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Evaluation of *Crescentia cujete* L. Extract as a Promoter of Skin Wound Healing in Sprague-Dawley Rats

Micco Joshua Apriano Pangaribuan[1,2], Bambang Sutrisno[3*], Sitarina Widyarini[3], Yos Adi Prakoso[2], and Ria Utami[1,2]

[1]*Master Program in Veterinary Sciences, Faculty of Veterinary Medicine, Gadjah Mada University, 55281, Indonesia*
[2]*Department of Pharmacology, Faculty of Veterinary Medicine, Wijaya Kusuma Surabaya University, 60225, Indonesia*
[3]*Department of Pathology, Faculty of Veterinary Medicine, Gadjah Mada University, 55281, Indonesia*

*Corresponding author's Email: bambang.sutrisno.patologi@gmail.com

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ABSTRACT

Wound is a tissue that easily disintegrates and requires proper management to promote its healing. One of the wound management techniques for effective healing is the use of herbal remedies such as *Crescentia cujete* (L.). The current study aimed to analyze the potential of *Crescentia cujete* (Buah berenuk extract, or BBE) in a cream-based formulation as a healing promoter in excision wounds in rat models. The present study involved 32 male Sprague Dawley rats, 3-month-old and weighing 251.81 ± 7.07 g. Two full-thickness circular wounds (6 mm diameter) were created on the shaved dorsal skin of each rat. The rats were divided into four groups, including untreated (K1), treated with 1% ascorbic acid cream (K2), 1% BBE cream (K3), and 2% BBE cream (K4). The biochemical compounds of BBE were tested using high-performance liquid chromatography (HPLC) for the level of ascorbic acid and conventional quantitative methods for other biochemical compounds, including flavonoids, alkaloids, tannins, saponins, and triterpenoids. Treatments were applied once daily for seven days. On days three and seven, four rats from each group were euthanized using cervical dislocation, and their skin samples were collected. The skin was tested using macroscopy, histopathology, collagen density analysis, and immunohistochemistry (IHC) against interleukin-6 (IL-6) and fibroblast growth factor (FGF). Statistical analysis was performed on the obtained data. The results demonstrated that BBE contained alkaloid, flavonoid, phenolic, saponin, tannin, and ascorbic acid. The current findings revealed that the use of 2% BBE cream had significant impacts on wound area in rat models, affecting histopathology, skin collagenization, and the immunoreactivity of IL-6 and FGF when compared to the other treatment groups.

Keywords: Collagen, *Crescentia cujete* L., Excision wound, Fibroblast growth factor, Interleukin-6

INTRODUCTION

Wounds are a common issue in humans and animals, which can cause tissue damage and lead to complications such as hemorrhage, blood loss, and infection, particularly in severe cases such as excision wounds (De La Tejera et al., 2023). Excision wounds create large open areas susceptible to contamination, potentially delaying healing (Quazi et al., 2022). The wound healing process involves overlapping phases, primarily inflammation and proliferation. Inflammation features the infiltration of inflammatory cells and different cytokines, including cyclooxygenase-2 (COX-2), tumor necrosis factor-α (TNF-α; Yen et al., 2018), and interleukin-6 (IL-6; Johnson et al., 2020).

The IL-6, a pro-inflammatory cytokine, plays a critical role in the acute phase of inflammation. This cytokine aids in the movement of leukocytes to the wound area (Widyarini et al., 2024). However, if inflammation persists for an extended period, it can cause significant tissue damage and impede the stages of tissue proliferation (Schilrreff and Alexiev, 2022). Hence, it is crucial to minimize inflammation to facilitate proper healing and encourage tissue proliferation, which is characterized by the presence of fibroblast growth factor (FGF; Yun et al., 2010). As a result, effective wound management has become a significant focus within the medical community.

Wound management involves preventing infection using wound dressing (Dumville et al., 2016), ensuring sanitation (Wilkins and Unverdorben, 2013), and providing appropriate treatment (Sussman, 2023). Wound treatments include either chemical agents or herbal compounds. Among herbal remedies, *Crescentia cujete* L. commonly known as buah berenuk, has shown promising effects in promoting wound healing in humans and animals (Trimanto et al., 2019). *Crescentia* cujete L. originates in South America and has been distributed to numerous countries, including Indonesia (Madhukar et al., 2013). Previous studies have identified several beneficial compounds in *Crescentia cujete* (Buah berenuk extract, BBE), including hydrogen cyanide, flavonoids, iridoids (Rivera-Mondragón et al., 2020), crescentic acid, choline (Wilujeng et al., 2023), and ascorbic acid (Wijayanti et al., 2024). Ascorbic acid, a water-soluble vitamin, plays an essential role in cellular metabolism, acts as an antioxidant, promotes iron absorption, and supports cell integrity (Jaros-Sajda et al., 2024). Additionally, ascorbic acid is frequently used in skin care products to induce collagen synthesis and facilitate wound healing (Boo, 2022). Given its biochemical properties, BBE is expected to promote healing in excision wounds in humans and animals. The present study explored the effectiveness of BBE in excision wound healing in rat models.

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MATERIALS AND METHODS

Ethical approval

The present study received approval from the Ethical Clearance Committee of the Faculty of Dental Medicine at Airlangga University under reference number 1022/HRECC.FODM/X/2024. The study was conducted from September to December 2024, with all animal experimentation taking place in the Department of Pharmacology at the University of Wijaya Kusuma Surabaya, Indonesia.

