

**Effects of post-operative vitamin C therapy on surgical wound and adrenal histopathology, serum electrolytes and haematological markers of stress in albino rats**

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**Abstract**

Vitamin C therapy is rarely integrated into routine post-surgical care despite its reported central role in redox regulation, collagen maturation, epithelial repair and adrenal stress modulation. The present study investigated the effects of short-term post-operative vitamin C administration on surgical wound and adrenal histopathology and selected serum biochemistry and haematological parameters of albino rats. Sixteen male rats (mean weight  $261.94 \pm 6.35$  g) were randomly assigned to two groups (n = 8). All the rats underwent a standardized incision procedure with subcutaneous polypropylene tube implantation ( $2.5 \times 0.25$  cm) in the left groin region. In addition to routine post-operative care, Group I received intramuscular vitamin C (200 mg/kg) injection daily for five days, whereas Group II received routine post-operative care only. On day 10 post-surgery, wound morphology, adrenal histology, haematological indices and serum electrolyte profiles were evaluated. Results showed that rats in the vitamin C-treated group had complete epithelial closure, well-organized collagen deposition and an expanded zona glomerulosa. Rats in the untreated control group exhibited irregular epidermal architecture, less mature collagen and a more vacuolated zona fasciculata. The vitamin C-treated rats maintained higher serum sodium levels and a more balanced Na:K ratio, whereas the untreated controls showed increased neutrophil-to-lymphocyte ratio and serum globulin, reflecting greater physiological stress. These findings demonstrate that post-operative vitamin C therapy enhanced wound healing, supported endocrine-electrolyte homeostasis and attenuated stress-related adrenal responses. These findings highlight the potential value of vitamin C therapy as an adjunct in surgical recovery.

**Keywords:** Vitamin C; Post-operative therapy; Wound healing; Albino rats; Histopathology; Neutrophil: Lymphocyte ratio.

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## Introduction

Impaired wound healing remains a major challenge in surgical practice, particularly in the presence of conditions such as diabetes mellitus, vascular disease, obesity, ageing and malnutrition (Sen, 2019). Although minimally invasive surgical techniques lead to only minor trauma, invasive procedures remain essential in many clinical and experimental contexts, especially in regions where advanced technology is limited.

Wound healing is a complex and dynamic process involving haemostasis, inflammation, proliferation and tissue remodelling. Successful repair requires coordinated epithelial regeneration, fibroblast proliferation, angiogenesis and extracellular matrix (ECM) maturation geared towards re-establishing structural integrity and function. Disruption of these events, whether by oxidative imbalance, ischaemia, nutritional deficiency or systemic co-morbidity, can delay closure, compromise tensile strength and increase susceptibility to infection or dehiscence (Cullen *et al.*, 2002). Oxidative stress, in particular, impairs keratinocyte migration, reduces fibroblast viability, inhibits angiogenic signaling and disrupts collagen synthesis (Ukaegbu *et al.*, 2025).

The adrenal glands are central to the regulation of physiologic response to surgical trauma. Through glucocorticoids, mineralocorticoids and catecholamines, the adrenal cortex and medulla modulate inflammation, metabolism, blood pressure and electrolyte homeostasis (Dutt *et al.*, 2023). Excessive glucocorticoid release following surgery reportedly promotes lymphocyte suppression, impairs angiogenesis and compromises fibroblast function, ultimately delaying wound repair (Tiganescu *et al.*, 2020). Conversely, appropriate aldosterone activity has been reported to support sodium balance, fluid stability and cellular transport systems

essential for tissue regeneration (Scott *et al.*, 2023).

Vitamin C (ascorbic acid) is an essential micronutrient with potent antioxidant properties and multiple roles in tissue repair. It serves as a cofactor for prolyl and lysyl hydroxylases in collagen synthesis, stabilizing the triple helix structure and improving the tensile strength of healing tissue (Cullen *et al.*, 2002). Beyond collagen maturation, vitamin C has been reported to promote keratinocyte proliferation, support endothelial cell function, enhance angiogenesis, modulate immune responses and help maintain adrenal redox balance (Alberts *et al.*, 2025). Under physiological stress, vitamin C is rapidly depleted from adrenal tissue, and deficiency has been associated with delayed wound healing in both clinical and experimental settings, while supplementation has been shown to accelerate recovery in both surgical and chronic wounds (Guo and DiPietro, 2010).

