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Assessment Of Tissue Healing Property Of Gel Of Urtica Dioica L. And Xanthium Strumarium L. Against The Excision And Incision Wound Model In Rats

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ABSTRACT

Urtica dioica L., a long lasting herb found in South Asia, is utilized for the treatment of many ailments owing to its antioxidant and antibacterial characteristics. *Xanthium strumarium* L., originating from North America, Europe, and Asia, possesses anti-inflammatory, antioxidant, and antibacterial characteristics. Furthermore, it has been examined for its potential in treating cancer, and certain molecules have demonstrated cytotoxic properties. Additional research is required in order to comprehensively comprehend their therapeutic uses. The aim of study to assess the tissue injury healing properties of a gel that contains *Xanthium strumarium* L. and *Urtica dioica* L. The evaluation was conducted using rat models of excision and incision wounds. The objective set for study to find out the immediate harmful reaction on the skin caused due to gel formulations used for promoting wound healing. The rats underwent depilation using a razor blade and were then left undisturbed for a duration of 24 hours. Following a 24- hour period, the experimental medicine was administered to the shaved areas, whereas the control group got a local application of sterile water on skin. The medication was administered consecutively for a duration of 14 days, with daily monitoring for any alterations in the patient's clinical condition. There were no negative clinical changes observed. The study assessed the immediate skin toxicity of gel formulations used for wound healing in rats. Following a 24-hour period, the experimental medicine was administered to the shaved areas, whereas the control group was given sterile water. The study saw no detrimental clinical alterations. The gel formulation of *Xanthium strumarium* L. exhibited superior tensile strength and a shorter epithelization period in comparison to the control group. The gel formulation exhibited superior histopathological recovery. Reactive oxygen species levels were comparable to those in the usual therapy group.

Keywords: *Urtica dioica, Xanthium strumarium , excision and incision wound model*

1. INTRODUCTION

Urtica dioica L., also called "stinging nettle," is a perennial herb used in treating various diseases, including nephritic syndrome, hematuria, liver disease causing jaundice, menses irregularities like menorrhagia, arthritis, and diseases like rheumatism. It contains valuable chemical compounds and has various pharmacological activities, including antibacterial, antioxidant, analgesic, anti-inflammatory, immunomodulatory, hepatoprotective, anti-colitis, and anticancer effects [1, 2]. *Xanthium strumarium* L., known by the name of cocklebur, is a plant species native to North America, Europe, and Asia, known for its various properties like anti- inflammatory, antioxidant, and antimicrobial properties. Many studies were done for its potential use in cancer treatment, with certain compounds showing cytotoxic effects on cancer cells. Further research is needed to fully understand its mechanisms of action and potential therapeutic applications [3, 4].

Wound healing is an intricate process that encompasses diverse cellular and molecular systems. Throughout history, natural materials have been utilised for an extended period to enhance the process of wound healing, owing to their inherent therapeutic characteristics. The current study assessed the tissue injury healing property of the petroleum extract of *Urtica dioica* L. dried seeds and *Xanthium strumarium* L. dried seeds in rats using experimental excision and incision models.

2. METHODOLOGY

2.1 Production of extract

The completely dried seeds of *Xanthium strumarium* & *Urtica dioica* was coarsely powdered and then defatted with non-polar solvent petroleum ether individually. Initially, 100 grams of pulverized drug were placed in a Soxhlet apparatus and defatted using 250 ml of Petroleum ether (40-60°C) in a 1liter Soxhlet apparatus. The extraction process continued until the solvent in the siphon was clear. To ensure complete defatting, a drop of the extracted material was tested on filter paper; the absence of an oily spot confirmed that defatting was complete. Following defatting, the extract was filtered, and the solvent was removed using a rotary vacuum evaporator at 50°C. The concentrated extract was then obtained under reduced pressure with a rotary evaporator to produce ethanolic extracts of *Xanthium strumarium* and *Urtica dioica.*

2.2 Animal experimentation

Healthy white wistar rats (150-200 gm), taken from animal house of Bilwal Research Laboratory, Jaipur, Rajasthan for the present study. Experimental protocol is approved by IAEC of Bilwal Research Laboratory (IAEC approval number-BMRL/IAEC/2023-15) Rats were fed with Amrut rat feed manufactured by Pranavagro ltd. Sangali, Maharashtra. Food and water were provided ad libitum. Clean sterilized husk is used as a bedding material for the animals.

