142 hr, 4.2, 0.3281gm/ml, 1.70. Similarly for the 50% (v/v) ethanolic extract were 192 hr, 4.3, 0.3926gm/ml, 3.83 and for the hexane extract were 264 hr, 4.3, 0.3671gm/ml, 2.30. It was concluded that 50% (v/v) ethanolic extract showed best results for the highest Chebulinic acid production and found to be 8.6 mg/ml. Similarly the concentration was 4.2mg/ml from the aqueous extract and the concentration was 7.5mg/ml from hexane extract.

Among the extraction process (Batch, Soxhlet & Fermentation process) the highest Chebulinic acid production was obtained from fermentation process. From the batch process the chebulinic acid concentration was observed to be 3.4mg/ml at 60 min and the concentration was increased to 6.6mg/ml at 75 min from soxhlet extraction. Similarly the chebulinic acid concentration was increased to 8.6mg/ml at 192 hrs from fermentation process. The partition coefficient for fermentation, soxhlet & batch extraction were found to be 3.83, 2.0 & 0.64. The extraction of Chebulinic acid from *Terminalia chebula* by Soxhlet extraction and purify the Chebulinic acid by using Column chromatography. From the Soxhlet extraction the Chebulinic acid concentration was 6.6 mg/ml and it was increased to 9.4mg/ml from the Column

ISSN PRINT 2319 1775 Online 2320 7876

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chromatography. The purity of Chebulinic acid was improved by Column chromatography. Chebulinic acid showed many bioactivities including inhibition of cancer cell growth, inhibiting the contractile responses of cardiovascular muscles, anti-fungal, anti-bacterial activities etc. *In-vitro* anti-cancer activity of Chebulinic acid on Colon adenocarcinoma HT-29 cancer cell lines by using MTT cell growth inhibition assay was stuied. The maximum percentage inhibition of cancer cell lines for Chebulinic acid was found to be 41.2% at a dose of 200µg/ml. Finally the Chebulinic acid extraction from *Terminalia chebula* plays a vital role in medicine, biotechnology and various pharmacological activities.[12]

2.3 Formulation and development of fruits of *Terminalia chebula*

Water-in-oil (w/o) emulsions were prepared by adding the aqueous phase to the oily phase under continuous agitation. The oily phase consisted of paraffin oil (14%). The surfactant Abil EM90 (5%) was heated up to 75 ± 5˚ C. At the same time, the aqueous phase consisting of water (q.s.) was heated to the same temperature before adding the *Terminalia chebula* (5%). After that, the aqueous phase was added to the oil phase drop by drop. Stirring with the mechanical mixer was continued at 2,000 rpm for about 15 min. until all of the aqueous phase was added; a few drops of lemon oil were added during this stirring time to give the formulation a pleasant fragrance. The speed of the mixer was reduced to 1,000 rpm for homogenization and until the emulsion had cooled to room temperature. The base was prepared by the same method and with the same ingredients except *Terminalia chebula* extract.

2.4 Properties of the formulation

Stability tests were performed at 8 ± 0.1˚ C (refrigerator), 25 ± 0.1˚ C, 40 ± 0.1˚ C, and 40 ± 0.1˚ C (incubation) with 75% relative humidity (RH). Physical characteristics (color, creaming, and liquefaction), electrical conductivity, and pH of the formulation were measured at various intervals for 4 weeks. The base and formulation were divided into 4 separate samples which were kept at 8 ˚C in the refrigerator, at 25 and 40˚C +75% RH in stability chambers. The samples were observed organoletically with respect to changes in color, liquefaction, and phase separation for a period of 28 days at definite time intervals. No change in color occurred in any of the base or formulation samples. While no liquefaction was observed in any of the samples kept at 8 ˚C and 25˚C during the observation period, slight liquefaction occurred in the base and formulation samples kept 40˚C and 40˚C + 75% RH; the samples of formulation remained stable at all temperatures. The electrical conductivity values of the fresh base and formulation as well as the samples kept under different storage conditions for 28 days were determined. No electrical conductivity was found in any sample throughout the study period. The pH of freshly prepared base and formulation was 5.34 and 6.0, respectively. Average changes in pH values of both base and formulation from the time of preparation up to week 4 of the study period were determined. The pH values were measured immediately after preparation, then after 12, 24, 36, 48 and 72 h, and after 1, 2, 3 and 4 weeks.[13]

2.5 Procurement of animals

30 Female Sprague Dawely rats and 3 New Zealand White rabbit were procured from In-vivo Biosciences, Katha No 3169, Assessment No. 154, Kodigehlli village, Magadi road, Bengaluru 560091 Reg. no. 1165/Po/ReBiBt-5/NRc-L/08/CPCSEA.

