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Evaluation of the effects garlic extract with low level laser on healing cutaneous wounds in rabbits model

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***Abstract*---**This study was intended to assess the healing activity of effect of garlic extract 15% combined with the 8j /cm2 diode lasers by topical application on the skin experimentally in full - thickness cutaneous wounds in a rabbit's model. For this reason, this investigation was conducted on sixteen adult and clinically healthy males' rabbits, full-thickness circular skin wounds (1 cm in diameter) were made on the back of each animal. The animals were arbitrarily divided into two equal groups of eight rabbit's for each. In control group, the injuries were left without treatment. While in treatment group, were treated by a topical application of the garlic extract 0.15% using garlic extract, for two times /day for 7 successivedays, involving the wound with combination irradiated by diode laser at a wave length of (820nm), frequency of (10 KHz), power output (50mW), spot size (1 cm), energy density (8 J/cm2) and exposure time (5 second) for seven consecutive days. The clinical assessment of treated wounds demonstrated that the injury healing process contraction%, Re-epithilization % and total wound healing % were signifecantly $P<0.05$ than that of control wounds at 14 days of the investigation. The histopathological studying on seven and fourteen days post- treatment demonstrated that treated wounds have reduces inflammation during 3 first days post-treatment and promotes epithelialization in two weeks of healing with increased vasculature than those in untreated wounds. This study concluded that depending on clinical, macroscopic, histopathological findings revealed that there was the combination group showed significant difference from control groups confirmed that the act of synergy between laser and 15 % garlic

extract in the combination group gave the best and better results at the level of wound healing.

***Keywords*---**garlic extract, low level laser, cutaneous wounds, rabbit's, acute woundshealing.

Introduction

Skin wound repair requires complex and highly coordinated interaction between different cells to restore the epidermal barrier and tissue architecture after injury (Marggio *et al.*, 2009). The wound healing is a multi-stage phenomenon that requires the activation, recruitment of numerous cell types as keratinocytes, endothelial cells, fibroblasts and inflammatory cells, and macrophages which appear to be central to this process (Li *et al.,* 2007). Damage to any tissue triggers a cascade of events that lead to rapid repair of the wound. Re-epithelization, development of granulation tissue, and contraction of the underlying connective tissue are all involved in skin healing. This concentrated effort by the damaged cell layers is followed by a powerful inflammatory response, in which neutrophils, macrophages, and mast cells move from adjacent tissue and the circulation, and this inflammatory response is critical for battling infection (Martin and Leibovich, *et al.,* 2005). Low levels of laser or non-coherent light termed low level light therapy (LLLT) have been reported to accelerate some phases of ound healing (Demir *et al.,* 2004). In past low level laser therapy used in the united states to investigational use only. Yet LLLT is used clinically in many other areas including Canada, Europe, and Asia for treatment of various neurologic, dental, and dermatologic disorders (Posten *et al.,* 2005). In wound healing, laser therapy can increase number of vessels, it can stimulate proliferation of fibroblasts and increase its number, can promote process of collagenization (Pallotta *et al.,* 2012). Although molecular mechanism of LLLT has not been elucidated completely, the anti-inflammatory effect of laser irradiation may be one of the most important therapeutic effects on many injuries and disorders including wound healing (Wanner *et al.,* 2016). In study by study Barak *et al.* (2007) showed garlic extract give angiogenesis effect and The distribution of mast cells (especially activated mast cells) enhances the angiogenesis ratio and indirectly inhibits the inflammatory stage of healing. The role of mast cells in enhancing cellular proliferation during wound healing has been reported previously. Mast cells are known to secrete vascular growth factor (VGF), which stimulates endothelial cell proliferation; this, in turn, has been shown to up regulate neovascularization during the healing process (Younan *et al.,* 2011). The aim of present study is to evaluate the effect of an 820-nm low level laser with combined Garlic extract on full thickness skin woundin rabbit.

Material and Method

Experimental Animals Design: Research consents to animals' use and care Committee College of Veterinary Medicine, University of AL- Qadisiyah, along the period of the study from 10 July to 10 November of 2021. Sixteen adult healthy males' were utilized in this study. They were haphazardly divided into two groups; wounds were left without treated as control group. While, were treated by Garlic

Extract with Low Level Laser as treated group. The rabbits were sacrificed at 7th, and 14th postoperative days for histopathological assessment.