Herbal preparation and cream formulation

Fresh *Crescentia cujete* L. fruits (Total of 2 kg) were opened using a machete. The fruit pulp was then mashed and macerated with 70% alcohol. The maceration process was repeated four times, and the mixture was evaporated using a Soxhlet evaporator (Buchi, Indonesia; Wilujeng et al., 2023). The resulting thick extract of BBE was collected and standardized for its ascorbic acid content using high-performance liquid chromatography (HPLC; Prakoso et al., 2024). Other biochemical compounds of BBE, such as flavonoids, alkaloids, tannins, saponins, and triterpenoids, were tested (Ajuru et al., 2017). The results of biochemical compounds are presented in Table 1. The thick extract was subsequently formulated into a cream base at 1% and 2% (w/w) concentrations following Prakoso et al. (2019). The cream concentration was made from weight/weight (w/w) of the extract/cream-based formulation.

Table 1. Phytochemical screening of *Crescentia cujete* L. extract using high-performance liquid chromatography and conventional quantitative tests

Parameter	Result (mg/kg)
Ascorbic acid	7.41 ± 1.62
Alkaloid	18.71 ± 4.22
Flavonoid	9.13 ± 2.17
Phenolic	1.58 ± 0.53
Saponin	0.61 ± 0.61
Tannin	6.46 ± 3.20

Study design and animal model

The present study involved 32 3-month-old male Sprague Dawley rats, weighing 250 g. The hair on the rats' backs was shaved using clippers, and 70% isopropyl alcohol was applied topically as an antiseptic. Two round full-thickness wounds, each 6 mm in diameter, were created on the rats' backs using a punch biopsy tool. The rats were then randomly divided into four groups, each consisting of eight rats, including untreated (K1), treated with 1% ascorbic acid (K2), treated with 1% BBE (K3), and treated with 2% BBE (K4). Treatments were administered once daily for seven days (El Ayadi et al., 2020).

Gross lesion measurement

The diameter of the wound lesion was measured on days 0 (before treatment), 3, and 7 using Vernier callipers (Prakoso et al., 2020). The data was reported as diameter 1 (D1) and diameter 2 (D2). The wound area (WA) was then analyzed using the formula WA (mm^2) = D1 × D2. Furthermore, the percentage of wound closure was measured using the formula. PC (%) = ($WA_0 – WA_x/WA_0$) × 100% (Prakoso et al., 2020), where PC is the percentage of wound closure, WA_0 is the wound area before treatment, and WA_x is the wound area on day x.

Histopathology and immunohistochemistry

Skin samples were collected on days three and seven following the treatment. Before skin collection, the rats were euthanized using cervical dislocation. Afterwards, the back skin of the rat was incised and cut. The skin was then stored in 10% formalin for 24 hours and processed for histopathology. The histopathology was performed using routine methods, specifically haematoxylin and eosin (H&E), as well as Mallory staining (MS). The H&E staining followed the protocol described by Tucker et al. (2016), while the MS was performed to demonstrate collagen deposition, following the methodology of Rosario et al. (2022). The immunohistochemistry (IHC) was performed against IL-6 and FGF. For IHC, the skin block was sectioned and mounted on a poly-L-lysine-coated slide (Biolab, Indonesia). The staining procedure adhered to the protocol established by Prakoso et al. (2020). The anti-IL-6 antibody was diluted in 3% BSA in PBS (Merck, China) at a concentration of 1:100, while the FGF antibody was diluted at 1:250.

Morphometry of histopathology and immunohistochemistry

A pathologist conducted the morphometric analysis of H&E, MS, and IHC. The H&E analysis included different parameters, such as inflammatory cells, neovascularization, epidermal thickness, and the ratio of wound area to normal skin. The analyses were conducted at 10×10 magnification using a microscope (Olympus CX33, Japan). The MS was assessed based on the number of fibroblasts, collagen bundle organization, and density, while the IHC focused on the percentage area of immunoreactivity for IL-6 and FGF.

Inflammatory cells and neovascularization were scored according to established criteria: 1 = normal, 2 = mild, 3 = minimal, 4 = moderate, and 5 = severe (van de Vyver et al., 2021). Fibroblasts, epidermal thickness, wound skin thickness (WST), and normal skin thickness (NST) were quantified using ImageJ software (BSD-2, NIH, USA). The

ratio of wound area to normal skin (W/N) was calculated using the following formula. W/N = WST (µm)/ NST (µm) (Kuo et al., 2022). Additionally, collagen bundle characteristics, density, and the immunoreactivity of IL-6 and FGF were analyzed using ImageJ. Using ImageJ, the collagen bundle was marked with a scale, and the results were reported in micrometers (µm). To measure collagen density and the immunoreactivity of IL-6 and FGF, the images were converted to 8-bit format. The contrast and pixel settings were adjusted until the desired area was accurately labelled. The area was subsequently analyzed, and the results were expressed as percentages (Rueden et al., 2017).

Data analysis

The data was analyzed using SPSS version 26, with a significance level set at a p-value of 0.05. Two types of data were considered, including numerical and categorical. The numerical data included wound area, percentage of wound closure, fibroblast count, epidermal thickness, ratio of wound area to normal skin, collagen bundle count, collagen density, and the immunoreactivity of IL-6 and FGF. This data was analyzed using ANOVA and further validated with the Duncan test with a p-value of 0.05. The categorical data comprised inflammatory cells and neovascularization, which were analyzed using the Kruskal-Wallis and Mann-Whitney U tests.

