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Experimental Research Article

# Preventive effects of topical catechin hydrate in a rabbit ear model of hypertrophic scar

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## ABSTRACT

**Introduction:** Hypertrophic scars (HSs) are the result of excessive collagen deposition at the wound area, which gives raised, pruritic, and erythematous lesions. Until now, there is no optimal treatment for abnormal scarring, including HSs. Because of the anti-inflammatory and anti-fibrotic effects of catechins, the aim of this work was to investigate the preventive potential of catechin hydrate (CH) against HS formation in rabbits.

**Methods:** Topical CH ointments were prepared by the levigation method. 10 New Zealand White rabbits in 5 groups were used in this experimental research as follows: negative control, Eucerin (ointment base) treated group, and CH 1, 2, and 4% w/w ointment-treated groups. The anti-scarring effects of CH were investigated by measuring the epidermal thickness index (ETI), the scar elevation index (SEI), the amount of collagen deposition, the type I and type III collagen, and the MMP1 levels in scar tissue.

**Results:** Our results showed that after treatment with topical CH, the scars formed in the wound site were almost normal and flattened to the surrounding skin level, while in the control and eucerin-treated groups, the scars were raised and had a darker color than normal skin. In addition, topical CH in all concentrations significantly ($p<0.05$) reduced ETI, SEI, the collagen deposition, and the levels of type I and type III collagen compared to the control group. Moreover, CH 4% significantly ($p<0.05$) enhanced the MMP1enzyme levels compared to the control group.

**Conclusion:** Based on our findings, CH has the potential for the prevention of hypertrophic scar formation.

**Keywords:**
Catechin hydrate
Hypertrophic scar
Rabbit ear model
Collagen
MMP1

## Introduction

Hypertrophic scars (HSs) are the consequences of an abnormal wound healing process that occurs following tissue damage by physical injury. HSs can be painful and itchy, causing functional disability and aesthetic concerns (Tamri et al., 2020). These scars are described by dermal proliferation, with excessive extracellular matrix (ECM) proteins, mostly collagen, over a long period of

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time, and by continuing inflammation and hyperfibrosis (Davies et al., 2022). HSs following surgery, trauma, and especially burns are the most important concern for patients and a great challenge in clinical practice, because they can be pruritic, painful, raised, and esthetically unacceptable (Chipp et al., 2017).

Currently, there are various treatments for abnormal scarring, including silicone gel sheeting, pressure garments, onion extract and heparin, intralesional corticosteroids, laser therapy, cryotherapy, and surgery (Ogawa 2022). However, none of these treatments can safely and effectively treat HSs or prevent abnormal scar formation; hence, the development of new treatment methods is essential for the prevention and treatment of HSs.

Catechins are polyphenolic flavonoids that occur in many plants. Green tea is an important source of catechins. Catechin, epicatechin, epigallocatechin, epigallocatechin gallate, epicatechin gallate, gallocatechin, catechingallate, and gallocatechin gallate are the eight members of the catechin family (Bae et al., 2020). Catechin exhibits many pharmacological activities, including antioxidant, anti-proliferative, and anti-inflammatory effects (Bae et al., 2020). Moreover, anti-fibrotic effects of catechins have been shown in several studies (Dos Santos et al., 2020). In addition, Song et al. (2023) revealed the anti-scarring activities of epigallocatechin gallate (EGCG) in the rabbit ear model. Their findings revealed that EGCG suppressed the expression of transforming growth factor-β1 (TGF-β1), type I and type III Collagen, Smooth muscle alpha-actin (α-SMA), and endothelial nitric oxide synthase (eNOS) at mRNA levels in scarring tissues (Song et al., 2023). Moreover, Kweon et al. (2023) evaluated the anti-fibrotic effects of CH on chronic pancreatitis in mice, and they concluded that CH inhibited inflammation and prevented tissue damage and production of ECM components such as collagen and fibronectin during chronic pancreatitis (Kweon et al., 2023).

Based on the above, this experimental research was carried out to investigate the anti-scarring potential of catechin hydrate (CH) by evaluating its preventive effects against scar elevation index, epidermal hypertrophy, and collagen synthesis and deposition in a hypertrophic scar model in rabbits.

## Materials and methods

*Preparation of CH ointment*

Catechin hydrate (CH) was purchased from Sigma Aldrich (CAS number: 225937-10-0).