2.6 Housing of animals

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After procuring, the animals were acclimatized for 7 days at normal laboratory condition. All animals were maintained under a 12 hr. light/dark cycle. Water *ad libitum* and food was provided twice a day. All animals were maintained at room temperature in polyethylene cages (Rat) and in stainless steel (Rabbit). Before conducting the experiments, permission was obtained from "Institutional Animal Ethics Committee", College of Pharmacy & Research Centre, Vijayapura.

2.7 Grouping and treatment

Group I: Control Group - 6 animals were treated with ointment base twice in a day, topically.

Group II: Treatment Group – 6 animals were treated with 20% *Terminalia chebula* fruit cream.

Group III: Treatment Group - 6 animals were treated with 40% *Terminalia chebula* fruit cream.

Group IV: Treatment Group - 6 animals were treated with 80% *Terminalia chebula* fruit cream.

Group V: Positive Control Group - 6 animals were treated with 80% Soframycin cream.

2.8 Wound induction method

First, the hair of the test animals' left side lower back was completely shaved. The animals were situated to stay in the standard crouching position. A metal template measuring 20 mm diameter, whose outline was traced by a fine-tipped pen, was placed on the stretched skin of each animal's lower back. The wound areas were anesthetized by 2% lidocaine subcutaneous injection on the square corners and sterilized using betadine. Full thickness wounds were made by bistouries blades, forceps, and kukher scissors. A draft was drawn around each wound site by transparent plastic sheets and fine tipped pen marks. Sterilized wounds were washed with normal saline and betadine immediately. The animals were kept in individual cages after dressing and returned to their standard situation [temperature of (23 ± 2) °C, humidity of (50%–55%)]. Topical cream was applied on wound sites twice a day. To reduce infection rate, wound sites were evaluated daily for infection. All dressings and animal maintenance followed the ethical rules of standard surgery processes.

2.9 Method of wound area calculation

Wound area was calculated using Vernier caliper paper. Healing processes were found to be dependent on general (oxygenation, nutrition and infection) and individual (sex hormones, obesity, and age) factors. Although we tried to create equal situations to minimize differences, the effect of individual characteristics was undeniable in the healing process. To minimize errors in the measurement of the wound area as well as achieve statistically sound results, wound healing percentage was replaced with wound area and calculated as follows: [14, 15]

$$\text{Wound healing \& in N day} = \frac{(\text{Wound diameter in first day} - \text{Wound diameter in N}^{\text{th}}\text{ day})}{\text{Wound area in first}} \times 100$$

2.10 Procedure of application of *Terminalia chebula* fruit cream on rat wound

An amount of 500 mg of *Terminalia chebula* fruit cream was applied over a wound created on dorsal site of rat. *Terminalia chebula* fruit cream applied on wound of rat was covered with semi-occlusive dressing (a gauze patch, secured by a non-irritating tape) for a period of 6

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hr, after which the residual *Terminalia chebula* fruit cream residual was removed with a piece of cotton, soaked in distilled water.

3. Results

Table 1: Effect of *Terminalia chebula* fruit cream on wound contraction on day-3 of treatment.

S. No.	Treatment	Diameter of wound (in mm)	Wound contraction (%)
1.	Group I-Control group	19.733 ± 0.137	1.333 ± 0.683
2.	Group II-Treatment group	17.217 ± 0.147	13.917 ± 0.736
3.	Group III-Treatment group	15.283 ± 0.075	23.583 ± 0.376
4.	Group IV-Treatment group	14.733 ± 0.587	29.083 ± 0.642
5.	Group V-Positive control group	14.594 ± 0.517	28.417 ± 0.585

Table 2: Effect of *Terminalia chebula* fruit cream on wound contraction on day-21 of treatment.