RESULTS AND DISCUSSION

The present study demonstrated that the macroscopic examination of the wound area in Group K4 on days three and seven revealed a significant difference compared to the other groups ($p < 0.05$). In contrast, no significant differences were observed among groups K1, K2, and K3 when compared with one another ($p > 0.05$). The current result was correlated with the percentage of wound closure in Group K4 during the same observation periods ($p < 0.05$; Table 2 and Figure 1).

Histopathological evaluation of the excision wounds in rats revealed that all treatment groups demonstrated a better score for inflammatory cells compared to the untreated group on days three and seven ($p < 0.05$). Nonetheless, groups K2, K3, and K4 indicated similar scores for inflammatory cells when compared to K1 ($p > 0.05$). Additionally, Group K4 exhibited a thicker epidermis, denser neovascularization, and a higher ratio of wound tissue to normal skin on days three and seven compared to the other groups ($p < 0.05$). Group K4 was followed by groups K3, K2, and K1 regarding the parameters of epidermal thickness, neovascularization, and ratio of wound tissue to normal skin (Table 3). The qualitative assessment of the excision wounds after treatment is presented in Figure 2.

The histopathological findings were further supported by MS analysis, which assessed fibroblast presence, collagen bundle organization, and density (Figure 3). Group K4 displayed the highest density of fibroblasts and collagen in terms of number and density on days three and seven compared to groups K1, K2, and K3 ($p < 0.05$), consistent with the histopathological results. However, there was no significant difference in collagen bundle organization across all treatment groups ($p > 0.05$), K2, K3, and K4 indicated higher values in comparison to Group K1 ($p < 0.05$; Table 4). The immunoreactivity of IL-6 and FGF increased from day three to seven in all groups. The highest levels of immunoreactivity for IL-6 and FGF were observed in groups K3 and K4 compared to K1 and K2 ($p < 0.05$). Group K2 displayed a similar pattern of increase in IL-6 and FGF but did not perform as well as groups K3 and K4 ($p < 0.05$; Figure 4).

The study demonstrated that BBE contains several bioactive compounds, including alkaloids, flavonoids, phenolic compounds, saponins, tannins, and ascorbic acid, all essential for wound healing, especially ascorbic acid, which was the third most abundant compound in BBE. Li et al. (2021) reported that ascorbic acid beneficially promotes the growth of new vascular tissue and reduces inflammation. The neovascularization and reduced inflammation directly and indirectly support the skin in synthesizing collagen (Yin et al., 2022). Moreover, ascorbic acid enhances the activity of neutrophils and macrophages to eliminate debris and necrotic tissue from the wound in the first stage of inflammation, which is crucial for maintaining the wound septicity for the next stages of the wound healing process (Chow and Barbul, 2014). The wound asepticity supports the healing processes to move on, and the advanced healing processes become faster and shorter, especially with the change of IL-6 and FGF immunoreactivity. Hence, ascorbic acid has become a widely used antioxidant in skin products due to its capacity to promote wound healing (Pullar et al., 2017).

Table 2. Macroscopy findings of the excision wound in the rat skin model on days three and seven after treatment using *Crescentia cujete* L. extract

Parameter	Day	Group (mean ± standard deviation)			
		K1	K2	K3	K4
Wound area (mm^2)	3	29.66 ± 7.01^a	20.88 ± 13.01^a	26.22 ± 8.56^a	15.98 ± 5.07^b
	7	15.26 ± 6.52^a	11.79 ± 4.82^a	13.98 ± 7.29^a	1.73 ± 0.36^b
Wound closure (%)	3	22.60 ± 10.55^a	41.98 ± 36.14^b	30.48 ± 17.60^a	55.59 ± 14.09^b
	7	57.59 ± 18.13^a	67.48 ± 13.39^a	61.14 ± 20.25^a	95.18 ± 1.01^b

K1: Untreated group, K2: Treated with 1% ascorbic acid cream, K3: Treated with 1% BBE cream, K4: Treated with 2% BBE cream. ^{a,b} different superscript letters on the same row indicate significant differences ($p < 0.05$).

