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The Effect of Ascorbic Acid Supplementation on the Time of Healing of Rats Submitted to Neurosurgical Procedures

Efeito da suplementação de ácido ascórbico no tempo de cicatrização de ratos submetidos a procedimentos neurocirúrgicos

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Abstract

Introduction Vitamin C is an essential nutrient for both humans and rats and has been noted for its beneficial properties, among them, healing.

Objective To verify the effect of oral and subcutaneous vitamin C supplementation on the healing time of surgical wounds of rats skulls.

Statistical Methodology Thirty male Wistar rats were divided into 3 groups: 10 from the control group (GI), 10 from the group treated with oral vitamin C (GII), and 10 from the group treated with subcutaneous vitamin C (GIII). Vitamin C was administered to GI and GIII animals from the 3rd to the 7th postoperative day, totaling 10 days of administration at a dose of 100 mg/kg/day. On the 4th day of the study, the rats were submitted to a surgical procedure consisting of a 2-cm incision of the skin of the animals' heads and suturing with single stitches. After a determined period, the rats were killed and submitted to the collection of material for study by the picrosirius red technique for the evaluation of collagen types I and III, the degree of hematoxylin and eosin healing, and the rate of contraction of the wound on subsequent days. The results were described in averages, medians, minimum and maximum values, and standard deviations. For the comparison of the three groups, the analysis of variance with one factor (one-way ANOVA) or Kruskal-Wallis non-parametric test was used. The normality of the variables was evaluated by the Shapiro-Wilk test. Values of $p < 0.05$ indicated statistical significance. The data were analyzed using the IBM SPSS Statistics for Windows, v.20.0. software. (IBM Corp., Armonk, NY, USA).

Results The amount of collagen type III was higher in the groups that received vitamin C, however, without significant difference ($n = 0.292$). In relation to the rate of

Keywords
- scalp
- healing
- ascorbic acid
- Wistar rats

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contraction of the surgical wound, it was higher in the groups treated with vitamin C, with a significant difference between groups I and II ($p = 0.001$), and between groups I and III ($p < 0.001$). No significant difference was found between the groups that were treated with vitamin C ($p = 0.227$).

Conclusion Healing was more effective in the groups treated with vitamin C than in the group that did not receive vitamin supplementation. There was no significant difference in healing between the groups receiving oral or subcutaneous vitamin C.

Resumo

Introdução A vitamina C é um nutriente essencial tanto para humanos quanto para ratos e tem-se destacado por suas propriedades benéficas, entre elas, a cicatrização.

Objetivo Verificar o efeito da suplementação de vitamina C oral e subcutânea no tempo de cicatrização de feridas cirúrgicas do crânio de ratos.

Metodologia estatística: Foram utilizados 30 ratos *Wistar*, machos, divididos em 3 grupos, 10 do grupo controle (GI), 10 do grupo tratado com vitamina C oral (GII) e 10 do grupo tratado com vitamina C subcutânea (GIII). A vitamina C foi administrada aos animais de GII e GIII, do 3° dia ao 7° dia pós-operatório, totalizando 10 dias de sua administração, na dose de 100 mg/kg/dia. No 4° dia do estudo, os ratos foram submetidos ao procedimento cirúrgico, que consistiu na incisão de 2 cm da pele da cabeça dos animais e sutura com pontos simples. Após um período determinado, os ratos foram mortos e submetidos a coleta do material para estudo por meio da técnica de *picrosirius red* para avaliação do colágeno tipos I e III, o grau de cicatrização pela hematoxilina e eosina, e pela taxa de contração da ferida nos dias subsequentes. Os resultados foram descritos por médias, medianas, valores mínimos e máximos, e desvios padrões. Para a comparação dos três grupos, foi usado o modelo de análise da variância com um fator (ANOVA) ou o teste não-paramétrico de Kruskal-Wallis. A condição de normalidade das variáveis foi avaliada pelo teste de Shapiro-Wilk. Valores de $p < 0,05$ indicaram significância estatística. Os dados foram analisados com o programa computacional IBM SPSS Statistics for Windows, v.20.0. (IBM Corp., Armonk, NY, EUA).