1, 2, and 4 grams of catechin powder were dispersed in 100 g of Eucerin, and the 1%, 2% and 4% ointments were prepared by the levigation method.

*Animals*

10 New Zealand white rabbits of both sexes with a weight of 2 to 3 kg were obtained from the Center for Care, Reproduction and Breeding of Laboratory Animals of Hamadan of Medical Sciences. The animals were provided an acclimation period of 7 days, allowing them to stabilize to the laboratory conditions. The rabbits were maintained under standard conditions of 22±3 °C, 55% relative humidity, and 12 12-hour light/dark cycle. Animals were provided standard food and water. All considerations for working with laboratory animals were followed according to the guidelines prepared by the ethics committee of Hamadan University of Medical Sciences.

*Wound creation and hypertrophic scar establishment*

In this study, the rabbit ear HS model was used (Zarei et al., 2022). To create the wounds, the animals were anaesthetized by ketamine 80 mg/kg and xylazine 10 mg/kg injection (IP). After disinfection of the wound site, four full-thickness wounds of 1cm diameter were made on the internal side of each rabbit ear by a biopsy punch (wound area surface = 0.785 square cm) (Kryger et al., 2007). 10 rabbits with 80 wounds were used for evaluation of the preventive effects of topical administration of CH on hypertrophic scar formation. The animals were randomly divided into 5 equal groups (each group consisted of 2 animals and 16 wounds), and the first group received no treatment, the second group was treated with Eucerin, and the third, fourth, and fifth groups received 1%, 2% and 4% CH ointments, respectively. The treatments were applied twice a day, starting from 1 day after wound creation and continuing for 35 days, then scar tissue samples were collected from each group.

*Scar elevation index assessment*

Scar elevation index (SEI) assessment is a valid method to evaluate the degree of scar elevation (Feng et al., 2020). Hematoxylin–Eosin staining images of scar tissue samples were used for the calculation of SEI. The SEI was calculated by dividing the height of the scar tis-

sue by the height of the adjacent normal tissue. ImageJ[R] software was used for the measurement of SEI.

*Epidermal thickness assessment (ETI)*

The ETI was calculated by dividing the height of the epidermis of the wound tissue by the height of the epidermis of the adjacent normal tissue. Five fields of cross-sections of the scar and five fields of normal tissue nearby the scar (400X) were studied to estimate the mean epidermal height in scar and normal tissues, respectively. The ETI >1 represents epidermis hypertrophy (Shi et al., 2013).

*Collagen deposition assessment*

The collagen fiber arrangement was evaluated by Masson's trichrome stain procedure. Collagen was quantified in scar tissues by taking four images (100X) of dermis in each scar, and the mean collagen area was measured by Deconvolution ImageJ software (Chen et al., 2017).

*Type I and type III Collagen and MMP1 assay*

Rabbit collagen type I, type III and MMP1 concentrations in tissue samples from different experimental groups were quantified using rabbit-specific ELISA kits (Shanghai Crystal Day Biotech Co. China). All measurements were performed using the kit manufacturer's instructions.

*Statistical analysis*

The data were analyzed using the one-way analysis of variance (One-Way ANOVA) technique followed by multiple comparisons. GraphPad Prism 8 software was used for data analysis. The results were shown as Mean ± SEM. P values less than 0.05 were considered statistically significant.

## Results

*Macroscopic features of wound tissue after treatment in experimental groups*

Scars formed at the wound site of CH-treated animals were almost normal and flattened to the surrounding skin level. In the non-treatment and Eucerin-treated groups, HSs were formed in the wound site, and they were raised and showed a dark-red color (Figure 1).

*CH reduced the ETI and SEI*

Topical CH significantly reduced ($p<0.001$) the ETI in a dose-dependent manner compared with the non-treatment and Eucerin-treated groups (Figures 2 and 3). In addition, SEI was reduced significantly ($p<0.01$) in wound tissue samples obtained from animals treated with topical CH ointments (1%, 2% and 4%) (Figures 2 and 4).

*Collagen deposition was decreased in CH-treated scar tissues*

Investigation of the effects of CH on collagen deposition levels revealed a significant reduction in the amount of collagen deposition in tissue samples obtained from animals treated with topical CH 1%, CH 2% ($P<0.05$), and CH 4% ($P<0.01$) compared to control and Eucerin-treated groups (Figures 2 and 5).