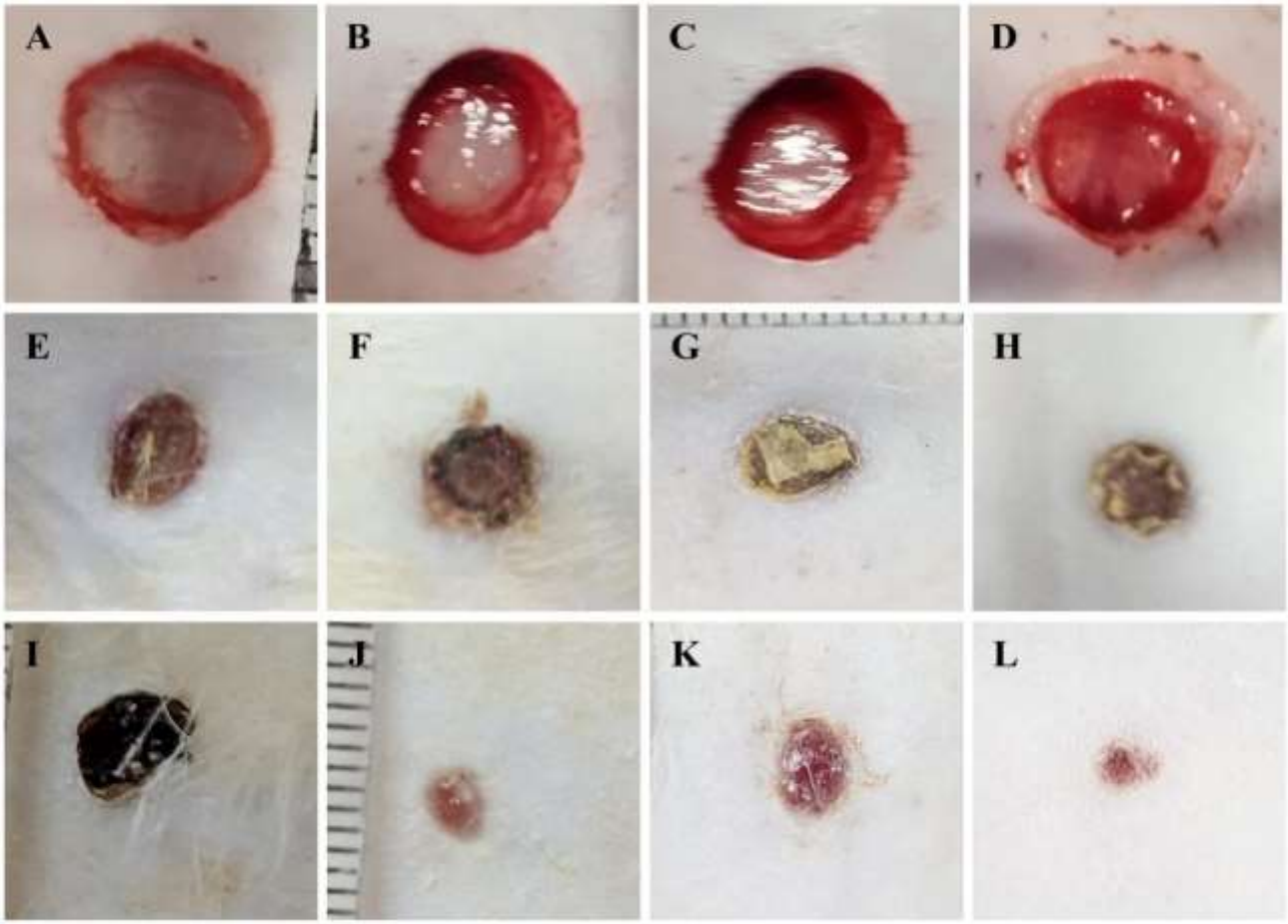

Figure 1. Macroscopic appearance of excision wounds in Sprague Dawley rats on days 0, 3, and 7 after treatment using *Crescentia cujete* L. extract. **A**: Wound on day 0 in group K1, **B**: Wound on day 0 in group K2, **C**: Wound on day 0 in group K3, **D**: Wound on day 0 in group K4, **E**: Wound condition on day three, scabs were observed on the wound surface of skin in group K1, **F**: Wound condition on day three in group K2, **G**: Wound condition on day three in group K3, and **H**: Wound condition on day three in group K4, **I**: Wound condition on day seven in K1 showed wide area of wound with the scab still present, **J**: Wound condition on day seven in group K2, **K**: Wound condition on day seven in group K3 indicated small wound area and the scab had fallen off the wound, and **L**: group K4 showed the smallest wound area with the scab already fallen off from the wound.

Table 3. Histopathological findings of the excision wound in rat skin on days three and seven after treatment with *Crescentia cujete* L. extract

Parameter \ Group (mean ± standard deviation)	Day	K1	K2	K3	K4
Score of inflammatory cells	3	4.00 ± 0^{a}	2.50 ± 0.57^{b}	2.50 ± 0.57^{b}	2.25 ± 0.50^{b}
	7	3.50 ± 0.57^{a}	2.25 ± 0.50^{b}	1.75 ± 0.50^{b}	1.25 ± 0.50^{b}
Epidermal thickness (µm)	3	0 ± 0^{a}	6.33 ± 7.57^{b}	7.98 ± 5.49^{b}	10.12 ± 1.66^{c}
	7	10.28 ± 7.29^{a}	15.72 ± 10.60^{a}	20.93 ± 3.91^{b}	49.15 ± 11.84^{c}
Score of neovascularization	3	0 ± 0^{a}	1.75 ± 0.50^{b}	1.50 ± 0.57^{c}	1.75 ± 0.50^{b}
	7	1.50 ± 0.57^{a}	2.75 ± 0.50^{b}	2.00 ± 0.81^{c}	3.25 ± 0.50^{b}
Ratio of wound/normal skin	3	0.18 ± 0.02^{a}	0.24 ± 0.02^{b}	0.22 ± 0.01^{c}	0.28 ± 0.01^{d}
	7	0.30 ± 0.02^{a}	0.42 ± 0.02^{b}	0.37 ± 0.02^{c}	0.45 ± 0.01^{d}

K1: Untreated group, K2: Treated with 1% ascorbic acid cream, K3: Treated with 1% BBE cream, K4: Treated with 2% BBE cream. ^{a,b,c,d} different superscript letters on the same row indicate significant differences ($P < 0.05$)

Table 4. Fibroblast and collagen deposition of excision wound in rat skin on days three and seven after treatment with *Crescentia cujete* L. extract