S. No.	Treatment	Diameter of wound (in mm)	Wound contraction (%)
1.	Group I-Control group	12.317 ± 0.117	38.417 ± 0.585
2.	Group II-Treatment group	8.333 ± 0.103	58.333 ± 0.516
3.	Group III-Treatment group	3.150 ± 0.105	84.250 ± 0.524
4.	Group IV-Treatment group	0.383 ± 0.075	98.083 ± 0.376
5.	Group V-Positive control group	0.250 ± 0.105	98.750 ± 0.524

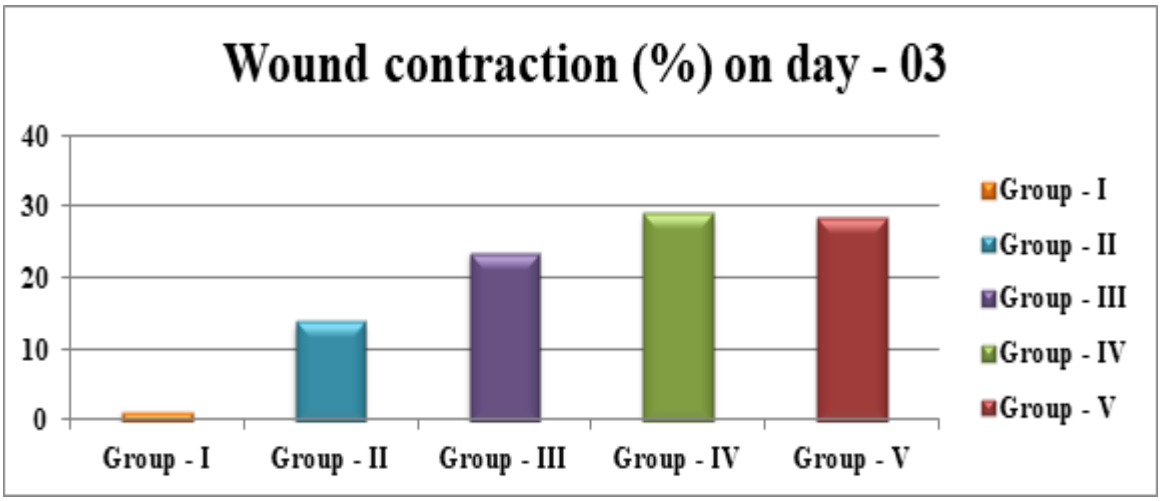

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Figure 2: Effect of *Terminalia chebula* fruit cream on wound contraction on day – 3 of treatment.

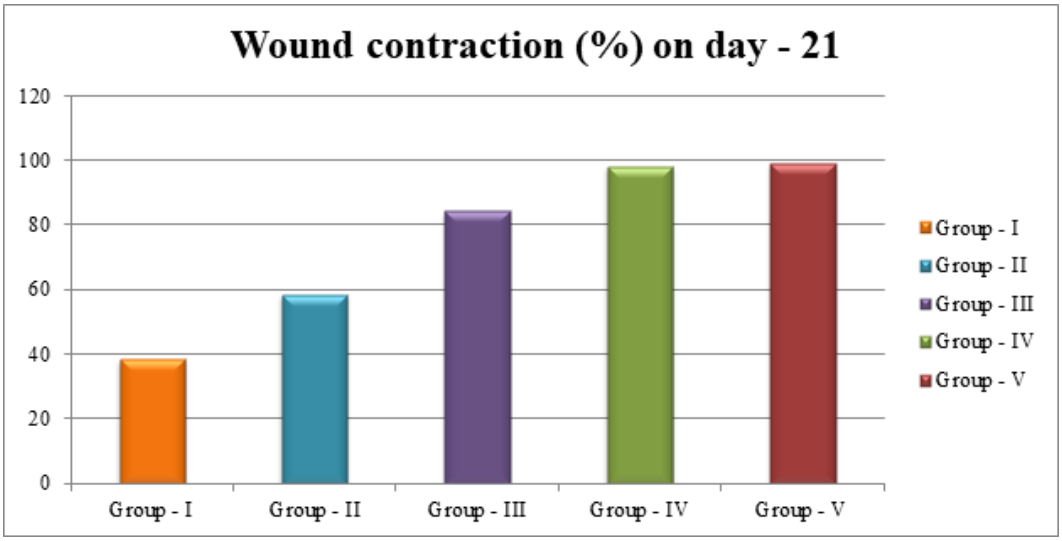

Figure 3: Effect of *Terminalia chebula* fruit cream on wound contraction on day – 21 of treatment.