2.3 Acute Dermal Reaction

Acute dermal reaction was determined in accordance with OECD 404. The study investigated acute dermal toxicity in rats by shaving 10% of their body surface area on their dorsal positions. Then, rats were categorized to 2 groups: test and control. The test group given a drug dose of 2000mg/kg, while the control group received sterile water. The application was repeated for 14 days, with daily observation for clinical changes [5].

2.4 Assessment of Wound healing activity of gel of Urtica dioica L. (GUD) and Xanthium strumarium L. (GXS)

- **Incision Wound Model**

The study involves 24 white laboratory rats that categorized to 4 groups, each group having six animals. Group 1 acted like the Control group and received a topical application of a simple gel base. Group 2 considered as the Standard group and was managed with topical application Povidone iodine ointment.Group 3 acted like the Test group for Gel formulation *Xanthium strumarium*, while Group 4 served like the Test group for Gel formulation *Urtica dioica*. Prior to the treatment, the rats were administered ketamine to induce sedation, and their dorsal hairs were then removed. An incision measuring 6 cm in length was created on the shaved back, and the separated skin was then brought together at a distance of 1 cm. Stitching was performed using suture material and a suturing needle. Following injury, those animals were housed in separate enclosures to reduce harm and the risk of infection. The objective of the study was to investigate the impact of gel formulations on the process of wound healing [6]. On the ninth day after the injury, the stitches were taken out from the animals that were under anesthesia. Day 10, measured the tensile strength. A sedated rat was positioned on a board, secured, and suspended

on a pulley. The board was positioned to deliver moisture to the wound until it burst. The water content in the bag was quantified and assessed for its tensile strength [7]. The study quantified the hydroxyproline content in excised tissue from an incision wound model, which is a constituent of collagen. The tissue was dehydrated, subjected to hydrolysis in hydrochloric acid, and subsequently oxidized using Chloramine-T. The measurement of absorbance was conducted using a UV-visible spectrometer [8].

- **Excision Wound Model Study**

The study comprised 24 white laboratory rats, which categorized to 4 groups, each group having six animals. Group 1, Control group, received a topical application of a simple gel base. Group 2, Standard group, was managed topically with Povidone iodine ointment. Group 3 and Group 4 served as the Test groups for Gel formulation *Xanthium strumarium* and Gel formulation *Urtica dioica*, respectively. Each animal was rendered unconscious by intraperitoneally injecting 1ml ketamine hydrochloride at a dosage of 10 mg per kilogram according to body weight. Their dorsal hairs were shaved with a razor blade. An excision wound was made by removing a section of tissue measuring 500 mm 2 in length and 0.2 cm deep from shaved area. This artificially made wound was left unprotected in the open air. Then these wounds of the individual groups were managed with daily administration of medication for a period of fifteen days or until complete epithelialization occurred starting from the day of initial injury. Percentage wound closures were recorded on days 0, 3, 6, 9, 12, and 15 for all groups. The time it took for epithelization and the decrease in scar size were observed. Wound contraction refers to the decrease in the size of a wound in relation to its original size. The previous wound mass was approximated as a percentage alteration [9].

2.5 Estimation of Antioxidants

To measured Catalase (CAT), Superoxide Dismutase (SOD) and reduced Glutathione (GSH) level in granulated tissue, after 15 days of post wounding, wound tissue were excised and collected in phosphate buffered saline (pH 7) [10].

2.6 Histopathological study

For histopathological study, Firstly excised granulated tissue and immediately preserve in 10% neutral buffered formalin, then embedded in paraffin wax and after that cut into 5µm thick sections and mounted on slides and with hematoxylin and eosin solution were used to stained and now examine the tissue sections under the microscope [11].

2.7 Statistics

The study parameters were executed in triplicate. This study data was expressed in form of mean ± standard deviation. Analysis of variance was conducted using Graph Pad Prism Version 7 to make comparison of the groups. This $p<0.05$ defined data is significant in terms of statistics.