Parameter	Day	Group (mean ± standard deviation)			
		K1	K2	K3	K4
Fibroblast	3	20.0 ± 2.82^{a}	93.25 ± 19.37^{b}	106.75 ± 19.77^{b}	164.0 ± 46.31^{c}
	7	48.25 ± 14.24^{a}	256.0 ± 36.35^{b}	232.75 ± 29.72^{b}	354.0 ± 54.86^{c}
Collagen bundle (µm)	3	5.75 ± 1.10^{a}	9.34 ± 3.22^{b}	10.18 ± 2.01^{b}	11.72 ± 2.40^{b}
	7	12.64 ± 1.74^{a}	16.77 ± 0.94^{b}	13.36 ± 2.54^{b}	17.40 ± 1.30^{b}
Collagen density (%)	3	15.99 ± 5.28^{a}	38.31 ± 6.61^{b}	32.22 ± 5.85^{b}	49.25 ± 8.37^{c}
	7	40.92 ± 2.92^{a}	75.79 ± 5.71^{b}	68.15 ± 2.17^{b}	88.48 ± 3.30^{c}

^{a,b,c} Different superscript letters on the same row indicate significant differences ($p < 0.05$)

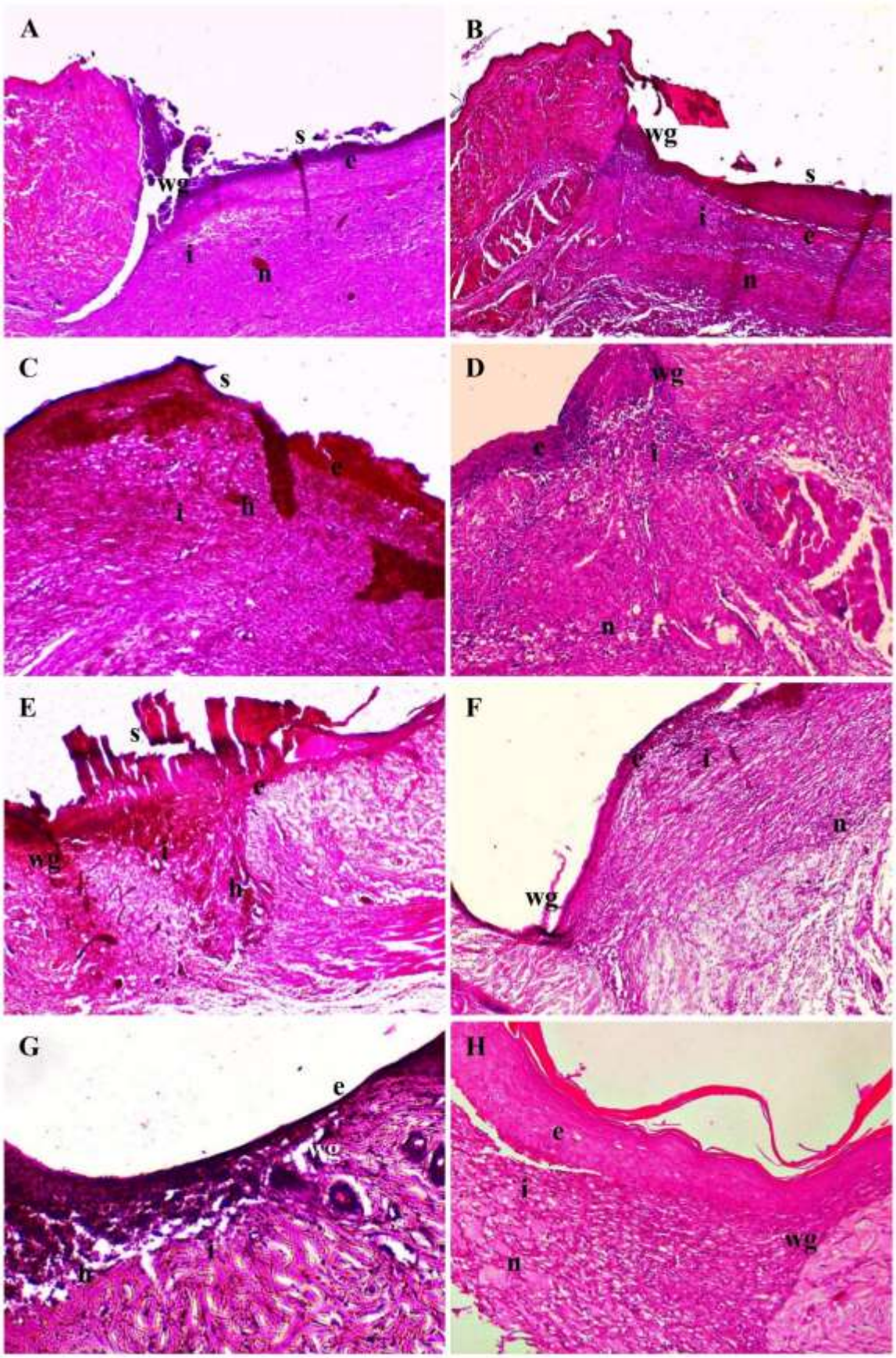

Figure 2. Histopathological findings of the excision wound in rats after treatment using *Crescentia cujete* L. extract on days three and seven. **A**: The histopathology of K1 demonstrated no significant change in the wound condition on day three, **B**: The histopathology of Group K1 on day seven, **C**: Group K2 indicated better wound condition on day three, **D**: Group K2 indicated minimal inflammation and epithelialization on day seven, **E**: Group K3 showed similar qualitative conditions to K2 on day three, **F**: Group K3 showed minimal inflammation and epithelialization on day seven, **G**: Group K4 revealed thin epithelialization of skin on day three, **H**: histopathology of Group K4 improved on day seven. **e**: Wound epithelial, **i**: Inflammatory cells, **n**: Neovascular, **s**: Wound scab, **wg**: Wound gap. H&E, 100× (A-H).

