Original Article

Wound Healing Properties of *Cyperus Papyrus* Ethanolic Extract in Wister Albino Rats

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ABSTRACT

Background: *Cyperus papyrus* has been used to treat various health problems, such as ulcers and inflammation. Its possible use to promote healing, especially in the case of wounds, is still a topic that lacks thorough research.

Objectives: This study assessed the wound-healing effect of the ethanolic extract of *C. papyrus* in Wistar rats using an excision wound model.

Methods: The ethanolic extract of *C. papyrus* was made into gels at concentrations of 5% and 10%. Excisional wounds were induced on the dorsal of Wistar rats, which were treated topically with the gels twice daily. The effects of the treatment were evaluated through macroscopic measurements of wound contraction daily, histopathological analysis, and regeneration assessment by day 14.

Results: Wounds treated with *C. papyrus* gel at both concentrations of 5% and 10% showed increased rates of wound contraction when compared to the control group and the 10% formulation showed the most pronounced effect. Staining identifiable changes were noted that suggested enhanced epithelialization, advanced collagen maturation, inflammatory exocytosis within lower ranges of collagen deposition in the left interstitial tissue, and a decrease in the remaining inflammatory cells.

Conclusion: Using a 10% gel of *C. papyrus* extract through a topical procedure may increase the rate of wound healing in rats. This is probably due to the anti-inflammatory and antioxidant effects of the extract. These results confirm its effectiveness for further development of herbal therapeutic medicines to promote wound healing.

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Introduction

Wounds are physical injuries that result in an opening or break of the skin, causing a disturbance in normal skin anatomy and physiology, and resulting in the loss of continuity of the epithelium, with or without loss of underlying connective tissues, according to the Wound Healing Society (WHS) [1]. Wounds affect a large number of people and seriously reduce the quality of life [2]. Generally, there are three phases of wound healing: Inflammatory, proliferative, and remodeling [1]. Several agents have been used in the management of wounds and a wide variety of treatment modalities are available for wound repair. Among these medicines, those of herbal origin have a significant impact on the treatment and healing of wounds [3]. Medicinal plants offer significant benefits for wound treatment, not only because they are inexpensive and affordable but also because they are generally safe and do not typically cause hypersensitivity reactions [4].

The increasing demand and availability of medicinal products have created a need to isolate and identify the principles responsible for their therapeutic activities and effectiveness. For instance, the stimulation of fibroblasts by plant extracts has been observed as one of the mechanisms, by which medicinal plants enhance the wound healing process [5].

Cyperus papyrus belongs to the family Cyperaceae, which comprises monocotyledonous graminoid flowering plants known as sedges, that superficially resemble grasses or rushes [6]. It contains small amounts of sesquiterpenes relative to monoterpenes obtained by gas chromatography; it also contains phenolic compounds, as well as Na, K, Mg, Fe, I, and proteins, which are considered micronutrients [7]. *C. papyrus* has traditionally been used in the treatment of painful spasms, eye diseases, ulcers, fever, diarrhea, and various inflammatory conditions.

Any pathophysiologic disturbances in the healing process result in delayed or halted healing and present problems that result in frustrating and expensive care, ultimately failing to meet patient and provider goals [8]. Wounds (both normal and diabetic wounds) can become significant issues and may respond poorly to medications [9].

This study was conducted to evaluate the wound-healing activity of *C. papyrus* ethanolic extract in experimentally induced excision wounds in rats.

Materials and Methods

Materials

Plant material

Fresh aerial parts were harvested from the Botanical Garden at the Medicinal and Aromatic Plants and Traditional Medicine Research Institute (MAPTRI), Khartoum, Sudan, in August 2019. Plant identification (specimen No. Y-2010-54-MAPTRI-H) was referenced at MAPTRI, Khartoum, Sudan. The freshly harvested leaves were then air-dried. A total of 100 g of the plant sample was coarsely powdered using a mortar and pestle, and a sample was extracted with 80% ethanol by soaking extraction, according to the method described by Sukhdev et al. [10]. The extract was air-dried in an evaporating dish until completely dry. The yield percentage was calculated, and the dried extract was weighed and stored in sealed plastic containers at 4 ˚C for subsequent experiments.