Preparation of 0.15 % Garlic Extract: Allium sativum cloves were hand-collected in the Iraq in March. Cloves were separated, sliced by hand, and approximately 500 g of fresh plant material was left to dry naturally on laboratory benches, at room temperature (23-24 °C).The resulting extract was oven-dried at 40 °C for 24 h, yielding 12 g of dry extract. The extracts were stored at -20 °C until use in the experiment. (Abad, Nouri, Tavakkoli,*el,at,* 2011;Prichoa Roman, Manfredini, *el,at,* 2011).Then he took 50 grams of crushed garlic and placed it in Soxhlet beaker No. A.While alcohol was added in beaker No. B with a capacity of 500 ml of 70% ethanol.Prepare to put in place In 100 mL of distilled water, dissolve 15 garlic extract.

Utilization of Garlic Extract with Low Level Laser in full-thickness wounds

Prior all surgical procedures, the animals were first tranquilized with Diazepam at a dose of 1mg/kg and a mixture of 5 mg/kg of xylazine hydrochloride, 35 mg/kg of ketamine hydrochloride was directed by intramuscular injection. The dorsa sides were prepared aseptically for the creation of 1cm in diameter a full-thickness skin incision by using punch machine under local anesthesia with 2% lidocaine hydrochloride (OUBARI-Pharma, Syria),were created in sixteen rabbits is illustrated in (Fig.1). These wounds were allocated, depending on the method of the treatment, into two groups; the eight rabbits were left without any treatment, Served as control group and did not receive garlic extract or laser treatment. The animals in treatment group were treated with topical application of the garlic extract twice in daily. The session include tainting the site of operation with 70% ethanol prior to make the skin incision; after creation of incision a topical application of the garlic extract 0.15% using garlic extract, for two times /day for 7 successivedays, involving the wound with combination irradiated by diode laser at a wave length of (820 nm), frequency of (10 KHz), power output (50mW), spot size(1 cm), energy density (8 J/cm2) and exposure time (5 second) for seven consecutive, days irradiation once daily was done immediately after finishing the surgical operation. The probe of laser irradiation was applied closely to the wound surface.

Fig. 1: : (A) Representative pictures of the size of induced wound (B) control wound (C) Diode laser device used in this experiment, wound was irradiated with laser, the probe placedclose and perpendicular on the wound.

Evaluation of wounds healingHistopathological evaluation

The rabbits were sacrificed at seventh, fourteenth, 7th and 14th postoperative days with ether and the tissues from the wound include the whole thickness of the skin and the rounding skin of the individual animal was removed. These samples were then separately fixed in 10% formalin, dehydrated through graded alcohol series, cleared in xylene, embedded in paraffin wax and cut transversely by a microtome into 5 ìm thin sections and tissue sections were stained with haematoxylin and eosin (H&E).

Healing follow-up: A general clinical assessment was performed in all animals (animal behavior, cardiorespiratory activity and body temperature). Digital photographs of the wounds were obtained at day 0, 3, 7, and 14. A standardized ruler was included in each photograph for digital calibration of the photographs. Gross evaluation of wound healing was performed based on the percent of wound contraction (WC %), area of epithelialization (AE) (cm2), and wound area (WA) (cm2). Wound Contraction was calculated using the formula: (W0 – WI) / W0* 100 W0 = the initial wound measurement; WI = the wound measurement on day of measurement. Statistical Analysis: The Statistical Analysis System-SAS was utilized to impact on various factors (treatment and days) in study parameters (percentage). The least significant difference(LSD) test was utilized to comparative between percentages in this examination.

Results

Acute Wound Analysis all created wounds of treatment and control group diminished rapidly in size along the examination, yet the close inspection of wounds images indicated that the rate of wound closure in treated wounds were significantly (*P≤0.05*) more along the period of the study as compared to untreated wound. Depending on the information in (Tab.1below),the mean ± SD of total wound area for treatment group in day7 was recorded (27.74± 3.65) cm; and Control group (13.19± 3.21) cm. There were some significant differences between groups. However, total wound area in day 14 in treatment group was significantly higher than in Control group (*P<0.05*) (43.16± 5.55), (29.84± 3.20) respectively. The mean ± SD of epithelialized area of each group in day 7 was as follows; in treatment and control group recorded (65.16± 3.56) (32.84± 3.20) cm respectively. There was significant differences weredetermined between treatment and control group (*P≤0.05*) and showed better wound contraction compared with group Control as follows; in treatment and control group recorded (55.31± 3.72) (35.28± 3.27) cm respectively illustrated in (Fig.2).