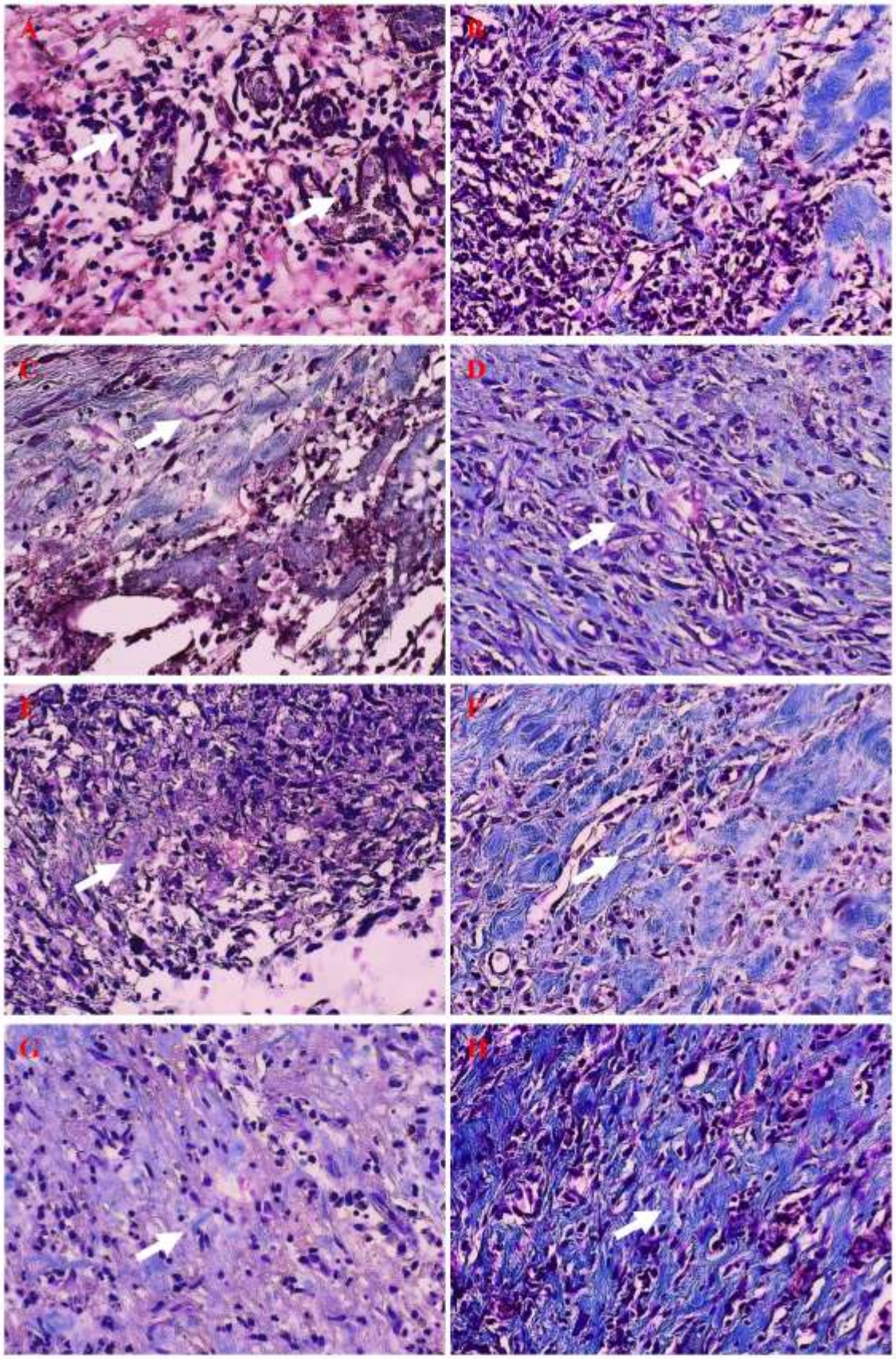

Figure 3. Collagenization of the excision wound in rats after treatment using *Crescentia cujete* L. extract on days three and seven. **A**: Group K1 demonstrated mild fibrogenesis and collagenization on day three, **B**: Group K1 showed minimal fibrogenesis and collagenization on day seven, **C**: Group K2 indicated mild fibrogenesis with no collagen appearance on day three, **D**: The fibrogenesis and collagenization became denser on day seven in Group K2, **E**: Group K3 showed minimal fibrogenesis and collagenization on day three, **F**: Group K3 showed denser fibrogenesis and collagenization on day seven, **G**: Group K4 had a better qualitative pattern compared to Groups K1, K2 and K3 regarding collagen and fibroblast on day three, **H**: Group K4 demonstrated denser collagenization and fibrogenesis on day seven compared to the other groups. The white arrow shows fibroblasts, and the deep blue arrow shows collagen. MS staining, 400× (A-H).