4. Discussion

Khan M and et al reviewed that, herbal drugs represent a major allocation of all the recognized systems of health in the world.[7] Numerous plants have been widely used in the treatment of variety of disorders and diseases but the use of traditional medicinal plants in improving the efficiency of wound has not been much applauded by scientists.[13] Many studies proved that, Terminalia chebula plant have lots of medicinal activities such as, antibacterial, anticancer, antifungal, antidiarrhoeal, antiviral, and so on. But this study was taken up to investigate and illustrate the wound healing activity of Terminalia chebula fruit cream in Sprague Dawley rat. The present study was depends on investigation of wound healing activity of Terminalia chebula fruit cream on Sprague Dawely rat. This study was included five groups of animals and each group contains six animals (Female Sprague Dawley rats). First group was control group, in which animals were treated with ointment base twice in a day topically. Second group was first treatment group, in which all animals were treated with 20% Terminalia chebula fruit cream. Third group was second treatment group, in which all animals were treated with 40% Terminalia chebula fruit cream. Fourth group was third treatment group in which all animals were treated with 80% Terminalia chebula fruit cream. Fifth group was standard treatment group in which all animals were treated with 80% Soframycin cream.

The wound was created in all animals of all groups by the procedure as mentioned in methodology to study the topically applied Terminalia chebula fruit cream on wound healing process. Increase in the wound healing activity was observed in all treatment groups treated with Terminalia chebula fruit group day wise, when compared with that of control group animals. 80% Terminalia chebula fruit cream treated group of animals showed highest recovery or wound healing activity than that of 20% and 40% of Terminalia chebula fruit cream on day 21. 80% Terminalia chebula fruit cream treated animals showed greater percentage of wound healing

ISSN PRINT 2319 1775 Online 2320 7876

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activity when compared with that of other groups. On day 3, 6, 9, 12, 15, 18 and 21 diameter of wound was measured of all animals of all groups and on every above days treatment group fourth that is 80% Terminalia chebula fruit cream treated animals showed greater wound healing activity compared to control, 20%, 40% Terminalia chebula fruit cream treated animals. Body weight was taken of all animals on day 7, 14 and 21 and observed that, in control and other two treatment group animals weight was abnormal or reduced but 80% Terminalia chebula fruit cream treated animals showed normal and increased in body weight. After getting positive results of wound healing activity from 80% Terminalia chebula fruit cream, it was necessary to test non-irritant effect of the cream. So, acute dermal irritation study was carried out by using three female New Zealand White rabbits. Based on this study findings proved that, 80% Terminalia chebula fruit cream is non-irritant according to OECD principles of GLP.

From these animal studies it can be concluded that, significant increase in the wound healing activity was observed in 80% Terminalia chebula fruit cream treated rats. In control group, 20% and 40% Terminalia chebula fruit cream treated animals showed decreased or slow wound healing activity when compared with that of 80% of Terminalia chebula fruit cream group animals. On day 21 control group animals showed 38.42%, 20% Terminalia chebula fruit cream treated animals showed 58.33%, 40% Terminalia chebula fruit cream treated animals showed 84.25% and 80% Terminalia chebula fruit cream treated animals showed 98.08% wound healing activity. The wound contraction results of 80% Terminalia chebula fruit cream treated animals were comparable with standard drug treated animals (98.75%).

5. Conclusion

Wound healing activity was compared with that of control and Soframycin cream as standard. Animals treated with 80% *Terminalia chebula* fruit cream exhibited 98.08% reduction in wound area as compared to the control animals (38.42%). 80% *Terminalia chebula* fruit cream treated wounds were found to induce contraction of wound faster compared to the control, 20% *Terminalia chebula* fruit cream and 40% *Terminalia chebula* fruit cream. In conclusion, 80% *Terminalia chebula* fruit cream promotes significant wound healing in rats, and further evaluation of this activity in humans is suggested.

Acknowledgements

The research was supported by the Pacific Academy of Higher Education and Research University, Udaipur; Dr. Jignyasha Amitkumar Raval, Professor, Pacific Academy of Higher Education and Research University, Pacific Hills, Pratapnagar Extn., Airport Road, Debari, Udaipur – 313003 and Dr. Vishal Bharat Babar, Chief Executive Officer, Professor & Principal, DKSS Institute of Pharmaceutical Science & Research for Girls, Swami-Chincholi, Bhigwan-413130, Maharashtra, India.

Declaration

Funding: None

Conflict of interest: None to declare

Ethical approval: The study was approved by the Institutional Ethical Committee, Pacific Academy of Higher Education and Research University, Udaipur.

ISSN PRINT 2319 1775 Online 2320 7876

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