3. RESULT ANALYSIS

3.1 Acute Dermal Reaction

Acute dermal toxicity was assessed in rats, with 10% of body surface area shaved and left as it is upto 24 hours. A drug which was testing, applied to shaved sites, while a control group received sterile water. No adverse clinical changes were observed, and gel formulations were found safe for therapeutic efficacy evaluation.

3.2 Assessment of Wound healing activity of gel of Urtica dioica L. (GUD) and Xanthium strumarium L. (GXS) using Incision Wound Model

Skin breaking force is a decisive factor in wound healing process and re-establishment of tissue strength is indication of repairing of damaged skin. Mechanical strength to the dermis is provided by Collagen and elastic fiber network. Tensile strength of granulated tissue is the minimal optimum force needed to rupture the incised part that reveals the effectiveness of drug, level of healing, processing of healing and strength of wounded tissue. In this wound model, progress of wound healing is determined by rupturing strength of this cured tissue treated with various preparations on 10th day (Table 1).

Table 1: Effect of Treatment on Tensile strength (g) and Hydroxyproline content (mg/g tissue) using Incision Wound Model

Groups	Tensile strength (g)	Hydroxyproline content (mg/g tissue)
Group 1	342.28 ± 10.21	36.40 ± 4.02
Group 2	576.20 ± 3.63***	80.61 ± 5.44**
Group 3	552.32 ± 4.72***	71.66 ± 2.97***
Group 4	519.44 ± 5.21**	62.19 ± 4.06***

Results are expressed as mean ± SEM; *P<0.001 when compared to NC and **P<0.01,

***P<0.05 when compared to Group II; Group I served as Control group was given simple gel base topically, Group II served as Standard group treated with Povidine iodine ointment topically, Group III served as Test group for Gel formulation Xanthium strumarium and Group IV served as Test group for Gel formulation Urtica dioica.

3.3 Assessment of Wound healing activity of gel of Urtica dioica L. (GUD) and Xanthium strumarium L. (GXS) using Excision Wound Model

Wound contraction is the reflection of healing of wounds. Better efficacy of medication depends upon speedy wound closure. The wound contraction expressed by excision wound model after topical application different gel formulation Xanthium strumarium and Urtica dioica (Table 2; Figure 1). An accepted level of free radicles in the body is not a serious consideration but their increased level not only cause damages to the tissues but may produce carcinogenic modifications (Table 3), which is further confirmed by the histopathological examination (Figure 2)

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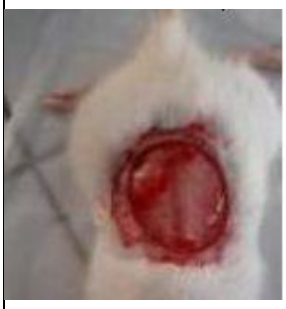
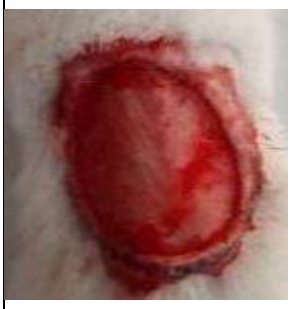
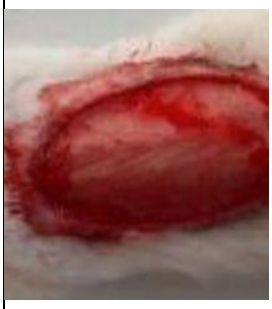
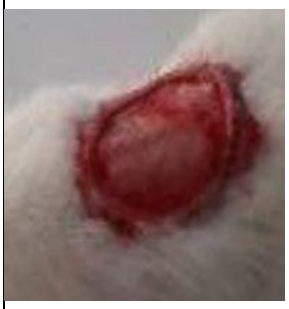
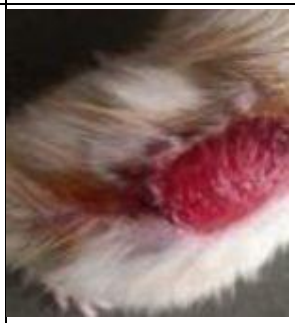
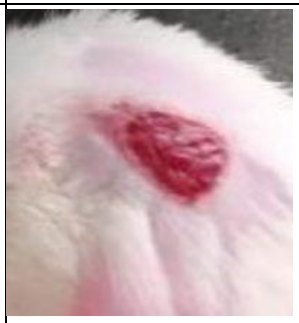
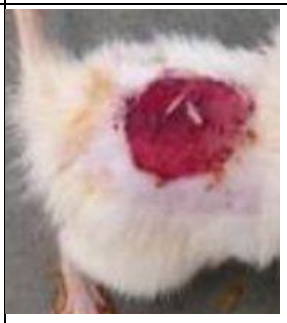
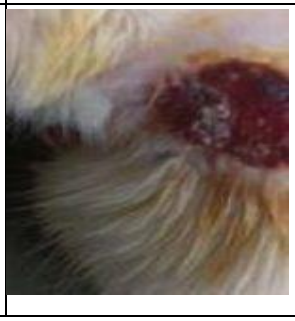
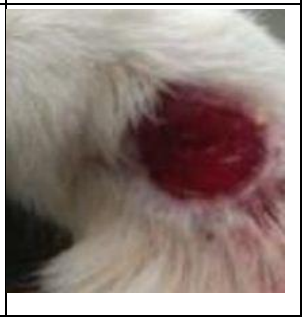
Table 2: Effect of Treatment on Wound contraction using Excision Wound Model

