

***Pfaffia Glomerata* IN THE HEALING PROCESS OF SKIN WOUNDS**

A utilização da Pfaffia glomerata no processo de cicatrização de feridas da pele

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ABSTRACT - Background - When tissue loss occurs in the skin comes the need for reconstruction and many devices and medication are proposed to increase the healing process. The use of herbal medicine, such as *Pfaffia glomerata*, aims to play an active role in the scar formation. **Aim** - To evaluate the results of the use of *Pfaffia glomerata* on healing process of surgical wounds in rats. **Methods** - A total of 40 rats, which were held surgical wounds with a punch of 3 mm in diameter at the upper right back where no substance was applied and another in the lower region, where the herbal extract was used. They were divided into four subgroups of 48 hours, one, two and three weeks in relation to sacrifice. Measures were taken on the circumference to analyze the macroscopic contraction of the wound. Microscopic data were analyzed using the hematoxylin-eosin staining to verify the inflammatory process; immunohistochemistry, factor VIII, to observe the vascular density, and Masson trichrome to study fibrosis. **Results** - Macroscopic plant group achieved better results than the control group. The analysis of factor VIII showed statistical significance in a week of herbal medicine. In fibrosis, it was found that during the 48 hours the control group showed 70% of cases with minimal fibrosis, whereas the plant, 90%. In one week, the control group showed 10% of cases with no fibrosis, 60% with minimal fibrosis and 30% with moderate fibrosis, while the group plant showed 70% of cases with minimal fibrosis and 30% with moderate fibrosis. In the period of two weeks, the control group maintained 60% of cases with minimal fibrosis and increased to 40% of those with moderate fibrosis, while the plant maintained its group average presented in the previous group. In three weeks both groups, control and plant, kept the percentages presented in the previous period. **Conclusion** - In relation to wound contraction, plant group showed better results than the control group. In regard to histological analysis, HE staining showed a greater presence of inflammation in the animals of control groups. The immunohistochemical technique showed a vascular density, higher in the plant during the period of one week and Masson's trichrome staining showed no significant changes

HEADINGS - Wound healing. Rats. Phytotherapy.

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RESUMO - Racional - Quando ocorre perda tecidual na pele surge a necessidade de reconstituir-se o tecido lesionado e atualmente cada vez mais surgem artificios que se propõem neoformá-lo. O uso de fitoterápicos, como a *Pfaffia glomerata*, tem a finalidade de buscar nestes produtos princípios ativos que desempenhem efetivo papel no processo de cicatrização. **Objetivo** - Avaliar os resultados da utilização da *Pfaffia glomerata* na cicatrização de feridas cirúrgicas em ratos. **Métodos** - Foram utilizados 40 ratos, nos quais realizaram-se feridas cirúrgicas com punch de 3 mm de diâmetro no dorso da parte superior direita onde nenhuma substância foi aplicada e nesses mesmos animais foram também realizadas feridas cirúrgicas na região inferior, onde foi aplicado o extrato do fitoterápico. Foram divididos em quatro subgrupos de 48 horas, uma, duas e três semanas em relação ao sacrifício. Foram tomadas as medidas na circunferência para analisar a contração da ferida macroscopicamente. Microscopicamente os resultados foram analisados utilizando-se a coloração de Hematoxilina-Eosina, para verificar o processo inflamatório; imunohistoquímica, fator VIII, para observar a densidade vascular; e tricrômio de Masson para estudar a fibrose. **Resultados** - Macroscopicamente o grupo planta obteve resultados superiores ao grupo controle. A análise da variável fator VIII mostrou significância estatística no grupo de uma semana do fitoterápico. Na variável fibrose, constatou-se que no período de 48 horas o grupo controle apresentou 70% de casos com fibrose mínima, ao passo que o da planta, 90%. Em uma semana, o grupo controle apresentou 10% de casos com ausência de fibrose, 60% com fibrose mínima e