Tab. 1: Shows the Rates of Total Wound Healing (WH %), (cm) in both groups

GroupDay	Mean ± SE		LSD
	Control Group	Treatment Group	Value
0 th	0.00 ± 00 a	0.00 ± 00 a	0.00 NS
3 th	0.00 ± 00 a	0.00 ± 00 a	0.00 NS
7 th	BC 13.19± 3.21 b	B 27.74± 3.65 a	7.103 *
14 th	AB 29.84± 3.20 b	A 43.16± 5.55 a	10.753 *

[a,b,c] Values (Mean ± SE) having with the different small letters in same row and big letters in samecolumn differed significantly at p ≤0.05.

Tab. 2: Shows the Rates of Wound Epithelization (AE %), (cm) in both groups

Group	Mean ± SE		LSD
Day	Control Group	Treatment Group	Value
0 th	0.00 ± 00a	0.00 ± 00a	0.00 NS
3 th	0.00± 00a	0.00 ± 00a	0.00NS
7 th	BC 19.11± 2.23 b	B 32.98± 4.26 a	8.107 *
14 th	AB 32.84± 3.20 b	A 65.16± 3.56 a	8.316 *

[a,b,c] Values (Mean ± SE) having with the different small letters in same row and big letters insame column differed significantly at p ≤0.05.

Tab. 3: Shows the Rates of Wound Contraction (WC %),(cm) in both groups

GroupDay	Mean ± SE		LSD
	control Group	Treatment Group	Value
0 th	0.00 ± 00a	0.0 ± 00a	0.00 NS
3 th	0.00± 00a	0.0 ± 00a	0.00 NS
7 th	BC 16.00± 3.21 b	B 27.00± 3.11 a	7.209 *
14 th	AB 35.28± 3.27 b	A 55.31± 3.72 a	8.822 *

a,b,c Values (Mean ± SE) having with the different small letters in same row and big letters insame column differed significantly at p ≤0.05.

Histopathological evaluation: In control group on 7day post-operatives showed, severeinflammatory cell infiltration, a large number of neutrophils and number of macrophages (neutrophils and macrophages) in the dermis and epidermis with congestion of the blood vessels, hemorrhage in the dermal layer and severe edema (Fig.3). While, on 14 days post- operatives indicated the presence of abscess with a thick crust, cracked, splintered scab was discovered beneath the scab, with irregular full epithelial layers made of numerous layers of cells. (Fig.5); also other section indicated the field was heavily infiltrated with inflammatory cells and just a little amount of granulation tissue with Partial stratum basal hyperplasia (Fig.7). However, at day 7, in treatment group, the most cases received Garlic Extract with Low Level Laser demonstrated immature granulation tissue described by dense less cellular regular collagen fibers (Fig.4). However, at day 14 post treatments indicated there is no crust and complete keratinized layer above the epidermis and the two edges of wound meet, marked downward hyperplasia of epidermal layers with formation of new hair follicles thin and regular arrangement of collagen fibers and formation of new blood vessels in the healed area (Fig.6). Also in another section showed Infiltration of inflammatory cells and formation of hair follicles with regular arrangement of collagen fibers in the dermis. Formation of new blood vessels which contain red blood cells with regular and acidophilic collagen fibers in the healed area likewise demonstrated dense thick mature granulation tissue and on the special stain appeared stained collagen fibers in the wound site (Fig.8).