Groups	Wound contraction (mm2) observation days						Epithilization period (Days)
	0 day	3 days	6 days	9 days	12 days	15 days	
Group 1	503.86 ±	487.38 ±	391.32 ±	363.47 ±	274.71 ±	183.56 ±	24.16 ± 1.47

	4.22	4.37	4.96	7.21	4.61	4.66	
Group 2	502.01 ± 6.39***	407.57 ± 6.23**	324.24 ± 4.47***	247.68 ± 3.95***	131.59 ± 3.31***	13.37 ± 2.44***	17.16 ± 1.16**
Group 3	503.99 ± 7.03**	417.79 ± 4.04***	333.67 ± 7.68**	261.55 ± 6.36**	144.38 ± 3.55***	18.47 ± 2.18***	19.66 ± 1.03***
Group 4	501.30 ± 4.78***	443.78 ± 4.33***	349.42 ± 4.50***	297.92 ± 4.82***	160.90 ± 4.50**	31.20 ± 4.05**	21.66 ± 0.81***

Results are measured in terms of mean ± SEM; *P<0.001 in comparision to NC and **P<0.01,

***P<0.05 in comparision with Group 2; Group1 served as Control group was given simple gel base topically, Group 2 served as Standard group managed with Povidine iodine ointment topically, Group 3 served as Test group for Gel formulation Xanthium strumarium and Group 4 served as Test group for Gel formulation Urtica dioica.