Resultados: A quantidade de colágeno tipo III foi maior nos grupos que receberam vitamina C, porém, sem diferença significativa ($n = 0,292$). Em relação à taxa de contração da ferida operatória, ela foi maior nos grupos tratados com vitamina C, com diferença significativa entre os grupos I e II ($p = 0,001$), e entre os grupos I e III ($p < 0,001$), não sendo encontrada diferença significativa entre os grupos que foram tratados com vitamina C ($p = 0,227$).

Conclusão A cicatrização foi mais efetiva nos grupos tratados com vitamina C em relação ao grupo que não recebeu suplementação da vitamina. Não houve diferença significativa na cicatrização entre os grupos que receberam a vitamina C oral ou subcutânea.

Palavras-chave
- escalpe
- cicatrização
- ácido ascórbico
- ratos Wistar

Introduction

The healing process is common to all wounds, regardless of the agent that caused it, it is systemic and dynamic and is directly related to the general conditions of the organism. It consists of a perfect and coordinated cascade of cellular, molecular, and biochemical events that interact for tissue reconstitution to occur.

Tissue damage, the initial stimulus for the healing process, puts blood elements in contact with collagen, synthesized by fibroblasts, and other substances in the extracellular matrix, causing platelet degranulation and activation of the coagulation and complement cascades. With this, the release of several vasoactive and chemotactic mediators that guide the healing process by attracting inflammatory cells to the wound region occurs.

According to the literature, ascorbic acid acts as an electron donor for the proline hydroxylation process, during collagen synthesis, a fact that leads to suspicion of its increased demand in tissue repair processes.

Methodology

The present research was performed in the vivarium and in the laboratory of operative technique and experimental surgery at the institute of medical research (IPEM, in the Portuguese acronym) of Faculdade Evangélica Mackenzie do Paraná (FEMPAR). Thirty male Wistar rats were used, divided into 3 groups, 10 from the control group (GI), 10 from the group treated with oral vitamin C (GII), and 10 from the group treated with subcutaneous vitamin C (GIII).

Vitamin C was administered to animals from GII and GIII, from the 3rd to the 7th postoperative day, totaling 10 days of its administration, at a dose of 100 mg/kg/day. On the 4th day of the study, the rats were submitted to a surgical procedure that consisted of a 2-cm incision of the skin of the animals' heads and sutures with simple stitches. After a determined period, the rats were killed and subjected to the collection of material for study using the picrosirius red technique to assess collagen types I and III, the degree of healing by hematoxylin and eosin (HE), and the rate of wound contraction on subsequent days. The results were described by means, medians, minimum and maximum values, and standard deviations. For the comparison of the three groups, the one-way analysis of variance (one-way ANOVA) model or the Kruskal-Wallis non-parametric test was used. The condition of normality of the variables was assessed by the Shapiro-Wilk test. Values of $p < 0.05$ indicated statistical significance. The data were analyzed with the computer program IBM SPSS Statistics for Windows, v.20.0. (IBM Corp., Armonk, NY, USA).

All ethical parameters were respected, and this research was approved by the Ethics Committee on the Use of Animals of Faculdade Evangélica Mackenzie do Paraná (CEUAs / FEMPAR).