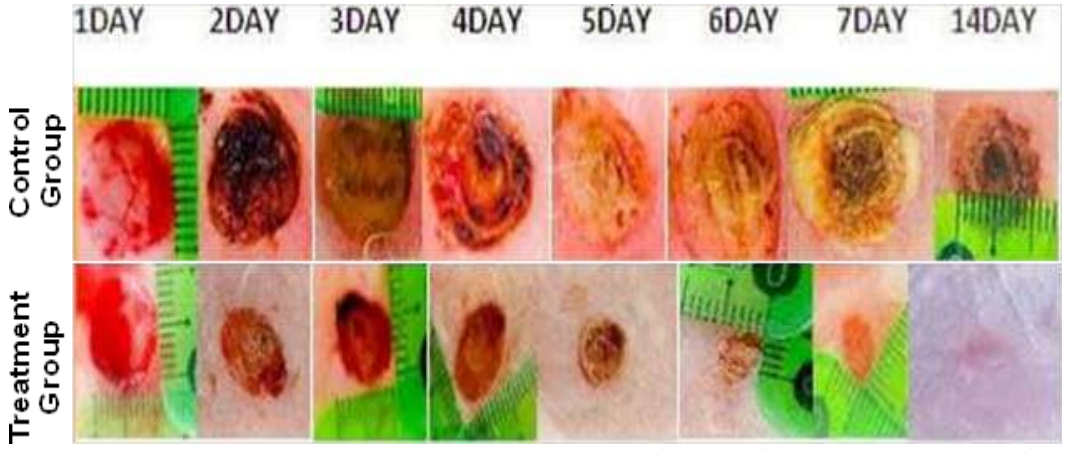

Fig 2: Representative pictures control group and treated group during period experimental study

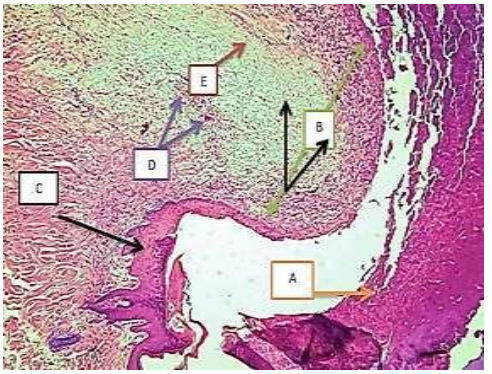

Fig 3: Histopathological segment of control group, at 7 days PO showed (A) thick crusts which attached closely with the surface of wound and wide scar tissue (B) Complete sloughing of keratinized layer, mild hyperplasia in the epidermis layer cells (C) Increased granulation tissue and profuse collagen fibers in the dermis (D) (H& E stain 100X).

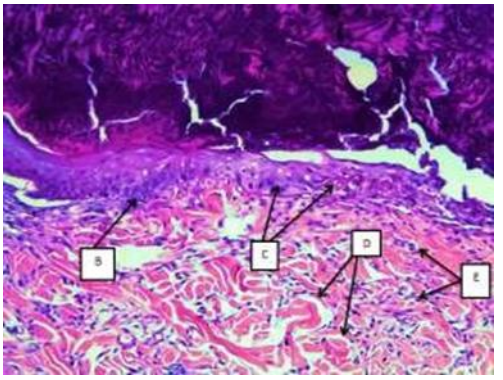

Fig 4: Histopathological segment of treatment group, at 7 days PO showed presence of thick crust (A) partially detached from the epidermis. Marked hyperplasia (B) basal cells in the stratum basal (C) thin or delicate and interlaced

collagen fibers with infiltration of inflammatory cells (E) formation of new and small hair follicles (H& E stain 100X).

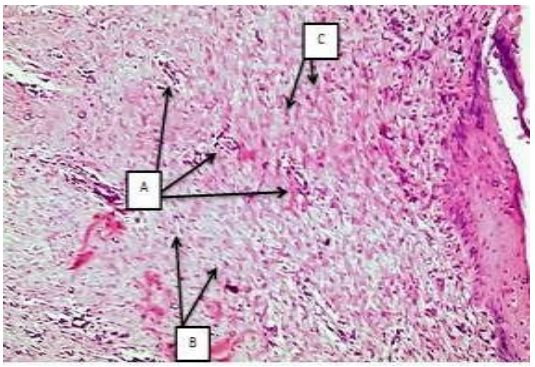

Fig 5: Histopathological segment of control group, at 14 days PO showed mild hyperplasia of stratum basale and few granulation tissues with new small blood vessels (A) and few fibrosis (B) Thin, coarse and interlaced collagen fibers in the dermis (C) (H& E stain 100X).