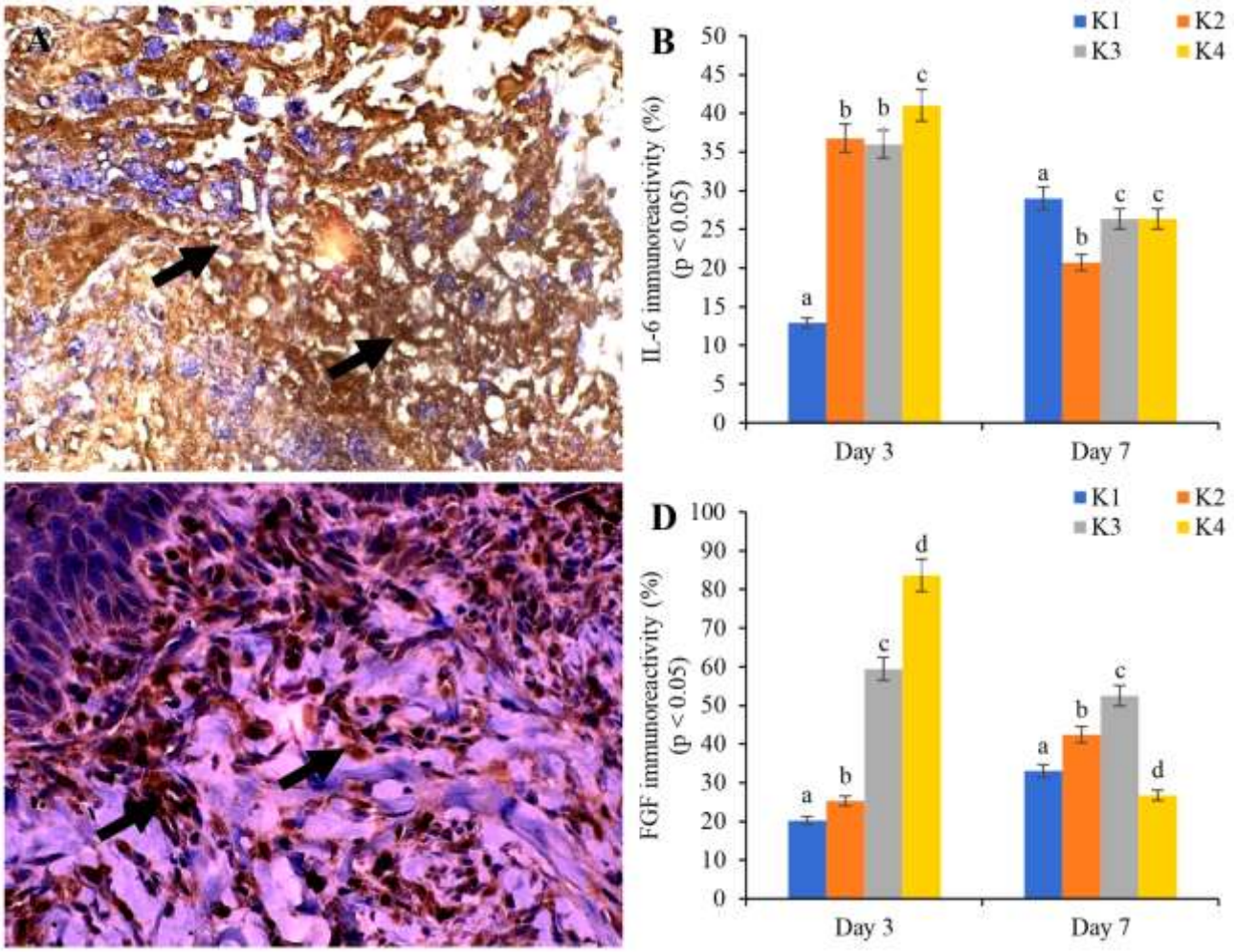

Figure 4. Immunoreactivity of interleukin-6 and fibroblast growth factor of excision wound in rats after treatment with *Crescentia cujete* L. extract on days three and seven. **A**: The IL-6 immune expression (black arrow), IHC antibody anti-IL-6, **B**: The immunoreactivity of IL-6 after treatment on day three indicated that K1 has the lowest immunoreactivity compared to K2, K3, and K4. However, the IL-6 decreased significantly on day seven in K2, K3, and K4, compared to K1. Group K2 demonstrated the lowest immunoreactivity of IL-6 compared to the other groups, **C**: The FGF immune expression (black arrow), IHC antibody anti-FGF, **D**: The trend of FGF after treatment on day three indicated K4 had the highest level of FGF compared to the other groups, followed by groups K3, K2 and K1, concomitantly. On day seven, K4 indicated the lowest FGF compared to the other groups; however, K2 and K3 indicated higher immune expression of FGF. The IHC staining using antibody anti-IL-6 (**A**) and antibody anti-FGF (**C**), 400× (**A** and **C**).