Despite its mechanistic relevance and therapeutic potential, vitamin C is not routinely incorporated into post-operative care protocols (Fukushima *et al.*, 2010). Targeted vitamin C micronutrient therapy may therefore represent an underutilized strategy for optimizing surgical recovery, particularly in circumstances characterized by oxidative stress and endocrine activation. This study investigated the effects of short-term post-operative vitamin C administration on wound and adrenal histopathology, serum electrolytes and haematological markers of stress in albino rats.

## Materials and methods

The study was carried out in the Department of Veterinary Pathology, College of Veterinary Medicine, Michael Okpara University of Agriculture, Umudike, Nigeria. Sixteen male albino rats (mean body weight;  $261.94 \pm 6.35$  g) were acclimatized for one month and then randomly assigned into two groups (Groups I

and II) of eight each. They were fed standard rat feed (Chikun® Finisher, Crown Flour Mills, Nigeria) and provided water *ad libitum* throughout the course of the experiment. Ethical approval for the study was obtained from the University Ethics Committee (Approval Reference Number: MOUAU/CVM/REC/202537). The Vitamin C used for the study was sourced from Embassy Pharmaceutical & Chemicals Limited, Lagos, Nigeria.

The rats were sedated with Diazepam (5 mg/kg body weight, intra-muscular) and anaesthetised with Ketamine hydrochloride (40 mg/kg body weight, intra-muscular) in preparation for the surgery. Each rat was placed on supine position and the left groin region was prepared for surgery following standard procedures. A nick was made on the prepared site and, akin to dead space wound model, a sterilized polypropylene tube (2.5 cm length by 0.25 cm diameter) was implanted subcutaneously (Ilango and Chitra, 2010). The wound was sutured by placing two interrupted sutures with nylon suture material. The day of implantation was designated post-surgical day (PSD) 0. Both rat groups were given standard post-operative care (Procaine penicillin and Streptomycin injection at the dose of 20 mg/kg body weight, intramuscular for 5 consecutive days post-surgery). Group I received additional treatment of Vitamin C at the dose of 200 mg/kg intramuscularly for 5 consecutive days post-surgery, but Group II was not treated with vitamin C (untreated control).

On PSD 10, the animals were euthanized and blood samples were collected for the following: serum total protein, albumin and globulin determination, differential white blood cell count, and serum sodium and potassium assays, using standard procedures. The serum total protein (Tietz, 1995) and serum albumin (Doumas *et al.*, 1971) were determined following standard procedures, and the globulin levels were calculated by

subtracting the albumin levels from the total protein levels. The differential WBC counts were done following the Leishman technique (Schalm *et al.*, 1975). The assay of serum sodium and potassium levels were done based on the colorimetric method (Henry, 1974; Young, 2001) using TECO® test kits (TECO Diagnostics®, U.S.A).

Samples of the healing wound (skin) and adrenal gland were also collected and fixed in 10 % phosphate buffered formal saline. They were dehydrated in ascending concentrations of alcohol, cleared in xylene for 1 hour 30 minutes, and embedded in paraffin wax. Sections (5 microns thick) were made and mounted on slides (Drury and Wellington, 1967). The slides were stained with haematoxylin, counterstained with eosin (H & E stain) and examined under a light microscopy.

Data obtained were analysed using IBM SPSS software® (Version 20). Student's t-test was used to compare results of the two groups. Significance was accepted at  $p < 0.05$ .

## Results

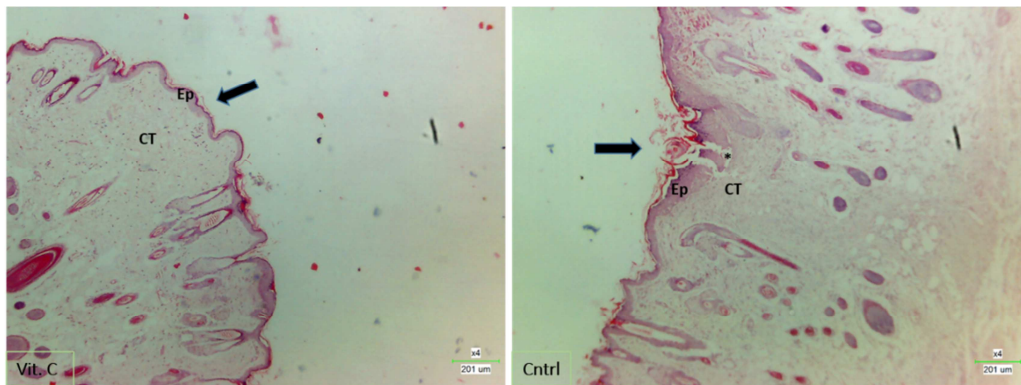
The histopathology photomicrograph showed that the epidermis at the incision site in the vitamin C-treated group was of normal thickness (Figure 1) and the incision site was not grossly apparent (Figure 2). In contrast, the incision site was excessively thickened in the untreated control due to epidermal proliferation and infolding (Figure 1) and remained visible as a thin scar (Figure 2).