DESCRITORES - Cicatrização de feridas. Ratos. Fitoterapia

30% com fibrose moderada, enquanto que o grupo planta apresentou 70% de casos com fibrose mínima e 30% com fibrose moderada. Já no período de duas semanas, o grupo controle manteve 60% dos casos com fibrose mínima e aumentou para 40% os com fibrose moderada, enquanto que o grupo planta manteve sua média apresentada no grupo anterior. No período de três semanas ambos os grupos, controle e planta, mantiveram as porcentagens apresentadas no período anterior. **Conclusão** – Macroscopicamente em relação à contração da ferida o grupo planta apresentou resultados superiores ao grupo controle. Com relação à análise histológica: a coloração de HE mostrou que houve a maior presença de processo inflamatório nos animais do grupos controle. A técnica de imunohistoquímica avaliada mostrou densidade vascular superior no grupo planta no período de uma semana sendo que na coloração de tricromio de Masson as alterações estudadas não se apresentaram significativas.

INTRODUCTION

Researchers and scholars have devoted themselves to the ancient understanding of healing that still remains unclear because of the many involved and interacted mechanisms. A small vascular abnormality or inadequate functioning of some cells, can generate significant changes in tissue healing. The healing process begun their study in Antiquity⁷. The earliest records are mentioned on Egyptian manuscripts dating from 3000-2500 BC. These were honey-based dressings, grease, flax and various kinds of droppings, which formed part of the principles of Egyptian pharmacopoeia.

The use of herbal medicines on the healing of surgical wounds has been growing in recent years of our era, with the search for active ingredients that play an effective role in accelerating the tissue recovery process.

There are many articles in the literature studying the action of *P. glomerata* (Spreng.) Pedersen in the healing process. It is a species belonging to the family *Amaranthaceae*, known as fafia, corange-evergreen and Brazilian ginseng⁹. It is found in the Brazilian territory, mainly in São Paulo, Paraná, Mato Grosso and Goiás

Neto AG, et al.⁸ studied the effects of the extract of *Pfaffia glomerata* in different animals showing anti-inflammatory and analgesic effects similar to those they could observe with nonsteroidal drugs, like indomethacin. These authors also stated that this suggests a mechanism of action associated with the inhibition of prostaglandin synthesis, as observed in many non-steroidal drugs, because they also have among its active sitosterol, stigmasterol and allantoin.

Antiedematogenic capability of this plant was also studied by Teixeira CGL, et al.¹¹ in 2006. They confirmed his ability and added that their results showed nitric oxide stimulation by some component present in the plant, activates soluble guanylate cyclase, which plays a role in the mechanism involved in anti-inflammatory effect of hydroalcoholic *Pfaffia glomerata*. This is a characteristic that the authors

considered an important factor to enhance their therapeutic potential.

This study aims to evaluate the results of using *Pfaffia glomerata* on the healing of surgical wounds in rats.

METHOD

This study was conducted at the Institute of Medical Research - Postgraduate Program in Principles of Surgery of the University Evangelical Hospital/Evangelic Faculty of Paraná in Curitiba, PR, Brazil. Was approved by the Ethics Committee of the Evangelical Theological Charity Society of Curitiba.

The hydroalcoholic extract of *Pfaffia glomerata* was obtained in the Laboratory of Pharmacognosy at the Federal University of Paraná, Curitiba, PR, Brazil.

After obtaining the extract, followed the toxicity test to ascertain if the concentration obtained was toxic.

A total of 40 rats, Wistar, male, came from the Technology Institute of Paraná, aged 120 days and average weight of 230 g.