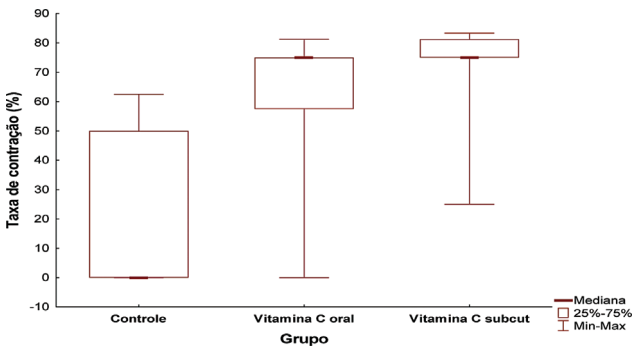
Results

A significant difference was found between the three groups in terms of the rate of contraction (►**Tables 1** and **2**). There was a significant difference between the control group and the groups treated with oral vitamin C ($p = 0.001$) and subcutaneous vitamin C ($p < 0.001$). No significant difference was found between the two groups treated with vitamin C ($p = 0.227$), the rates observed are shown in **Graph 1**.

►**Figs. 1**, **2**, and **3** demonstrate the evolution of the wound through the photos obtained on the 3rd, 5th, and 7th days of the study. It is possible to notice the reduction in the size of the wound and the gradual disappearance of the crust and granulation tissue.

Table 2 Comparison of the contraction rate in relation to each group

Compared groups	*p*-value*
Control versus oral vitamin C	**0.001**
Control versus subcutaneous vitamin C	< **0.001**
Oral vitamin C versus subcutaneous vitamin C	0.227



Graph 1 Contraction rate of the wound in percentage observed on day 7 of treatment.

Using the table adapted from the protocol created by Greenhalgh D. G. et al., the lesions were classified from 1 to 4, with 1 being the worst and 4 being the best degree of healing ►**Table 3**,**4**,**5**,**6**.

►**Figs. 4**, **5**, and **6** show the histological variation of the degree of healing in relation to each group.

The results indicate that there is no significant difference between the three groups in relation to the area of collagen I and the area of collagen III (**Graphic 2** and **Graphic 3**).

►**Fig. 7** shows the greater emphasis of type-I collagen fibers in relation to type-III collagen fibers in a control group rat. In the groups that received vitamin C, ►**Fig. 8** and **9**, especially in the group that received it subcutaneously, ►**Fig. 9**, the percentage of type-III collagen stands out in relation to type-I collagen.

Discussion

In our study, the highest amount of type-III collagen was identified in the groups that received vitamin C, however, with no significant difference. The rate of contraction of the surgical wound was higher in the groups treated with vitamin C, with a significant difference between groups I and II

Table 1 Comparison of percentage values of operating wound contraction rate in each group and among all groups

Variable	Group	N	Average	Median	Minimum	Maximum	Standard deviation	*p**-value*
Contraction rate	Control	10	20.8	0.0	0.0	62.5	26.5	
(%)	Oral vitamin C	10	64.9	75.0	0.0	81.3	23.4	**0.001**
	Subcutaneous vitamin C	10	70.0	75.0	25.0	83.3	18.4	

* Kruskal-Wallis non-parametric test, $p < 0.05$.

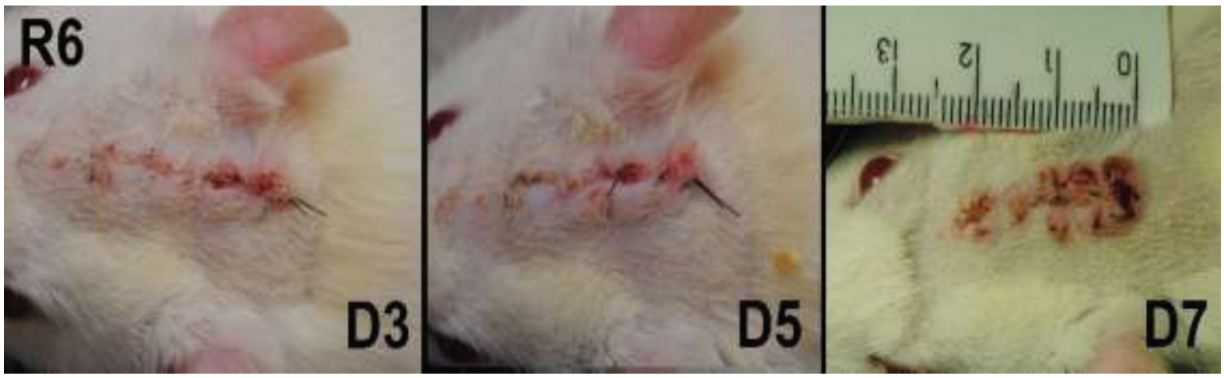


Fig. 1 Aspect of group I rat wound contraction (Control).