Chemicals and drugs

Carbopol 941 and triethanolamine were obtained from Spectrum Chemicals, Ltd, China. Povidone iodine 10% ointment was obtained from the Sudan market, Mathely Trading & Drugs.

Experimental animals

Healthy Wister albino rats of both sexes weighed between 100 and 120 g, were obtained from the animal house of MAPTRI, NCR. Each rat was housed alone in polypropylene (485×350×200 mm) cages at 25 ˚C and subjected to a 12:12h light–dark cycle, with free access to food and water ad libitum. The animals were allowed to acclimatize for one week before use.

Methods

Formulation of semi-solid preparations

As shown in Table 1, *C. papyrus* extract was dissolved in water, then mixed with glycerin, and the carbomer was added by sprinkling on the surface while constantly mixing at high speed. Triethanolamine was added with slow agitation until a clear viscous gel was formed.

Two concentrations were formulated from *C. papyrus* extract: 5% and 10%, whereas the gel base was prepared without the addition of the extract. The concentrations of 5% and 10% *C. papyrus* ethanolic extract were selected based on preliminary phytochemical screening, literature on topical herbal formulations, and pilot toxicity tests [11].

Table 1. Carbopol gel formulation according to United States Pharmacopeia

Component	Percentage (% w/w)
Carbomer 941	0.5
Glycerin	10
Triethanolamine	0.5
Water	89

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Wound-healing activity

The excision wound model was used to evaluate the wound-healing activity of ethanolic extract of *C. papyrus* [12]. The dorsal fur of the animals was shaved with an electric clipper. The anticipated area of the wound to be created was outlined on the back of the animals. A full-thickness excision wound with a circular area and a depth of 0.2 cm was created under sterile conditions. The entire wound was left open. The animals were closely observed for any signs of infection, and those that showed signs of infection were excluded from the study and replaced. The day of surgery was considered day zero. Wounded animals were randomly divided into five groups, with six animals in each group. Group I (control) did not receive any treatment; Group II received a simple Carbopol gel base; group III received povidone-iodine ointment 10% (no silver sulfadiazine or other advanced comparators were used due to availability at that time); group IV received 5% CP extract; and group V received 10% CP extract. Treatment was applied topically to all groups twice daily (every 12 hours), and the wound areas were measured each morning until complete closure was achieved.

The percentage of wound contraction was calculated, and the onset of healing was determined. Skin tissue was then collected from the wound area and placed in 10% formalin for histopathological examination.

Histopathology examination

The fixed tissues were dehydrated with 100% ethanol solution and embedded in paraffin. They were then processed into 6-8 microns using a microtone and then stained with hematoxylin-eosin. The samples were observed under a light microscope (40X) by a histopathologist, as previously described by [13]. The presence of the epithelial layer, fibrous tissues, inflammatory cells, and granulation tissues was assessed.

Statistical analysis

Statistical analysis was performed using GraphPad Prism software, version 5. Data were expressed as mean and contraction percentages. The two-way ANOVA (followed by Bonferroni's post-hoc test) was conducted to determine significant differences and $P<0.05$ were considered significant.

Results

Phytochemical screening

As shown in Table 2, the ethanolic extract of *C. papyrus* contains various secondary metabolites, including saponins, flavonoids, tannins, steroids, and other compounds, with different percentages.

Wound area and contraction percentage

Mean contraction percentage and wound area increased more rapidly in animals that received *C. papyrus* extract than in animals that received the standard drug, the base group, or the control group as demonstrated in Table 3 and Figure 1.

Onset of healing

Only two days were required to induce healing in animals that received *C. papyrus* extract formula, and four days for the standard drug when compared to the negative control group. Whereas the Carbopol gel base does not differ significantly in inducing healing compared to the negative control group as demonstrated in Table 4.

Histopathology findings

As shown in Figure 2, the histopathology changes in the wound area further demonstrated the wound-healing effect of the *C. papyrus* extract. The control group showed the presence of inflammatory cells (ICs) and granulation tissues, with an absence of the epithelial layer (EC) when compared to the CP extract-treated groups, which showed an absence of ICs and the presence of an intact epithelial layer and fibrous tissues.