Surgical wounds were performed in all with punch of 3 mm in diameter. On the back of the top of the animal no substance was applied and in the lower region was used to extract *Pfaffia glomerata*. So the leak do not alter, the result of the control upward wound. Thus, the control and the plant group were made in the same animals

Surgical procedure

The animals were weighed and underwent general anesthesia with intramuscular injection of ketamine (Ketalar[®] Ache) at a dose of 50 mg/kg and xylazine (2% Virbaxyl[®] Virbac) at a dose of 10 mg/kg. The mice remained in deep anesthesia until the end of operation and then positioned prone for shaving the dorsal region, made with stainless steel blade and liquid soap. Was prepared a model of transparency sheet with 8 mm wide at its center and a circumference of 3 mm that coincided with the size of the lesion; the demarcations were done with black pen in the skin. Antisepsis with an alcoholic

solution of 2% chlorhexidine was done prior to the surgical wound.

After surgery, the animals were placed in their respective boxes, room temperature, with light-dark cycle and free access to drinking water and commercial standard diet (Nuvilab Nuvital®). All cages were cleaned, and food and water changed daily. The animals received no postoperative dressings and remained under this regime until the date set for the measurements of the experiment.

According to the schedule, the rats were killed by lethal inhalation of sulfuric ether in 48 hours one week, two weeks and three weeks after surgery.

Once dead, they were placed in prone position and measured the extent of the central diameter of the wound. Later with the scalpel, the skin was incised in the demarcation area put in 10% formalin and sent to histological analysis.

Macroscopic and microscopic evaluation

Macroscopically, were taken measures in the center of the wound to analyse the wound contraction and recorded in a table for further evaluation.

Microscopic, data were analyzed using the hematoxylin-eosin staining to verify the inflammatory process; immunohistochemistry, factor VIII, to observe the vascular density, and Masson trichrome to study fibrosis.

Statistical analysis

The data, measured in millimeters, were organized in Excel spreadsheet and analyzed using the software Statistica, version 8.0. When comparing the groups, was adopted the nonparametric Wilcoxon test depending on the pairing. P values <0.05 indicated statistical significance.

TABLE 1 - Comparison of subgroups within the control group and p-values

Subgroup	n	Average	Median	Minimum	Maximum	Standart deviation
48 hours	10	2,9	3	2,5	3	0,2
1 week	10	2,6	3	2	3	0,5
2 weeks	10	2,1	2	2	3	0,3
3 weeks	10	2	2	2	2	0
Comparison made				p value		
48 hours x 1 week				0,116		
1 week x 2 weeks				0,043		
2 weeks x 3 weeks				---		

RESULTS

Macroscopic evaluation

Control Group

As shown in Table 1, there was no statistical

difference between subgroups in the control group and p values.

Plant Group

As shown in Table 2, the lowest average of this variable occurred in the subgroup of three weeks, but the difference was not statistically significant.

TABELA 2 - Comparison of subgroups within the group plan and p-values

Subgroup	n	Average	Median	Minimum	Maximum	Standart deviation
48 hours	10	2,7	2,75	2	3	0,3
1 week	10	2,4	2,25	2	3	0,4
2 weeks	10	2,2	2	2	3	0,4
3 weeks	10	1,8	2	1	2	0,4
Comparison made				p value		
48 hours x 1 week				0,050		
1 week x 2 weeks				0,418		
2 weeks x 3 weeks				0,109		

Comparison between groups

The lowest mean in control group was in three weeks time, whose value presented 2.0; in plant group, the lowest average value was 1.8 in the same time with no statistical significant difference.

Microscopic evaluation

Evaluation of the inflammatory process by HE staining

As can be seen in Table 3, groups had a greater number of variables with histological changes in the 48 hours subgroup. In subgroup one week, the group plant had the greatest number of cases with variation. In subgroup two weeks this relationship was different. In relationship to subgroup three weeks, the control group had one case with granulomas and an ulcerated epithelium with polymorphonuclear cells, whereas the group plan showed only one case with granulomas.

Immunohistochemical evaluation of factor VIII

Comparison of subgroups within each group

Control Group

As can be seen in Table 4, the difference was statistically significant when comparing the subgroups with 48 hours vs 1 week (p=0.011) and with 2 weeks (p=0.035).