Day	**Group 1**	**Group 2**	**Group 3**	**Group 4**
0 Day				
3 Day				
6 Days				

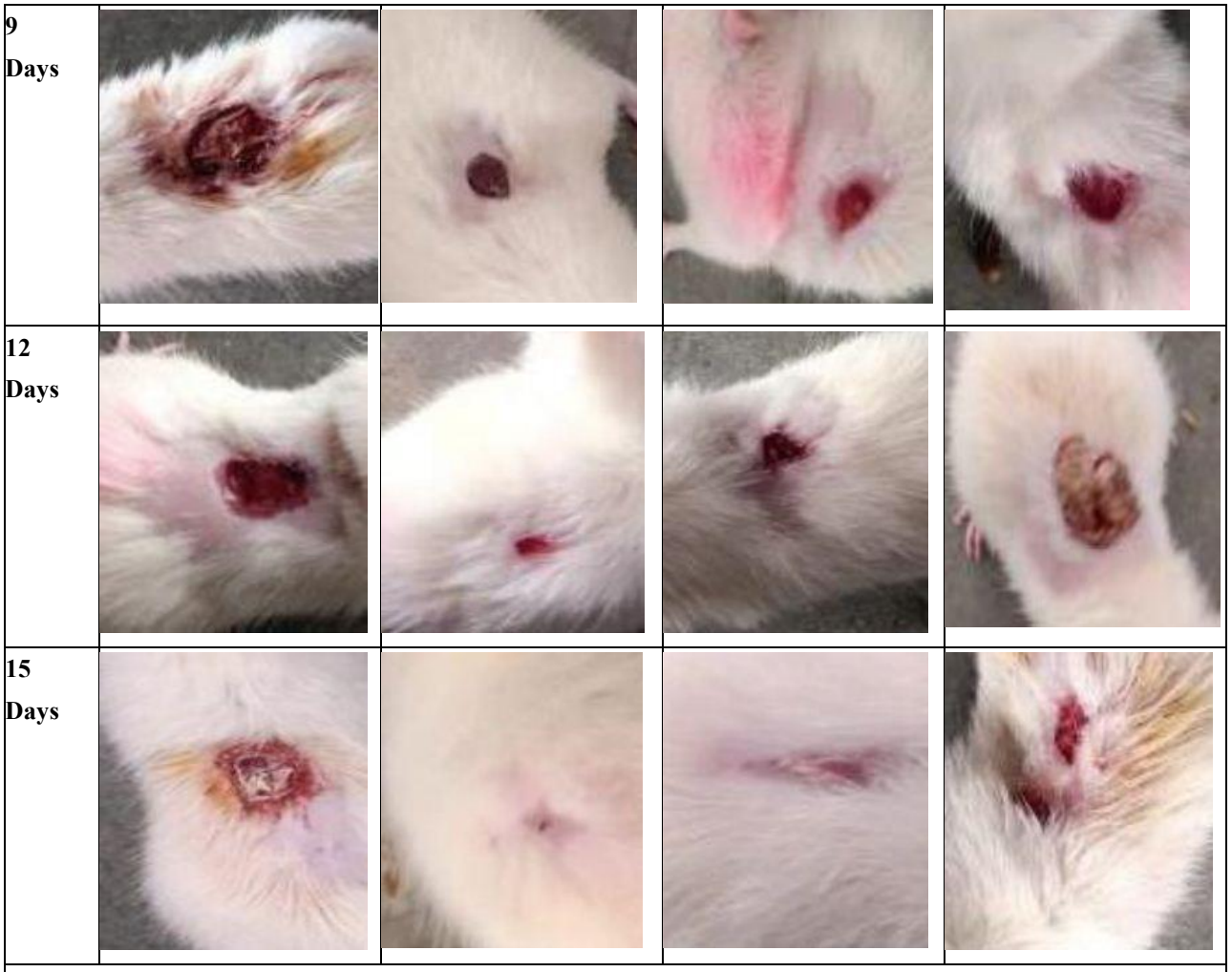


Figure 1: Effect of Treatment on Wound contraction using Excision Wound Model

Table 3: Effect of Treatment on Stress Parameters using Excision Wound Model

Groups	SOD (U/mg protein)	CAT (U/mg protein)	GSH (µg/g protein)
Group 1	6.26 ± 0.87	20.59 ± 1.46	10.63 ± 0.78
Group 2	10.90 ± 0.49***	32.14 ± 0.75**	21.22 ± 0.57***
Group 3	10.41 ± 0.61**	30.91 ± 0.70***	19.58 ± 0.63***
Group 4	8.09 ± 0.61**	26.25 ± 0.75**	17.39 ± 0.77**

Results are expressed in terms of mean ± SEM; *P<0.001 in comparisionto NC and **P<0.01,

***P<0.05 in comparision to Group II; Group I served as Control group was given simple gel base topically, Group II served as Standard group managed with Povidine iodine ointment topically, Group III served as Test group for Gel formulation Xanthium strumarium and Group

IV served as Test group for Gel formulation Urtica dioica.