Table 2. Phytochemical screening results of *C. papyrus* ethanolic extract

No.	Secondary Metabolite	Percentage
1.	Saponin	++
2.	Cumarin	-
3.	Alkaloids	-
4.	Flavonoids	+
5.	Tannins	++
6.	Steroids	++
7.	Triterpenes	-
8.	Anthraquinone	-

+: Trace, ++: Moderate, +++: High, -: Negative.

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Discussion

Wound contraction has a very crucial role in the closure of full-thickness skin wounds [14]. In the present study, the wound-healing potential activity of a gel containing *C. papyrus* ethanolic extract was investigated. The obtained results revealed a reduction in wound size in all rats; however, the animals treated with 5% and 10% *C. papyrus* ethanolic extract exhibited a significant ($P \leq 0.001$) reduction in wound area and consequently increased the contraction percentage more rapidly during the epithelization period compared to the control group. Results were expressed as Mean±SEM, with significant differences from the control group indicated. The onset of healing was found to be more rapid in the *C. papyrus* group compared to the other groups ($P<0.01$). Collagen, a main component of the extracellular matrix, significantly contributes to the wound strength. The wound-healing properties of *C. papyrus* extract are mainly due to its ability to enhance the formation of the epithelial layer (3 cross) and fibrous tissues (scant), as shown in Figure 3. In contrast, the negative control and base groups exhibited no healing due to the absence of epithelial and fibrous tissues, as well as the presence of inflammatory cells and granulation tissues at the end of the experiment (day 16), as demonstrated in Figure 3. The positive control (standard drug) showed partial healing, attributed to incomplete cross-linking of the epithelial layer and moderate fibrous tissue formation, as shown in Figure 3.

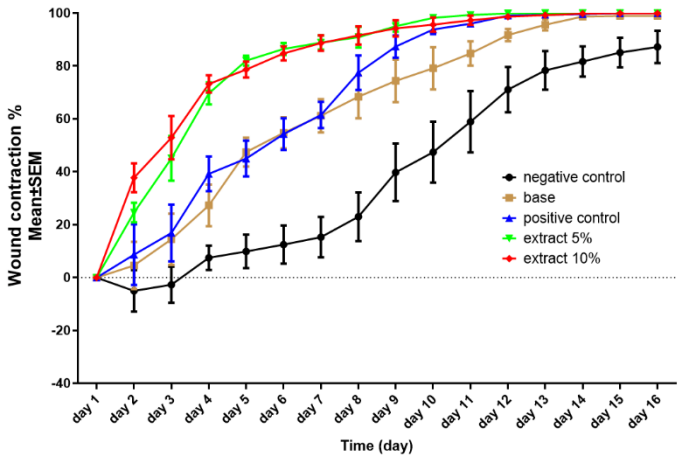

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Figure 1. Wound contraction percentage of the negative control, Carbopol gel base, positive control, and 5% and 10% *C. papyrus* extract groups

Note: Values are Mean±SEM.

Table 3. Wound healing effect of the ethanolic extract of *C. papyrus* in rats (excision wound model)