The outermost layer of the adrenal cortex, the zona glomerulosa, was very prominent in the vitamin C-treated Group I rats, but not so in the untreated Group II rats (Figure 3). Microscopic examination at a higher magnification confirmed clear-opaque secretory products/cytoplasmic accumulations, giving the cells a foamy appearance (Figure 4). The cells of the zona

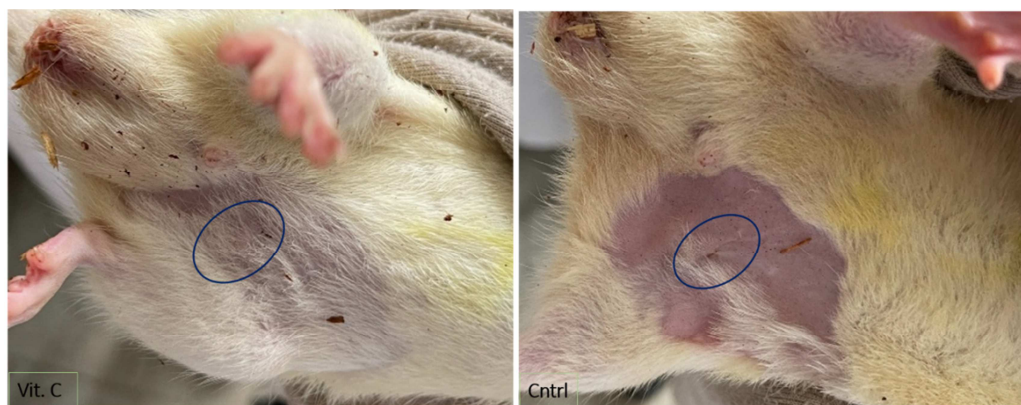
fasiculata, the middle layer of the adrenal cortex, in both groups were also observed to contain whitish droplets/globules within their cytoplasm (Figure 4). These globular cytoplasmic contents were more in the untreated Group II control rats than in the vitamin C-treated Group I rats.

The neutrophil-to-lymphocyte ratio (NLR) was not significantly different ( $p > 0.05$ ) between the two groups but was still lower in the vitamin C-treated Group I rats when compared to the untreated Group II rats (Figure 5).

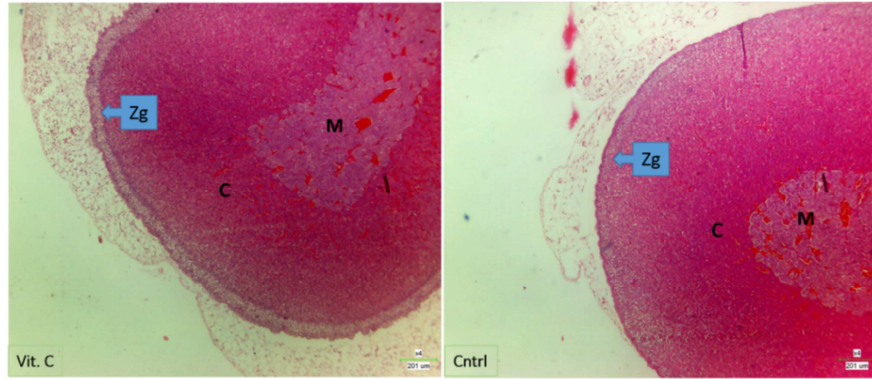
The vitamin C-treated Group I rats had significantly lower ( $p < 0.05$ ) serum globulin levels than the untreated Group II control rats (Figure 6). The serum sodium level of the vitamin C-treated Group I rats was also significantly ( $p < 0.05$ ) higher than that of the untreated control Group II rats (Figure 7). There was no significant ( $p > 0.05$ ) difference in the serum sodium to potassium ratio (Na:K) between the two groups, although the ratio was lower in the vitamin C-treated Group I than the untreated Group II control rats (Figure 8).