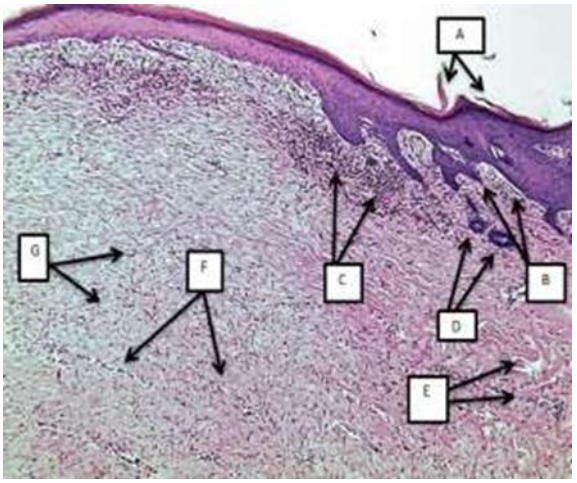

Fig 6: Histopathological segment of treatment group, at 14 days PO showed (A) hyperplasia of epidermal layers (B) Infiltration inflammatory cells in the dermis beneath the epidermis (C) new hair follicles (D) regular arrangement of collagen fibers, new blood vessels (F) few fibrosis in healed area (G). (H& E stain 100X).

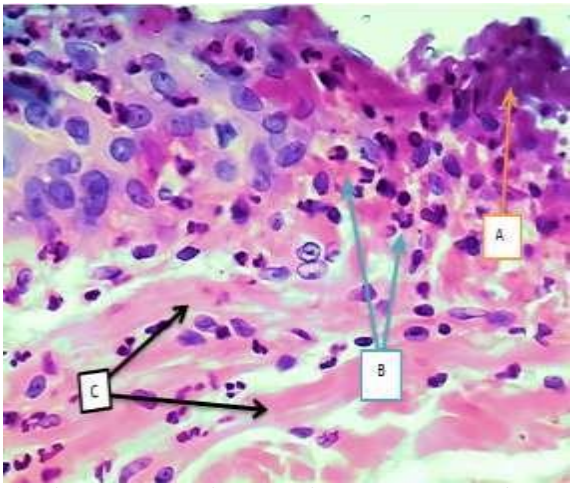

Fig 7: Histopathological segment of control group, at 14 days PO showed (A) mild hyperplasia of basal cells in the stratum basal Infiltration of neutrophils (B) thick and coarse collagen fibers (C) (H& E stain 400 X).

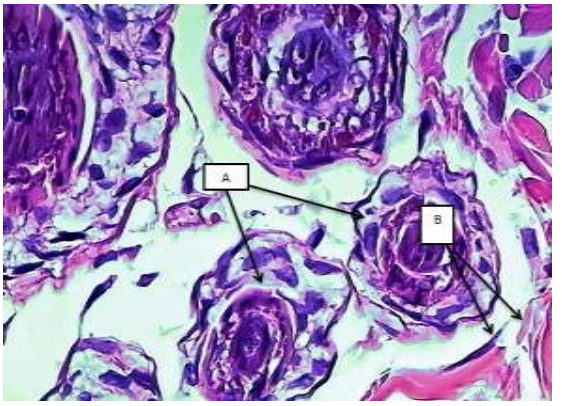

Fig 8: Histopathological segment of treatment group, at 14 days PO showed there were newly- formed and small hair follicles (A) in the dermis with coarse collagen fibers (B) (H& E stain 400X).

Discussion

The aim of this investigation was to assess the role of the LLLT and garlic extract on the wound healing processes of the skin of rabbits. In this study through the macroscopic and histological results that we obtained, it was proven that which assessed in vitro and effects on proliferation, migration and angiogenesis, and decided its ability to be resorbed in vivo rabbit full-thickness wounds model. When all is said in done, all injuries of treatment and control group diminished quickly in size along the period of this study, however, the close investigation of wounds images revealed that the rate of wound closure in treated wounds were significantly ($P<0.05$) more along the period of the study when contrasted with untreated injuries. However the results in treatment group demonstrated the wound contraction increased to much or reduction in size of the wound because laser and garlic combination effect compared with control