Days	Group	Mean±SEM/ %				
		Wound Area (cm²) and Percentage of Wound Extraction				
		Negative Control	Base	STD	5% CP Extract	10% CP Extract
0.	A	0.45±0.07	0.81±0.12	0.63±0.14	0.77±0.12	1.09±0.15
1.	A	0.45±0.07	0.81±0.12***	0.63±0.14	0.77±0.12**	1.09±0.15***
	C%	0.0	0.0	0.0	0.0	0.0
2.	A	0.46±0.06	0.77±0.11**	0.52±0.09	0.57±0.08	0.71±0.15
	C%	-5.032	4.57	8.68	24.6	37.77
3.	A	0.45±0.06	0.67±0.08**	0.49±0.09	0.46±0.14	0.55±0.16
	C%	-2.685	14.5	16.88	45.08	52.9
4.	A	0.42±0.07	0.58±0.08	0.36±0.07	0.25±0.07	0.31±0.07
	C%	7.5	27.3	39.21	69.65	73.18
5.	A	0.40±0.06	0.43±0.08	0.32±0.06	0.15±0.04	0.25±0.06
	C%	9.8	47.44	44.98	82.02	78.63
6.	A	0.38±0.06	0.36±0.06	0.28±0.07	0.11±0.03*	0.18±0.06
	C%	12.48	54.73	54.24	86.43	84.74
7.	A	0.36±0.046	0.31±0.06	0.25±0.06	0.10±0.04*	0.14±0.05
	C%	15.29	61.18	61.46	88.84	88.67
8.	A	0.33±0.04	0.26±0.06	0.17±0.06	0.09±0.05	0.11±0.06
	C%	23.03	68.4	77.44	90.9	91.54
9.	A	0.26±0.04	0.21±0.05	0.10±0.04	0.05±0.03	0.097±0.05
	C%	39.78	74.35	87.32	95.01	94.21
10.	A	0.23±0.04	0.16±0.04	0.05±0.02	0.02±0.02	0.07±0.04
	C%	47.4	79.14	93.78	98.19	95.6
11.	A	0.19±0.04	0.12±0.03	0.03±0.01	0.01±0.01	0.04±0.03
	C%	58.93	84.75	95.99	99.29	97.27
12.	A	0.13±0.03	0.07±0.01	0.01±0.0	0.001±0.0	0.02±0.02
	C%	71.07	91.7	99.03	99.85	98.66
13.	A	0.09±0.02	0.03±0.01	0.004±0.002	0±0	0.01±0.01
	C%	78.32	95.57	99.38	100	99.2
14.	A	0.07±0.02	0.01±0.002	0.003±0.001	0±0	0.003±0.002
	C%	81.68	98.7	99.6	100	99.83
15.	A	0.06±0.02	0.01±0.002	0.001±0.0	0±0	0.001±0.001
	C%	85.05	98.91	99.93	100	99.91
16.	A	0.05±0.02	0.01±0.003	0.001±0.0	0.0±0.0	0.001±0.001
	C%	87.2	98.91	99.93	100	99.91

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Abbreviations: A: Area of the wound, SEM: Standard error of the mean; C%: Contraction percent of the wound, CP: *Cyperus papyrus*.

*P≤0.05, **P≤0.01, ***P≤0.001,

Table 4. Onset of healing of *C. papyrus* extract groups and positive standard (STD) group compared to the negative control group

Group	Onset Day	P
5% CP extract	Day 2	≤0.01
10% CP extract	Day 2	0.001
Positive standard	Day 4	0.001

Positive standard: Povidone-iodine ointment.

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Although povidone-iodine was selected as the positive control in this study due to its well-established antiseptic and wound-healing properties, it is acknowledged that more advanced wound care agents, such as silver sulfadiazine, are commonly used in modern clinical practice for wound healing [15]. The absence of such a comparator limits the direct applicability of our findings to current clinical standards. Future investigations should include silver-based or other advanced wound-healing agents to comprehensively evaluate the relative efficacy of *C. papyrus* extract within a broader therapeutic context.

Flavonoids have important anti-inflammatory properties, as they reduce the levels of many inflammatory mediators, including PGE2, LTB-4, IL-1β, TNF-α, IL-6, IFN-γ, and COX while increasing anti-inflammatory mediators, particularly IL-10 [16]. They also produce an anti-oxidant effect and play a potential role in reepithelization in the wound area. Since the *C. papyrus* ethanolic extract contains flavonoids, it may contribute to its wound-healing properties.

The use of topical steroids, in conjunction with antibiotics and antifungals, can improve wound healing rates due to their anti-inflammatory action and ability to relieve pain associated with chronic wounds [17]. This can also be considered a major mechanism of wound healing properties observed in the *C. papyrus* ethanolic extract.

Medicinal plants that contain tannin (such as *C. papyrus*) indicate significant wound healing properties due to its function in the promotion of fibroblast proliferation and migration into wounds, as well as antibacterial activities [18].