*CH reduced the levels of collagen type I and type III in scar tissues*

The tissue levels of type I collagen were reduced ($p<0.01$) after treatment with topical ointments containing 1, 2, and 4% of CH. (Figure 6).

Also, CH significantly ($P<0.05$) reduced type III collagen levels compared to the control and Eucerin groups. (Figure 7).

*Effect of CH on MMP1 levels of scar tissue*

Topical treatment with CH 4% ointment significantly ($p<0.05$) enhanced the levels of MMP1enzyme in scar tissue compared to non-treatment and eucerin-treated groups. No significant differences were observed among other studied groups in MMP1 levels (Figure 8).

## Discussion

Hypertrophic scars are accompanied by functional and esthetics sequelae, and they still possess significant clinically relevant challenges. Currently, there is no universally accepted standard treatment for HSs, which is mainly due to not knowing the exact pathogenesis of this cutaneous condition. Therefore, the study of compounds that have the potential to prevent or treatment of hypertrophic scars is of particular importance.

In this experimental research, we evaluated the anti-scarring properties of catechin hydrate, which is an important phytochemical reported to have antioxidant, anti-inflammatory (Kim and Heo 2022), and antifibrotic effects.

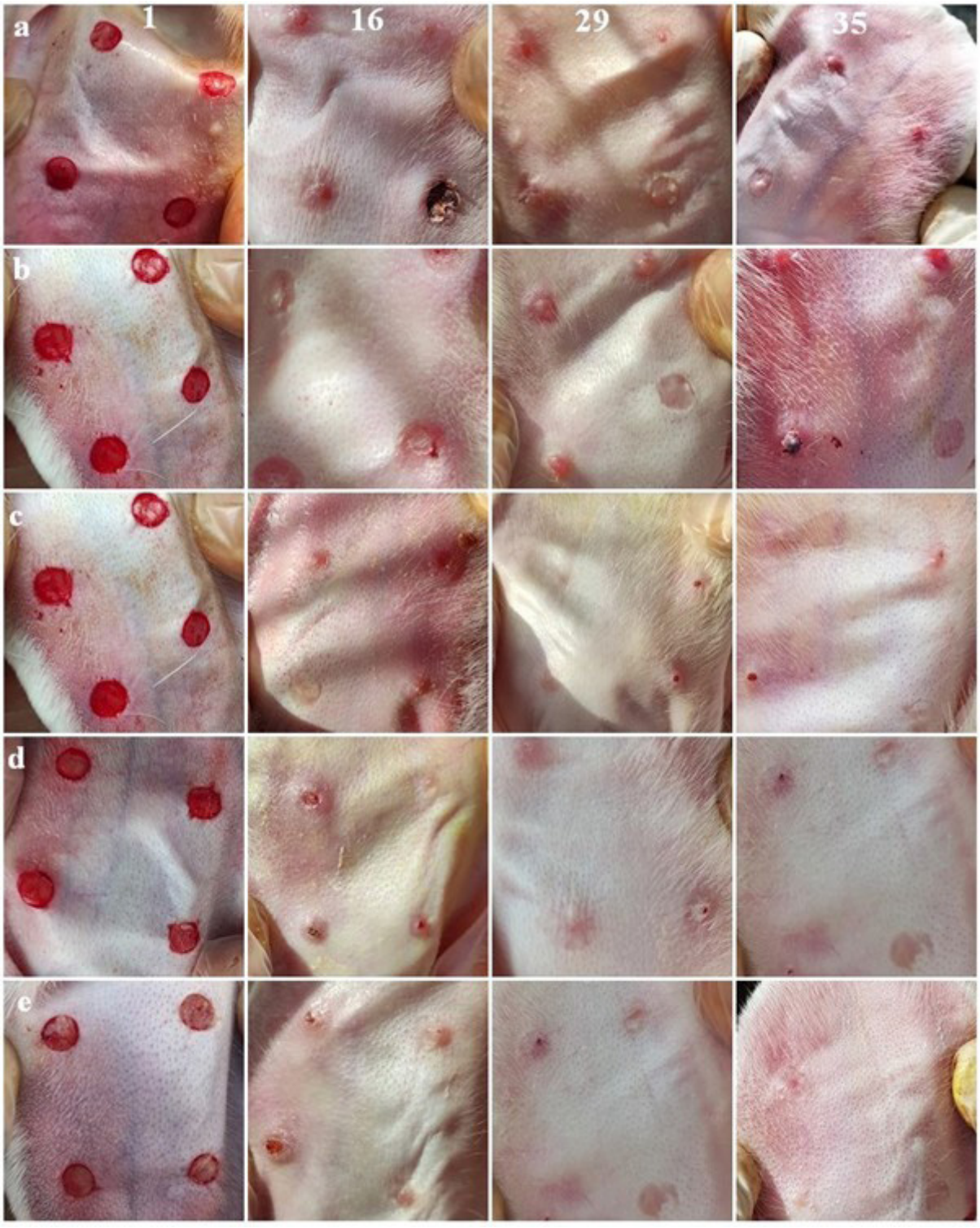


**FIGURE 1.** Macroscopic view of scar tissues on day 1, 16, 29, and 35 post injury. a; Control group, b; Eucerin group, c; CH 1% treated group, d; CH 2% treated group and e; CH 4% treated group.

The rabbit ear model was used to create HSs in this study. This model is a quantifiable, reliable, and responsive model, widely used to study the efficacy and mechanism of action of different therapeutic agents (Kloeters et al., 2007; Nabai and Ghahary 2017) .

The findings of this study revealed that CH improved the HSs by decreasing collagen deposition and type I and type III collagen levels. In addition, topical CH 4% ointment increased the MMP1 levels in scar tissue.

In hypertrophic scar, the ECM is different from that

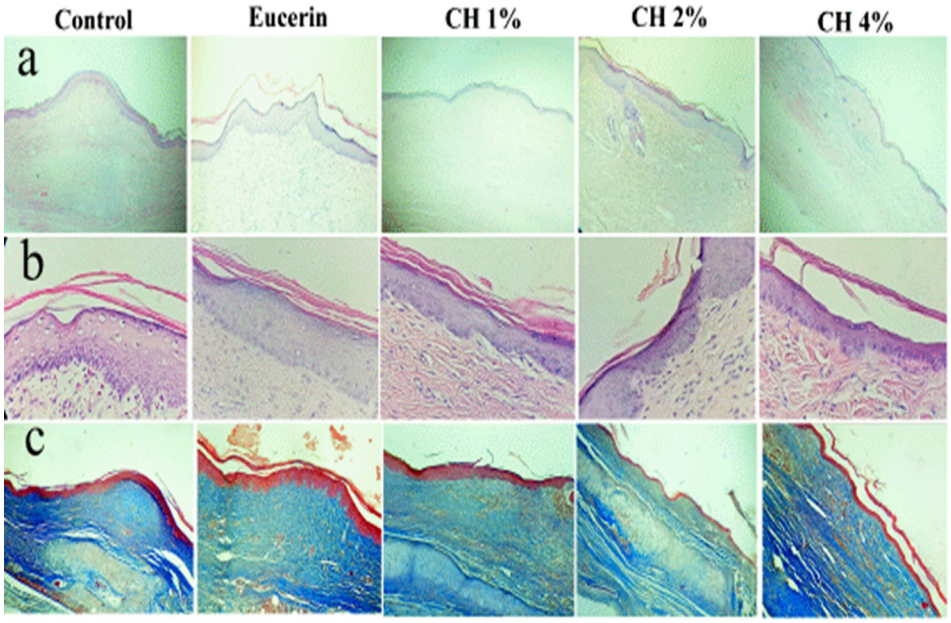


**FIGURE 2.** Microscopic view of scar tissue after 35 days of treatment with CH compared to the control and eucerin group. a. samples subjected to H&E staining (100X) for the measurement of SEI. b. samples subjected to H&E staining (400X) to determine the ETI. c. samples subjected to Masson's Trichrome staining (100X) to evaluate collagen deposition in tissue samples; in this staining method, collagen fibers are stained blue.