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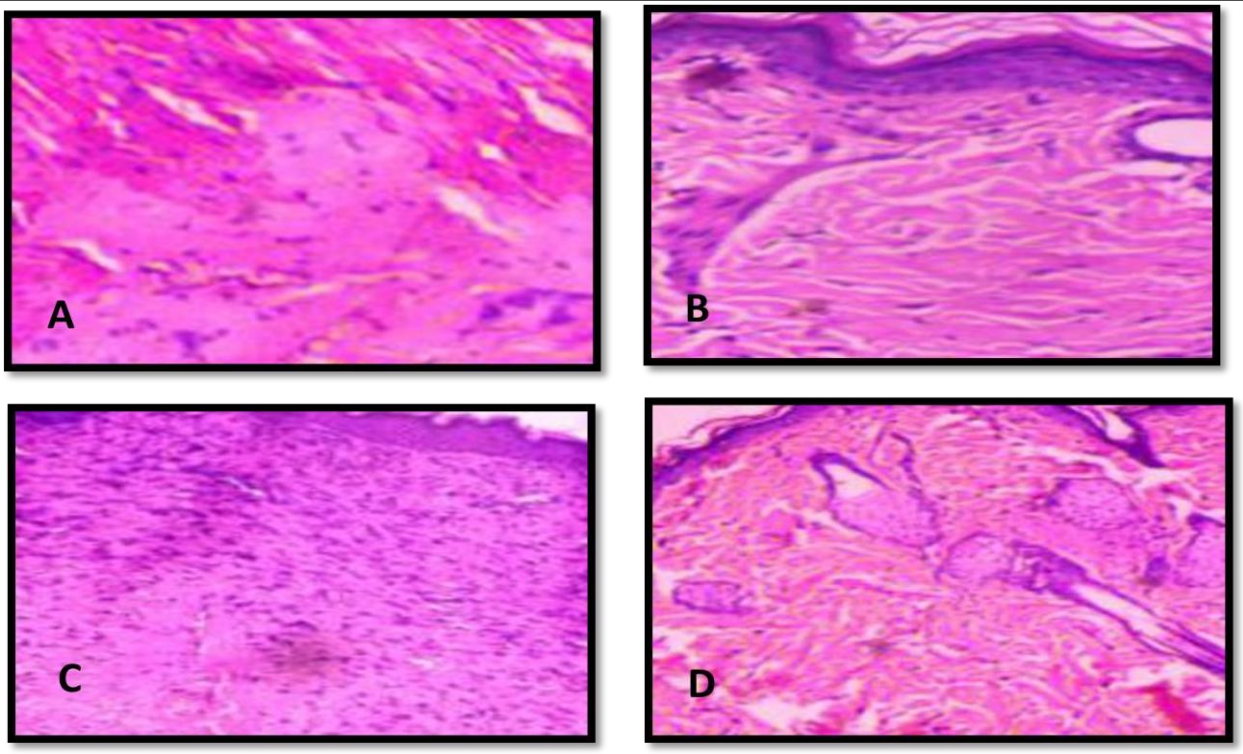

Figure 2: Histopathological Examination of Skin section in Excision Wound Model Group 1: No regeneration of epithelial layer, inflammatory cells, mild increase in fibrous tissue; Group 2: Complete and thick re epithelization and high number of fibroblast; Group 3: Well organized epithelization of thick layer with pre dominant collagen with few fibroblast, and Group 4: Regeneration of well defined epithelial layer and well formed granulation tissue and very few number of inflammatory cells

4. CONCLUSIONS

The objective of this study was to find out the immediate harmful reaction on the skin caused due to gel formulations used for promoting the healing of wounds. The rats underwent depilation using a razor blade and were subsequently left undisturbed for a duration of 24 hours. Following a 24-hour period, the experimental medicine was administered to the shaved areas, whereas the control group got a local skin application of sterile water. The medication was administered for a duration of 14 days, with daily monitoring for any alterations in the patient's clinical condition. There were no negative clinical changes observed. The study assessed the immediate skin toxicity of gel formulations for wound healing in rats. Following a 24-hour period, the experimental medicine was administered to the shaved areas, whereas the control group was given sterile water. The study saw no detrimental clinical alterations. The gel formulation of *Xanthium strumarium* exhibited superior tensile strength and a shorter epithelization period in comparison to the control group. The gel formulation exhibited superior histopathological recovery. The levels of free radicles were comparable to those in the usual therapy group.

5. CONFLICT OF INTEREST

None

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