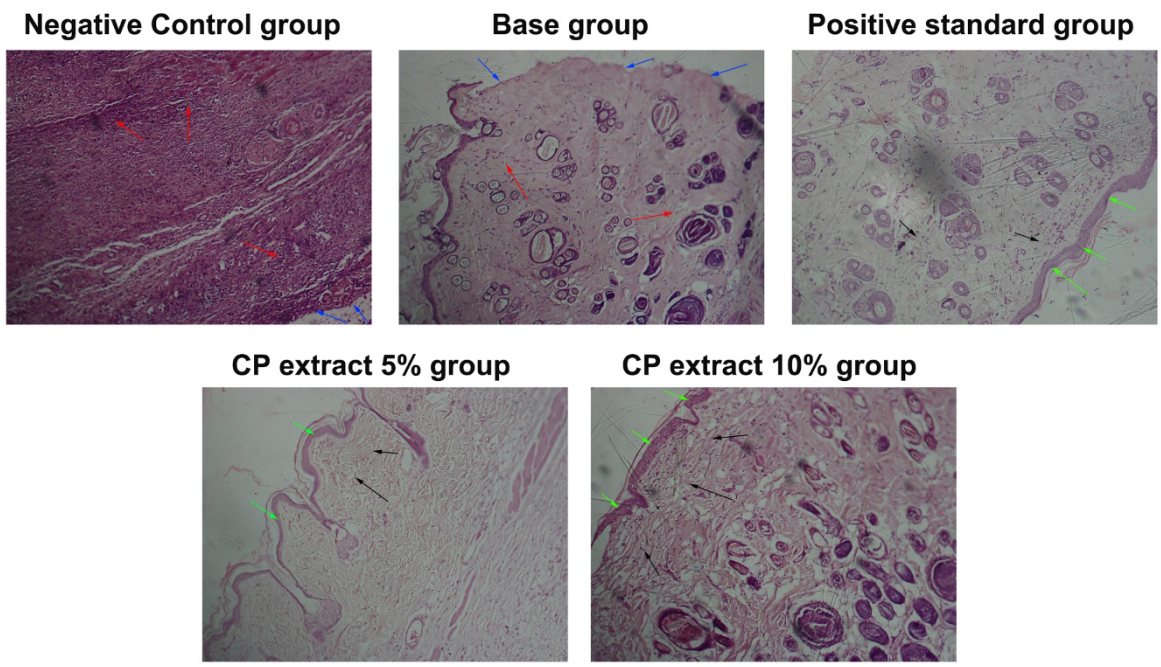

Figure 2. Histopathology of wound tissues (H&E, ×40)

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Note: Negative control (↑IC: Inflammatory cells, ↑GT: Granulation tissue "red arrow"; no epithelium "blue arrow"), CP 5% and positive standard (↑E: Intact epithelium "green arrow", ↑F: Fibrous tissue "black arrow"), and CP 10% (↑E: Thick epithelium; minimal ICs).