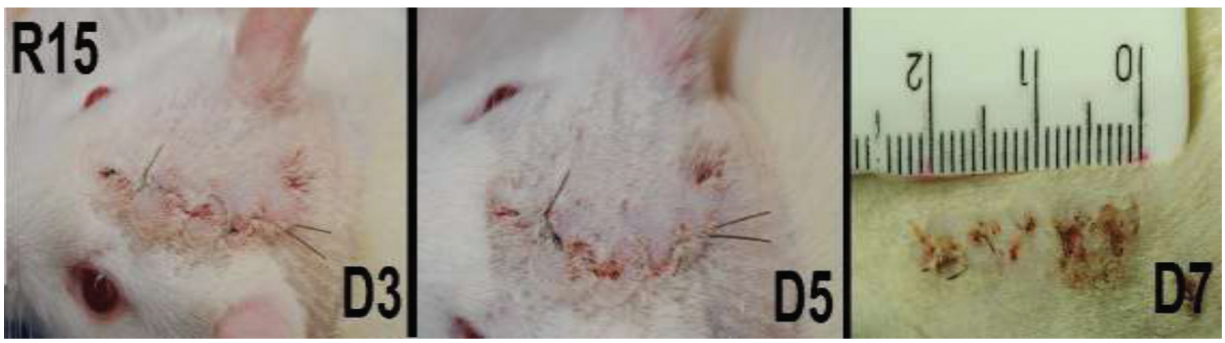


Fig. 2 Aspect of group II rat wound contraction (oral vitamin) for 7 days of observation.

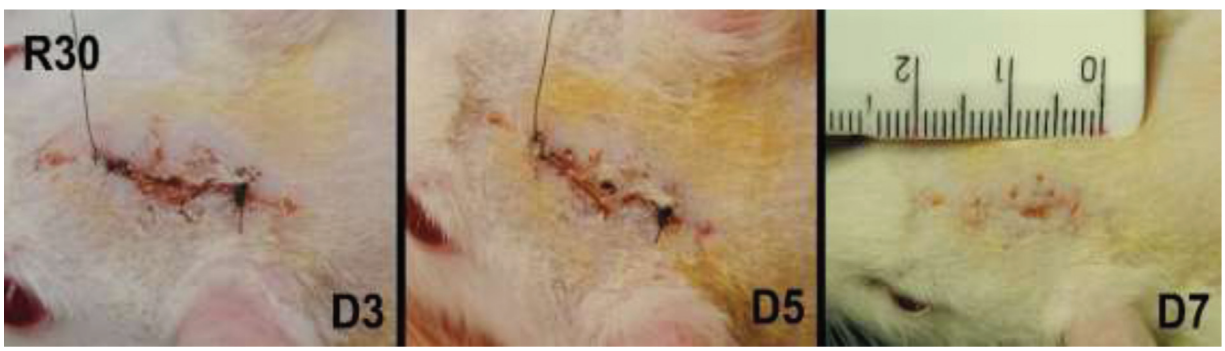


Fig. 3 Aspect of Group III Rat Wound Contraction (Subcutaneous Vitamin C) DURING 7 DAYS OF OBSERVATION. NOTE: Animals in the control group on days 3, 5, and 7 of the study.

($p = 0.001$), and between groups I and III ($p < 0.001$). No significant difference was found between the groups that were treated with vitamin C ($p = 0.227$).