group. To repair a wound, there are a variety of vascular and cellular changes, epithelial and fibroblast multiplication, collagen and elastin manufacturing, revascularization, wound contraction, and collagen and elastin accumulation (Chaves *el at.*, 2014). In addition, notable that the trophic regeneration, anti-inflammatory facts, and analgesic properties, all of which remain important for completing the healing process (Behm *el at.,* 2011), Although this low-level laser therapy is said to help increase mitochondrial activity, thereby increasing ATP, vasodilation, protein synthesis, the reduction in the anti-inflammatory prostaglandins, cell mitosis, and relocation and multiplication of keratinocytes, there are also claims that it stimulates the creation of new capillaries, this analgesic properties of the laser improves the effect of garlic extract in protecting the wound from irritation of garlic, which improves wound healing, and these findings of current study agrees with many researchers (Sfaxi *et al.* 2009).These results are close to the results obtained by Wardlaw *et al.* (2019) revealed that used laser on the healing process of incisions that were made to Dachshund dogs. The results indicated promoting the healing process in a fast way with restoring epithelization by day seven, not only that, but the effects continue to improve the healing process until the end of the experiment (Lopes et *al.,* 2010). These results supported by (Wardlaw *et al.,* 2019) confirm that our findings that show decreases in time needed for completing a good epithelial and collagen restoring wound healing process .Laser phototherapy, a photosensitive dye, was shown to have an antibacterial effect that may enhance tissue regeneration (Woo and Heil, 2017). Our outcome the histopathological assessment of treated wound sections seemed a high incidence of mature granulation tissue, myofibroblasts and new blood vessels, at the same time with few myofibroblasts were scattered through fibrous connective tissue containing congested blood vessels were notice in the sections of control wounds. The results of this investigation might be related to the effect of a synergistic effect between garlic and laser which could be played an important role in the enhancement and acceleration of cutaneous wound healing. This determination is in a harmony with other many studies, in which the LLLT was used to repair tissue defect directly. They have indicated that a synergistic effect between garlic and laser could induce specific tissue regeneration in vivo. In study by Demidova-Rice *et al.* (2007) who discovered that LLLT stimulates wound contraction by promoting fibroblast, myofibroblast differentiation as well as fibroblast motility. Myofibroblasts are the consequence of fibroblast development and are responsible for bringing the wound's margins together by cell contraction. This plays a critical function in transporting blood vessels that are rapidly regenerated under the influence of angiogenetic substances released by fibroblasts, supplying nutrients and oxygen to the area Simunovic *et al.* (2000) and Tabakoğlu *et al.* (2016) who have also found, at day 14 of their experiment, that there were focal pattern of epithelial proliferation and over tissue of granulation, However, these changes were less in the laser group with healed epidermal tissue covering a granulation tissue with a high degree of development. Collagen rich fibers arranged in a parallel way to the wound surface. Investigation on skin wounds exposed to aged garlic extract show an increase in the re-epithelialization and profuse dose-dependent neovascularization (Ejaz *et al.,* 2009). In this study, important garlic component for tissue healing and wound contraction were the re-arrangement of ground collagen fibers, which can ultimately influence the quality of scars, The extracellular matrix functions as a reservoir for growth factors and signaling which endothelial cells can migrate

during angiogenesis (Li *et al.,* 2003). The mechanism of tissue contraction during wound healing is not completely understood. There are two theories proposed to explain this process. The first theory suggests that the contraction forces are generated by myofibroblasts which are transmitted to other cells and surrounding connective tissues. This hypothesis suggests that the myofibroblasts would act as a multicellular unit to contract the tissue (Ottl *et al.,* 1998).

The second theory proposes that the contraction is caused by the re-arrangement of collagen fibers through the action of fibroblasts. The dislocation forces created by these cells within the connective tissue would lead to the re-orientation of collagen fibers into thicker bundles as well as their contraction (Ehrlich and Rajaratnam, 1990, and Berry *et al.* 1998). In conclusion wound healing is a complex process, resulting from the interaction between a large number of cell types, extracellular matrix proteins, and mediators such as cytokines and growth factors. Depending on clinical, macroscopic, histopathological findings revealed that there was the combination group showed significant difference ($P \leq 0.05$) from control groups confirmed that the act of synergy between laser and 15 % garlicextract in the combination group gave the best and better results at the level of wound healing. Also use topical of laser and 15 % garlic extract allows us to increase and improvethe therapeutic approach to the cutaneous wound.

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