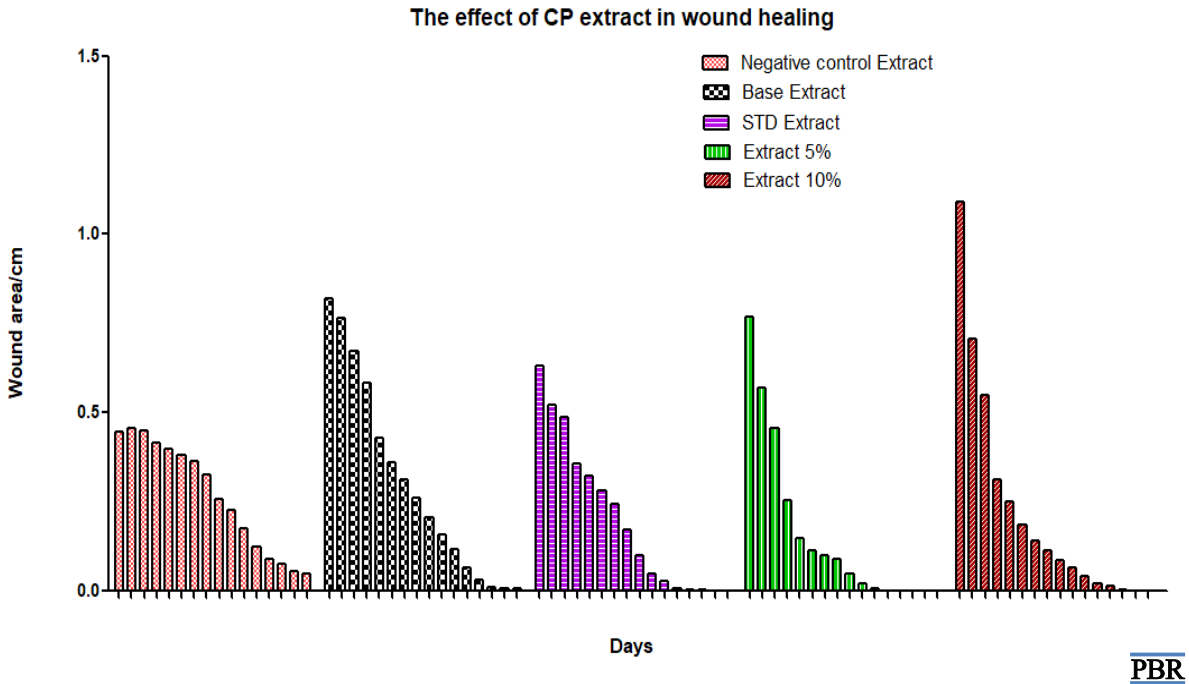

Figure 3. Wound area of the negative control, Carbopol gel base, positive control, and 5% and 10% *C. papyrus* extract groups
Note: Values are Mean±SEM.

Saponins have multiple mechanisms in wound healing. They effectively suppress inflammatory reactions during the early phase, promote re-epithelialization of the wound, and promote matrix synthesis throughout the wound healing process [19]. This is another additional wound-healing mechanism of the ethanolic extract of *C. papyrus*.

The wound healing effects observed in this study are due to the anti-inflammatory, antioxidant, and fibroblast-stimulating properties of phytoconstituents, such as flavonoids, tannins, and saponins present in the *C. papyrus* extract. However, it should be noted that these mechanistic insights are extrapolated from established literature, and our study did not include direct biochemical assays (e.g. cytokine profiling, oxidative stress markers) or molecular investigations (e.g. gene expression studies) to confirm these pathways. Future research should incorporate such analyses to elucidate the precise molecular and cellular mechanisms underpinning the wound-healing activity of this plant extract.

Conclusion

The *C. papyrus* ethanolic extract has wound healing activity, as the onset of healing was faster than in all other groups, and the closure percentage was higher in the *C. papyrus* extract group compared to the positive control, base, and negative control groups. The histopathology examinations revealed that in the *C. papyrus* ethanolic extract and positive control groups, a sufficient amount of epithelial layer and fibrous tissues were formed without the presence of inflammatory cells or granulation tissues. These results suggest that sericin has wound-healing effects without causing allergic reactions.

Limitations

Lack of modern comparators: Only povidone-iodine was used as a positive control, and the inclusion of advanced treatments (e.g. silver sulfadiazine) would strengthen clinical relevance. Mechanistic gaps: while phytochemicals (flavonoids and tannins) were identified, biochemical/molecular data (e.g. cytokine levels, antioxidant assays) are lacking to confirm their role. Animal model constraints: Results from healthy Wistar rats may not fully translate to chronic or diabetic wounds in humans. Short-term evaluation: The long-term effects of CP extract (e.g. scar quality, recurrence) were not assessed. Dose optimization: Only two concentrations (5% and 10%) were tested; a broader dose range might reveal optimal efficacy.

Sample size: Small group sizes (n=6) may limit statistical power for detecting subtle effects. It should be noted that the assumptions of normality and homogeneity of variances, which are prerequisites for ANOVA, were not formally tested in this study. Future analyses should incorporate these checks to strengthen the robustness of statistical inferences.

Ethical Considerations

Compliance with ethical guidelines

All experiments involving animals were performed with the permission and under the strict guidance of the Institutional Animal Ethical Committee for research on small animals at the Faculty of Pharmacy, Omdurman Islamic University, Omdurman, Sudan (Permission letter number: OIU/IAEC/Exp.Ph.2019/6). Although no anesthesia / analgesia was used due to the superficial nature of the wound model, every effort was made to reduce distress and ensure animal welfare.

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Authors' contributions

All authors contributed equally to the conception and design of the study, data collection and analysis, interception of the results and drafting of the manuscript. Each author approved the final version of the manuscript for submission.

Conflict of interest

The authors declared no conflict of interest.

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