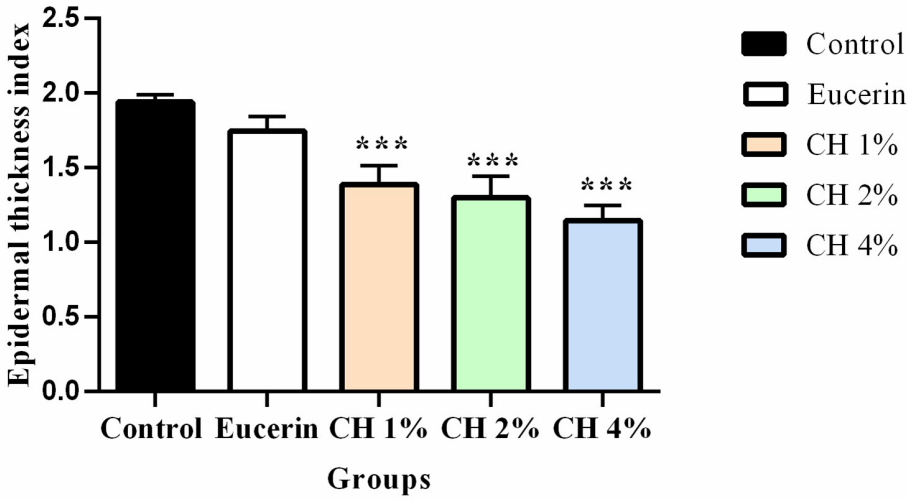


**FIGURE 3.** Comparison of the epidermal thickness index (ETI) of the studied group after treatment with topical CH. Data are shown as Mean±SEM. *** ($p$<0.001) indicates the significant differences from the control group.

present in normal skin and normotrophic scar, both in terms of quality and quantity. The total collagen is increased in HSs, and collagen fibrils are narrower and disorganized compared to normal skin and normotrophic scars, in which the collagen fibers align in a direction parallel to the surface (Grieb et al., 2011). Therefore, one of the most important strategies to prevent abnormal scar formation is reducing collagen synthesis.

Lonati-Galligani et al. (1979) examined catechin effects on the synthesis of collagen in cultured human fibroblasts. Their results showed that catechin significantly reduced collagen synthesis (Lonati-Galligani et al., 1979). The findings of this research support our results. The results of another research revealed that green tea extract and EGCG suppressed type I collagen production, probably by interference in the PI-3K/Akt/mTOR signaling pathway (Zhang et al., 2006). The antifibrotic effects of catechin on hepatic fibrosis were reported by

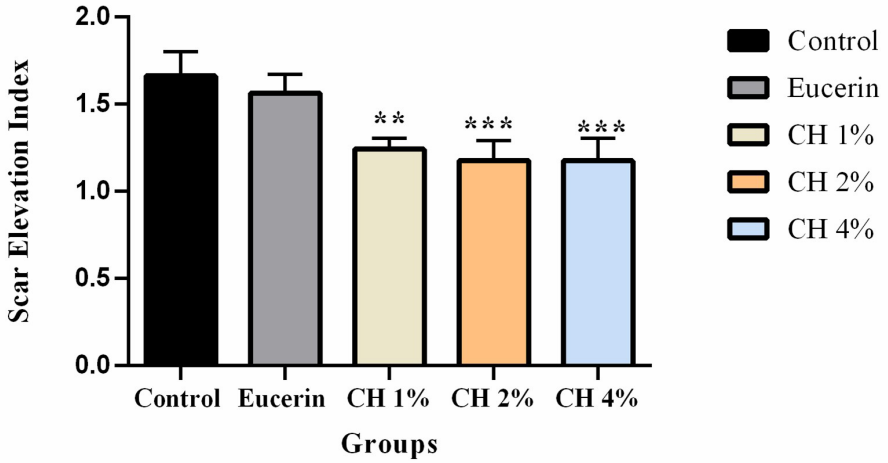


**FIGURE 4.** Comparison of scar elevation index (SEI) of the studied groups after treatment with topical CH. Data are shown as Mean±SEM. ** ($p$<0.01), *** ($p$<0.001) indicate the significant differences from control group.