The dose of ascorbic acid was chosen based on previous studies, which verified that these are the minimum concentrations capable of affecting wound healing and that can be used in humans without leading to toxic and harmful effects when administered.

The surgical technique was chosen, as it is a technique that is easy to reproduce and standardize, based on previous works, which opted for the incision in the cranial region, of ~ 20 mm in length. The technique used to assess the intensity of the inflammatory process was HE, which is considered the main means of analysis when the objective of the study is the epithelial tissue.

Regarding the intensity of healing, the most advanced form was found in groups II and III in relation to the control group, that is, in the group that did not receive vitamin C supplementation, there was a greater degree of inflammation and a more pronounced granulation tissue.

The present study was based on previous works, cited in the bibliographic reference,[1–55] for the choice and organization of groups, surgical technique, dose of ascorbic acid, technique for evaluating the inflammatory process and intensity of healing, which observed a greater number and better arrangement of fibroblasts in animals in groups II and III when compared with the control group, since the use of ascorbic acid maintains an adequate concentration of the vitamin in the skin, which stimulates the proliferation of dermal fibroblasts.

Table 3 Comparison of percentage values according to hematoxylin and eosin groups and classifications

HE (degree)	Group (treatment)		
	Control	Oral	Subcutaneous
0	–	–	–
	–	–	–
1	–	–	–
	–	–	–
2	3	–	–
	30%	–	–
3	4	3	3
	40%	30%	30%
4	3	7	7
	30%	70%	70%
Total	10	10	10

Table 4 Comparison of the percentage values of healing assessed by hematoxylin and eosin

Hematoxylin and eosin (degree)	Group (treatment)		
	Control	Oral	Subcutaneous
0, 1, or 2	3	0	0
	30%	0%	0%
3 or 4	8	10	10
	80%	100%	100%
Total	10	10	10

The degree of scarring assessed by hematoxylin and eosin was higher in the groups that received vitamin C, however, with no significant difference between the groups.

The macroscopic evaluation was necessary since the wound contraction process is the fourth phase of the healing process and consists of the centripetal movement of the edges. The phase that precedes the contraction of the wound is that of proliferation, responsible for the closure of the lesion itself, and it is divided into three subphases, which are reepithelization, fibroplasia, and angiogenesis. Finally, the

Table 5 Comparison of the percentage values of the degree of healing in the groups assessing the statistical significance

Groups compared	*P*-value*
Control x oral	0.214
Control x subcutaneous	1
Oral x subcutaneous	0.472

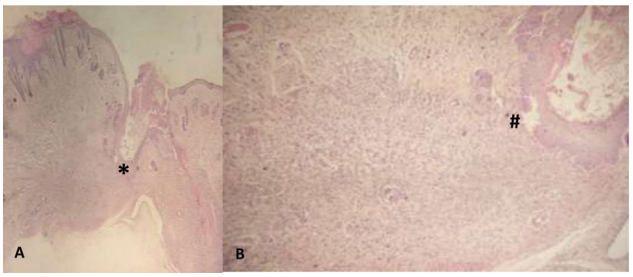


Fig. 4 Photomicrograph of the wound healing area in the Control group. LEGEND: (A) (HE 40x) - Granulation tissue of the epithelium (B) (HE200x). NOTE: (A) (*) Epithelium partially covering the surgical wound. (B) (#) Thin and immature granulation tissue, predominantly inflammatory cells, with few fibroblasts, capillaries, and collagen deposition.