The present study resulted in similar findings, demonstrating that 1% ascorbic acid cream promotes wound healing via its ability to inhibit inflammation, promote neovascularization, fibrogenesis, and collagenization. Shortening the inflammation phase may reduce the immunoreactivity of IL-6 (Nash et al., 2023). Inhibition of IL-6 in the early stage of inflammation is expected to attract the fibroblast to proliferate via activating the FGF pathway in the wound area (Farooq et al., 2021). The FGF is a protein with a biomechanism that stimulates regeneration. The increased immunoreactivity of FGF occurred on day seven in wound healing or after the decrease of IL-6. Moreover, the FGF influences fibrogenesis, and through this process, collagen can be synthesized to fill the wound area (Park et al., 2015). In the current study, ascorbic acid enhanced the formation of collagen bundles and density in the wound area after treatment. Interestingly, using BBE in excision wounds indicated a similar mechanism trend with ascorbic acid as monotherapy. However, higher BBE concentrations indicated better improvement in skin wound healing, supported by 2% BBE cream, which showed lower inflammatory cell counts, greater epidermal thickness, increased neovascularization, a better wound ratio, more fibroblasts, enhanced collagen, and accelerated FGF levels in the early stages of wound healing compared to the other groups.

The present study explained that BBE, which contains ascorbic acid and other compounds such as alkaloid, flavonoid, phenolic, saponin, and tannin, can interactively promote wound healing. According to the present study, the inflammatory cell inhibition and enhancement of FGF in the early stage of inflammation are the most essential processes in wound healing. The significant increase of FGF in an early healing stage may influence the other healing factors set on the wound area (Chen et al., 2022).

Additionally, other biochemical compounds, such as alkaloids and flavonoids, support wound healing through their antioxidant activity (Criollo-Mendoza et al., 2023). Moreover, flavonoid acts as an antibacterial agent in the wound area,

and tannins can promote wound contraction and relieve pain (Zulkefli et al., 2023). As Prakoso et al. (2020) stated, pain relief in the wound specifically decreases prostaglandin and COX-2, elevating the lymphocytes cluster differentiation 4+ (CD4+) to penetrate and promote the wound area. Phenolic and saponin are also essential factors in promoting wound healing. Phenolic is a crucial factor in promoting the secretion of vascular endothelial growth factor (VEGF) and platelet-derived growth factor (PDGF; Vitale et al., 2022). The VEGF maintains the wound's local circulation, and PDGF promotes tissue proliferation. In addition, saponin has been proven to promote wound remodeling and contraction by enhancing pro-collagen and collagen activity (Kim et al., 2011). Meanwhile, tannin reduces scar tissue and scavenges free radicals (Chokotho and van Hasselt, 2005).

The present study demonstrated that BBE can serve as an effective herbal treatment for excision wounds, which is consistent with a previous study by Hartati et al. (2018). For instance, Jahandideh et al. (2017) demonstrated that a polyherbal paste (PHP) containing *Aloe vera*, *Boswellia carteri*, and *Commiphora myrrha* promoted wound healing in a rat model. However, the PHP formulation employed a higher concentration of herbs, particularly at 10%, which is five to ten times greater than the concentration of BBE used in the present study. Notably, BBE resulted in a shorter healing time for excision wounds compared to prior studies employing other types of herbal products (Hartati et al., 2018; Priyanka et al., 2024). In a study by Priyanka et al. (2024), an herbal gel containing *Barringtonia acutangula* fruit at a 10% concentration facilitated excision and incision wound repair over 20 days. However, the wound gap remained, indicating a more extended healing period than observed in the present study, where the 2% BBE treatment resulted in wound closure and complete epithelial layer restoration within seven days.

Additionally, Boakye et al. (2018) reported that *Phyllanthus muellerianus* extract promoted wound healing in 13 days using a concentration of 0.4% (w/w) in an incision wound model, which differs from the current results. Incision wounds, unlike excision wounds, do not create an open wound area, rendering them less prone to contamination and infection. Furthermore, the use of BBE for wound healing therapy has not been tested on diabetic wound models. A former study indicated that ultra-micro powder composed of *Angelica sinensis*, *Radix rehmanniae*, *Calcined gypsum*, and *Calamine* (ARCC) could be effective for treating diabetic wounds in adults aged 55 to 70 years (Zhong et al., 2022).

CONCLUSION

Based on the present findings, the utilization of BBE as a wound therapy demonstrated superior efficacy in promoting wound healing compared to ascorbic acid cream monotherapy due to its biochemical compounds, including alkaloid, flavonoid, phenolic, saponin, tannin, and ascorbic acid. The compounds in *Crescentia cujete* L. extract can interactively promote wound healing by inhibiting inflammatory cells and enhancing fibroblast growth factor in the early stages of inflammation. The *Crescentia cujete* L. extract could treat excision wounds in rat models. However, further research is needed to establish the biosafety of *Crescentia cujete* L. and explore its potential roles in managing different wound types and chronic wounds, including incision wounds and diabetic wound models, to validate its effectiveness as a healing promoter across different wound conditions.

DECLARATIONS

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Authors' contributions

Yos Adi Prakoso and Bambang Sutrisno supervised, designed, and implemented this study. Micco Joshua Apriano Pangaribuan was responsible for performing the study, data collection, and analysis. Manuscript writing and ensuring all necessary revisions were made by all authors. Yos Adi Prakoso, Bambang Sutrisno, Sitarina Widyarini, and Ria Utami assisted in checking the manuscript and then provided suggestions on the research test. All authors checked and approved the findings of this study and the last edition of the submitted manuscript.

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Competing interests

The authors declare that they have no conflicts of interest.

Ethical considerations

This paper is original, conducted solely by the authors; it has not been published elsewhere.

Availability of data and materials

The data supporting the findings of this study are available from the corresponding author upon reasonable request.

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