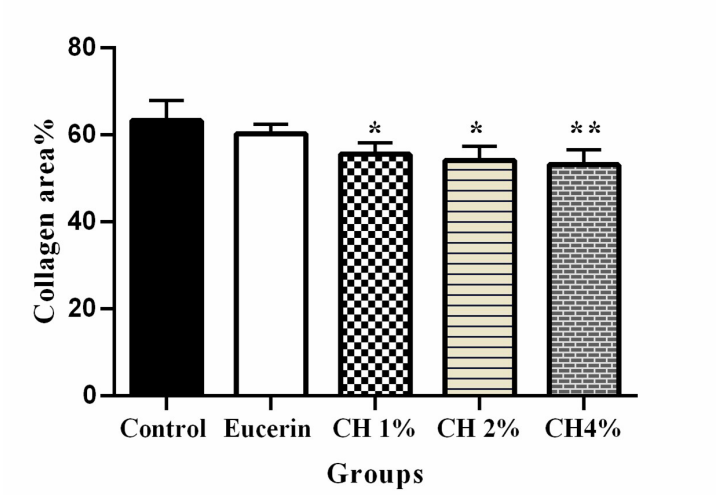


**FIGURE 5.** Comparison of collagen deposition in scar tissues in the studied groups after treatment with topical CH. Data are expressed as Mean±SEM. * ($p$<0.05) and **($p$<0.01) indicate the significant differences from control group.

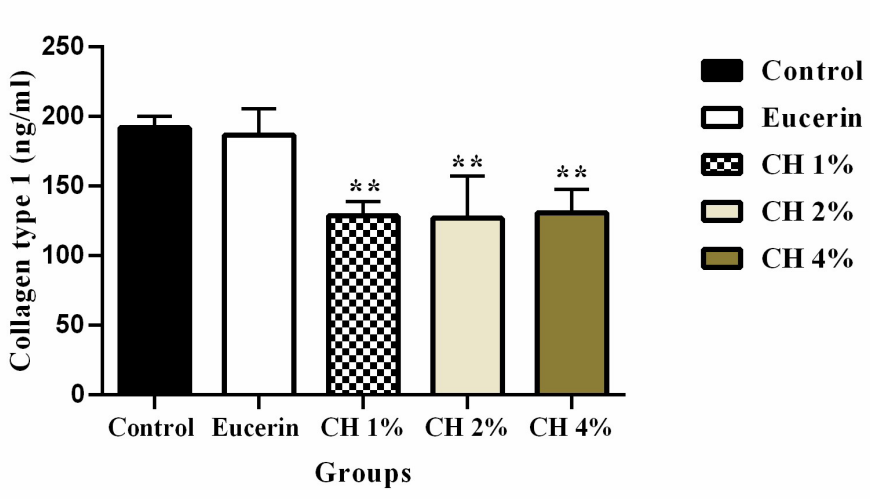


**FIGURE 6.** Comparison of collagen type I levels of scar tissues after treatment with topical CH. Data are shown as Mean±SEM. *($p$<0.05), ** ($p$<0.01 indicate the significant differences from control group.

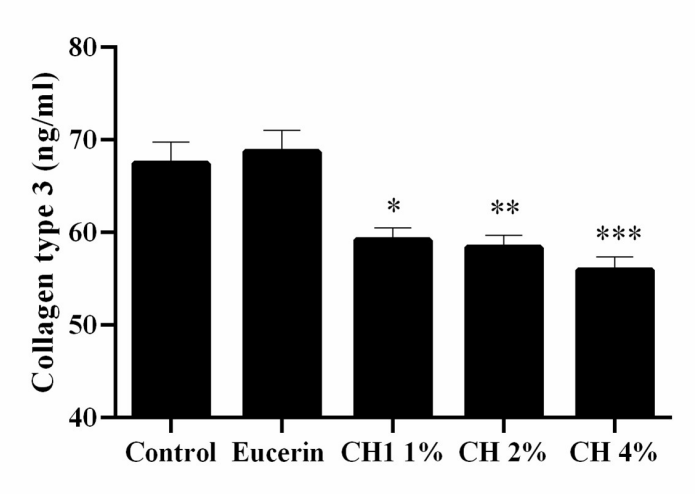


**FIGURE 7.** Comparison of collagen type III levels of scar tissues after treatment with topical CH. Data are shown as Mean±SEM. *($p$<0.05), ** ($p$<0.01) and *** ($P$<0.001) indicate the significant differences from control group.