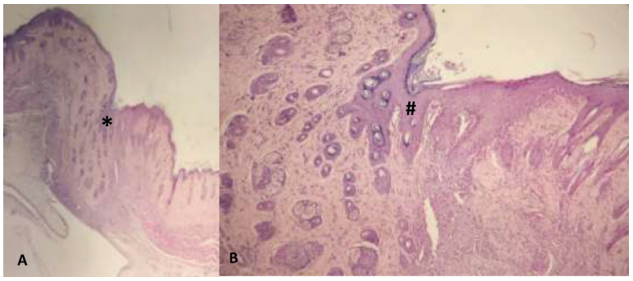


Fig. 5 Photomicrograph of the wound healing area of the subcutaneous vitamin C group. (A) (HE 40x) - Granulation tissue of the epithelium (B) (HE200x). NOTE: (A) (*) Epithelium fully covering the lesion. (B) (#) Medium-thickness granulation tissue, with few inflammatory cells with a predominance of fibroblasts with collagen deposition. Neovascularization present in good quantity.

Table 6 Comparison of the percentage values of the collagen I and Iii area in each group and among all groups

Variable	Group	N	Average	Median	Min	Max	Standard deviation	P-value*
Collagen I area	Control	10	50.7	50.0	12.3	84.0	23.7	
(%)	Oral vitamin C	10	48.5	45.3	31.7	82.0	15.0	0.292
	Subcutaneous vitamin C	10	37.5	39.0	1.7	64.8	20.1	
Collagen III area	Control	10	49.3	50.0	16.0	87.7	23.7	
(%)	Oral vitamin C	10	51.5	54.7	18.0	68.3	15.0	0.292
	Subcutaneous vitamin C	10	62.5	61.0	35.2	98.3	20.1	

*one-way ANOVA, $p < 0.05$.

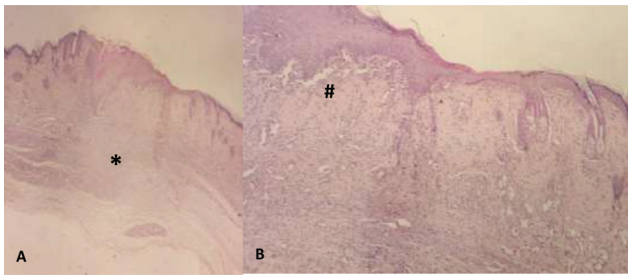
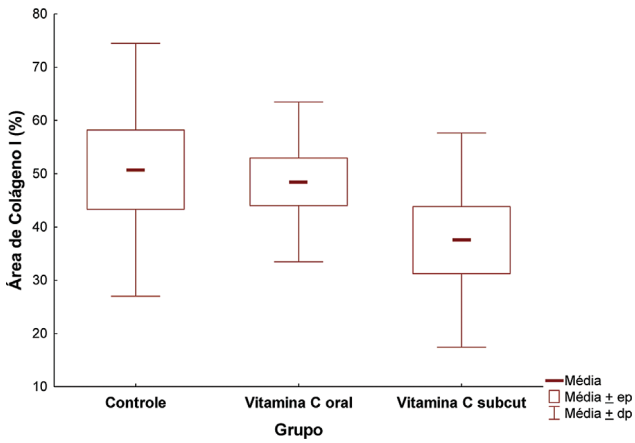
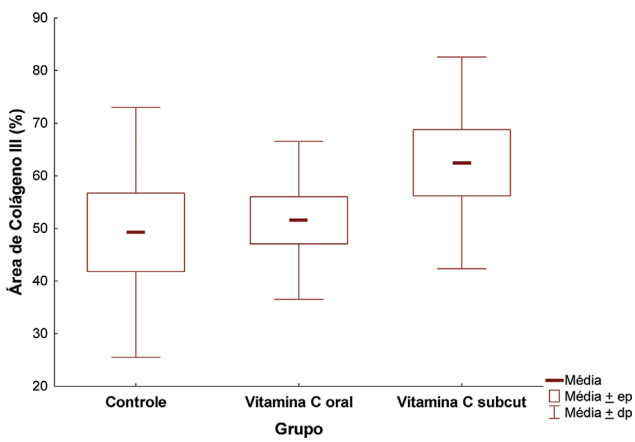


Fig. 6 Photomicrograph of the wound healing area of the oral vitamin C group. (A) (HE 40x) - Granulation tissue of the epithelium (B) (HE200x). NOTE: (A) (*) Epithelium fully covering the lesion with little crust formation. (B) (#) Thick and vascularized granulation tissue, predominance of fibroblasts and large collagen deposition.