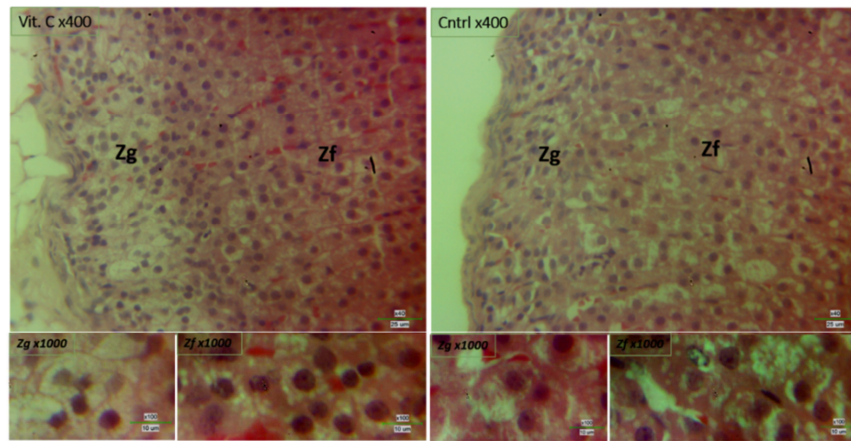
**Figure 1.** Photomicrograph of the section of the incision wound (arrows) on the albino rats at day-10 post-surgery. Epithelium (Ep) is normal in the vitamin C group, but thickened with infolding in the untreated control (Cntrl), with underlying connective tissue (CT) proliferation in the dermis. Note the pockets of dead spaces (\*) formed in the untreated [H & E;  $\times 100$ ]



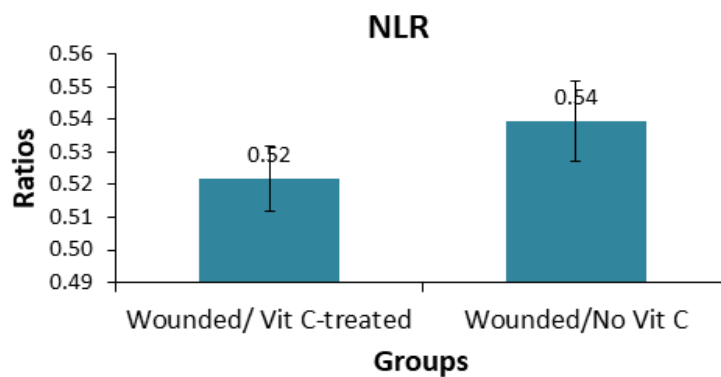
**Figure 2.** Gross pictures of the healing status of skin incision (circled) of rats at day-10 post-surgery; the surgical incision was not grossly apparent in the vitamin C-treated group but was visible as a scar in the untreated control (Cntrl).



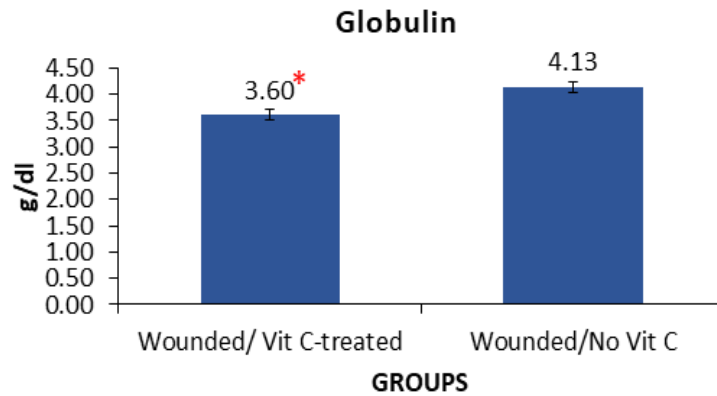
**Figure 3.** Histopathology photomicrograph of section of the adrenal glands of the rats at day-10 post-surgery; the zona glomerulosa (Zg) of the cortex (C) was very distinct in the vitamin C-treated group compared to the untreated control. The medulla (M) of the two groups were morphologically similar [H & E;  $\times 100$ ]