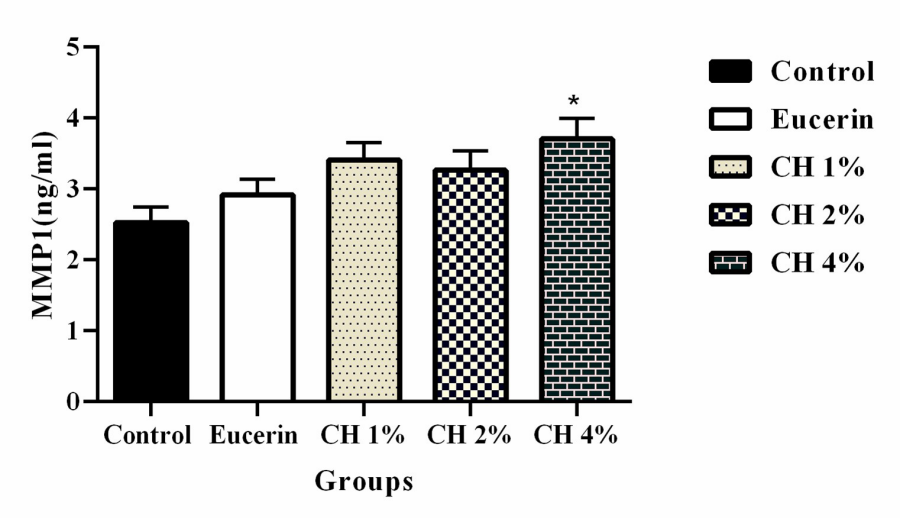


**FIGURE 8.** Comparison of the MMP1 levels of scar tissues after treatment with topical CH. Data are expressed as Mean±SEM. * ($p$<0.05) indicates the significant differences from the control group.

Bragança de Moraes et al. (2014), their findings revealed that catechin downregulated collagen type I mRNA expression (Bragança de Moraes et al., 2014).

Our study did not include investigating the mechanism of action of CH in the prevention of hypertrophic scars; however, one of the possible mechanisms is to deactivate the transforming growth factor-beta /alpha-smooth muscle actin differentiation (TGF-β/Smad) signaling pathway, which has been reported in some studies(Kweon et al., 2023; Panji et al., 2021). Approximately in the second week of the wound healing process, fibroblasts differentiate into myofibroblasts, which, like fibroblasts, secret ECM proteins such as type I and type III collagen. Myofibroblasts are α-Smad-positive cells activated by TGF-β. Myofibroblasts have a vital role in wound contraction. TGFβ is the most representative cytokine to enhance fibrosis and scar tissue formation (Shirakami et al., 2020). Therefore, inhibiting the TGF-β/Smad signaling pathway by CH can justify its anti-scarring effects. In this regard, various studies have shown that flavonoids reduce the production of TGF-β1 and IL-1β, control the ECM secretion, and prevent extravagant collagen deposition (Zhang et al., 2022). However, more studies are needed to prove this hypothesis. In this study, we evaluated the effect of CH on MMP1 levels in scar tissue, and the results showed that topical CH at 4% concentration significantly increased the MMP1 levels compared with the control groups. MMP1 (collagenase), an enzyme responsible for collagen degradation, is reduced in hypertrophic scars. The aim of many new

therapeutic approaches is to increase MMPs, intending to reduce excessive collagen (Lee et al., 2015).

Appropriate degree of inflammation is necessary for wound healing; however, excessive inflammation results in hypertrophic scar formation, and the intensity of inflammation is directly related to the final size of the scar. (Wang et al., 2020). Compounds that show anti-inflammatory effects on wound healing seem to be useful in preventing abnormal scar formation. Catechins, including CH, possess anti-inflammatory properties and several studies have revealed their anti-inflammatory and their radical scavenging activities (Shahid et al., 2016). The possible mechanism underlying the anti-scarring activity of CH could be attenuation of inflammation and acceleration of wound closure.

## Conclusion

In summary, the findings of this experimental study indicated that CH could prevent hypertrophic scar formation, it mainly attributed to its antioxidant and anti-inflammatory effects, as well as its effect on accelerating wound healing. The efficacy of treatment using CH was demonstrated in the rabbit ear model of HS. The rabbit ear model is a practical, reliable, and responsive model to evaluate candidate anti-scarring therapies. Therefore, CH can be considered as a candidate for preventing abnormal scar formation during the wound healing process.

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## Conflict of interest

The authors declare there is no conflict of interest.

## Ethics approval

The experimental protocol was approved by the research ethics committee of Hamadan University of Medical Sciences (IR.UMSHA.REC.1399.944).

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