Graph 2 Collagen I area (%) between the groups on the seventh day of treatment.



Graph 3 Collagen III area (%) between groups on the seventh day of treatment.

remodeling phase follows that of contraction of the wound and is the last stage of healing.

Conclusion

Ascorbic acid supplementation achieved more effective cranial healing compared with the group that did not receive

Fig. 7 Photomicrography of type-I and -III collagen fibers. 400x in histological staining with pricosirius red in an animal in the control group (GI) on the tenth day.

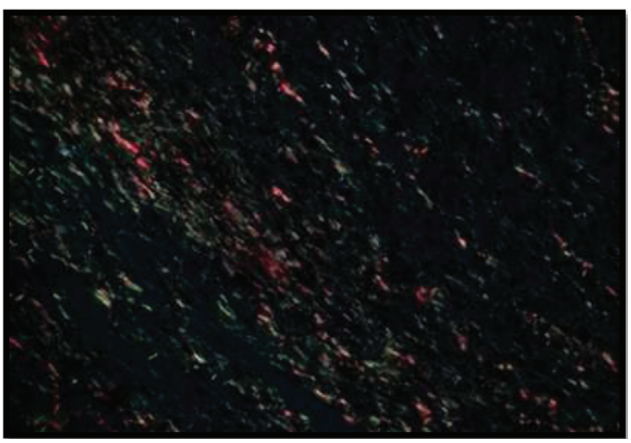


Fig. 8 Photomicrography of type I and III collagen fibers. 400x in histological staining with pricosirius red in an animal in the group treated with oral vitamin C (GII) on the tenth day.

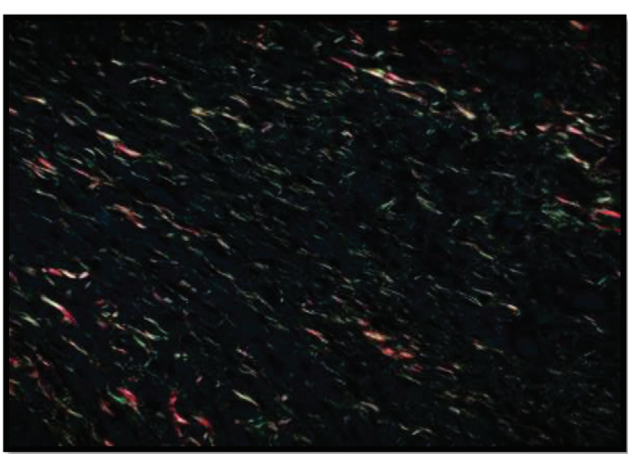


Fig. 9 Photomicrography of type-I and -III collagen fibers. 400x in histological staining with picrosirius red in an animal in the group treated with subcutaneous vitamin C (GIII) on the tenth day. Type-I collagen represented by the symbol #. Type-III collagen represented by the symbol *

vitamin C supplementation. There was no significant difference in healing between the groups that received oral or subcutaneous vitamin C.

Regarding the rate of wound contraction, there was a significant difference between the control group and the groups treated with oral vitamin C ($p = 0.001$) and subcutaneous vitamin C ($p < 0.001$). No significant difference was found between the two groups treated with vitamin C ($p = 0.227$).

The degree of healing assessed by HE was higher in the groups treated with vitamin C, but without significant difference between oral and subcutaneous.

The amount of type-III collagen was higher in the groups that received vitamin C, with a significantly greater difference in the group that received it subcutaneously.

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Conflict of Interests
The authors have no conflict of interests to declare.

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