**Figure 4.** Histopathology of sections of the adrenal glands of rats at day-10 post-surgery. The highly foamy cytoplasm of the cells of the zona glomerulosa (Zg) made it very conspicuous in the vitamin C-treated group; while the cells of the zona fasciculata (Zf) in the untreated control contained more pre-secretory steroidal globules than the vitamin C-treated group [H & E;  $\times 400$ ; inserts  $\times 1000$ ]

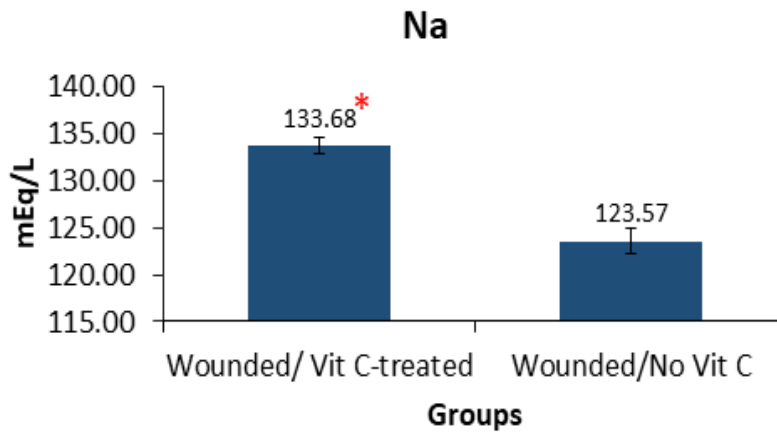


**Figure 5.** Blood neutrophil-to-lymphocyte ratio (NLR) of rats at day-10 post-surgery following post-surgical vitamin C administration, compared with the control that was not treated with vitamin C.



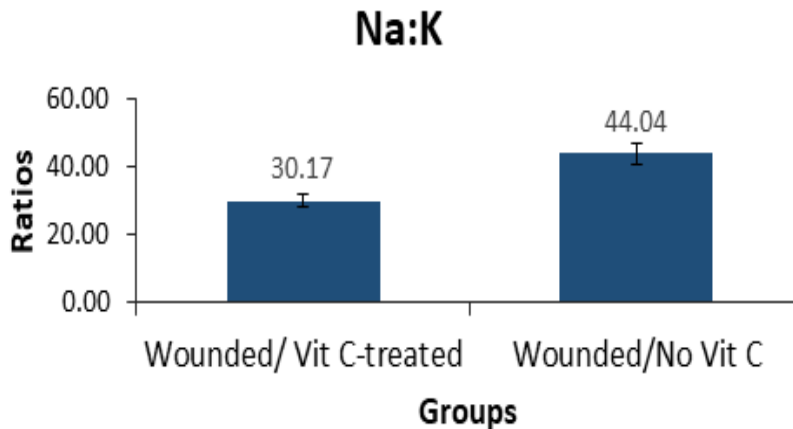
\* Superscript indicates significant difference between the means ( $p < 0.05$ ),  $n=8$

**Figure 6.** Serum globulin levels of albino rats at day-10 post-surgery following post-surgical vitamin C administration, compared with the levels in untreated control rat group that was not given vitamin C.



\* Superscript indicates significant difference between the means ( $p < 0.05$ ),  $n=8$

**Figure 7.** Serum sodium levels of rats at day-10 post-surgery following post-surgical vitamin C administration, compared with the levels in untreated control rat group that was not given vitamin C.



**Figure 8.** Serum sodium to potassium ratio of rats at day-10 post-surgery following post-surgical vitamin C administration, compared with the ratio in untreated control rat group that was not given vitamin C.

## Discussion and Conclusion

The presence of a continuous, normal thickened epidermis at the incision site in the vitamin C-treated rats implied a more complete and organized healing process compared with untreated controls. Enhanced epithelial proliferation and alignment suggest efficient closure of wound edges and restoration of skin integrity, whereas wounds of the untreated (no vitamin C treatment) group exhibited irregular thickening and infolding of the epidermis, leading to pockets of dead space and delayed closure. Such structural disorganization predisposes to surgical site infection and reflects suboptimal tissue repair. Apparently at the early stages of healing, proper alignment and closure of the edges of the epidermis were hampered in the absence of vitamin C.

There are known factors that affect healing of wounded tissues and they include nutrition, immunity and (oxidative) stress (Guo and DiPietro, 2010). Vitamin C treatment in the present study is believed to have promoted organized collagen deposition and fibroblast proliferation, both of which are essential for structural stability of newly formed tissue. These effects align with its established role as a cofactor in the hydroxylation of collagen precursors, ensuring proper cross-linking and tensile strength of healed wounds (Alberts *et al.*, 2025). By mitigating oxidative stress, vitamin C has been reported to further enhance fibroblast function and angiogenesis, processes known to be inhibited under oxidative conditions (Lopes *et al.*, 2024).

Adrenal histomorphology in this study revealed distinct differences between vitamin C-treated and the untreated control rat group. The prominent, foamy zona glomerulosa observed in vitamin C-treated rats suggested increased secretory activity and enhanced aldosterone synthesis, while the more vacuolated zona fasciculata in the untreated controls indicated elevated cortisol

production, consistent with a greater physiological stress response. The adrenal cortex has been reported to play a crucial role in modulating systemic adaptation to surgical trauma through glucocorticoid and mineralocorticoid balance (Stamou *et al.*, 2023; Zennaro *et al.*, 2015). The pattern recorded in the present study suggests that vitamin C therapy supports a shift towards homeostatic, rather than stress-dominant, endocrine regulation.

Increased aldosterone secretion may have contributed to improved wound repair by maintaining electrolyte and fluid balance necessary for cellular proliferation and migration (Funder, 2017). After surgery, increase in hypothalamic-pituitary hormone secretion causes increased cortisol and ADH release leading to hyponatraemia. Post-surgical hyponatraemia remains the commonest post-operative electrolyte abnormality encountered by surgeons (Cuesta and Thompson, 2015). In this study, administration of vitamin C mitigated this condition. Aldosterone reportedly also promotes macrophage infiltration, fibroblast proliferation and extracellular matrix synthesis (Ferreira *et al.*, 2021; Pohl *et al.*, 2021). The higher serum sodium concentration and the balanced Na:K ratio in the vitamin C-treated group indicate efficient sodium retention and potassium conservation, maintaining the ionic gradients required for Na<sup>+</sup>/K<sup>+</sup>-ATPase activity and cell function. Oxidative stress impairs this enzyme's activity (Srikanthan *et al.*, 2016); thus, the antioxidant effect of vitamin C likely contributed to its preservation.

In contrast, the untreated control group may have elevated cortisol activity, as suggested by higher neutrophil-to-lymphocyte ratio and globulin levels. Excess cortisol delays wound closure by suppressing lymphocyte proliferation, impairing angiogenesis and inhibiting fibroblast function (Tiganescu *et al.*, 2020), while cortisol binding globulin (CBG) is essential for the transport of cortisol in blood

(Henley et al., 2016). Vitamin C, which is concentrated in the adrenal cortex, has been shown to buffer excessive corticosteroid release during stress by reducing hypothalamic–pituitary–adrenal (HPA) axis activation and replenishing adrenal antioxidant capacity (Choi et al., 2011).

Taken together, vitamin C therapy probably reduced oxidative stress, stabilized keratinocyte and fibroblast function, enhanced collagen cross-linking and angiogenesis and modulated adrenal endocrine output towards homeostasis rather than stress dominance. The combined cellular, biochemical and endocrine effects resulted in superior epithelial regeneration, more mature granulation tissue and more favourable post-operative electrolyte patterns in the treated group.

Translational limitations of this study includes a modest sample size and assessments performed at a single time point (day 10), limiting temporal resolution of early healing events. Additionally, rats heal predominantly by contraction, unlike humans and some veterinary patients. Nonetheless, the incision model used here reliably reflects key phases of stromal and epithelial repair and is widely accepted for evaluating interventions that influence collagen synthesis and oxidative balance.

In conclusion, short-term post-operative vitamin C treatment enhanced structural and systemic aspects of wound repair by promoting epithelial continuity, collagen maturation and endocrine-electrolyte homeostasis while reducing stress-related haematological responses. These findings support the potential adjunctive use of vitamin C in post-surgical veterinary medical care and justify further investigation across species, wound types and dosing regimens in both clinical and translational settings.

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## Conflict of interest

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