Plant Group

In this group the difference was statistically significant when comparing the subgroups with 1 and 2 weeks, with p value less than 0.001 (Table 5).

Comparison of groups within the subgroups

The control group had the highest average time in 1 week (value=12.9). The group plan, also presented its highest average in 1 week's time with

TABLE 3 - Overview of changes observed by HE staining in control and plan groups

Control Group	48 hours	week		
		1	2	3
Edema	3 cases			
Presence of polymorphonuclear cells		1 case		
Edema and polymorphonuclear cells	1 case			
Edema, polymorphonuclear cells, abscesses and granulomas	1 case			
Granulomas				1 case
Granuloma due to focal foreign body reaction		1 case	1 case	
Shallow ulcer	1 case			
Deep ulcer	4 cases			
Giant cell reaction		2 cases		
Epithelium reepithelization			2 cases	
Ulcerated epithelium with polymorphonuclear cells and giant cell reaction			1 case	1 case
Plant Group	48 hours	week		
		1	2	3
Edema	4 (2 cases mild and 2 moderate)			
Presence of polymorphonuclear cells		1 case		
Edema and polymorphonuclear cells				
Edema, polymorphonuclear cells, abscesses and granulomas				
Granulomas	1 case	2 cases (1 with only one granuloma)	1 case	
Shallow ulcer	1 case			
Deep ulcer	4 cases			
Granulomas and polymorphonuclear cells				1 case
Ulcerated		1 case		
Ulcerated epithelium with presence of polymorphonuclear cells		2 cases		
Ulcerated epithelium with polymorphonuclear cells and giant cell reaction		2 cases		

TABLE 4 - Comparison of subgroups within the control group and p-values

Subgroup	n	Average	Median	Minimum	Maximum	Standart deviation
48 hours	10	9,9	9,5	7	17	2,88
1 week	10	12,9	11	10	17	2,81
2 weeks	10	9,5	8	7	17	3,31
3 weeks	10	8,9	7	5,5	15	3,13
Comparison made		p value				
48 hours x 1 week		0,011				
1 week x 2 weeks		0,035				
2 weeks x 3 weeks		0,631				

a value of 14.1, but with no significant difference (Table 6).

Evaluation of fibrosis

The analysis consisted of fibrosis microscopic

TABLE 5 - Comparison of subgroups within the group plan and p-values

Subgroup	n	Average	Median	Minimum	Maximum	Standart deviation
48 hours	10	11,8	13	8	14	2
1 week	10	14,1	13	11	19	3,11
2 weeks	10	9,4	9	7	13	1,78
3 weeks	10	9	9	7	15	2,39
Comparison made		p value				
48 hours x 1 week		0,247				
1 week x 2 weeks		< 0,001				
2 weeks x 3 weeks		0,436				

TABLE 6 - P values in comparisons between subgroups of groups

Subgroups	Plant X Control
48 hours	0,093
1 week	0,374
2 weeks	0,726
3 weeks	0,933

observation of greater or lesser presence of collagen fibers compared to normal tissue and, based on it, was found that the subgroup 48 hours, control group, showed 70% of cases with minimal fibrosis, whereas the group plan, 90%. In subgroup 1 week, the control group showed 10% of cases with no fibrosis, 60% with minimal fibrosis and 30% with moderate fibrosis, while the group plant showed 70% of cases with minimal fibrosis. In subgroup 2 weeks, the control group maintained 60% of cases with minimal fibrosis and increased to 40% the number of cases with moderate fibrosis, while the plant maintained its group average presented in the previous subgroup. And finally in subgroup 3 weeks, plant and control groups maintained their percentages presented in the previous subgroup.

DISCUSSION

The animal chosen, the Wistar rat^{2,3,4,5,10} is of simple acquisition in our country, they do not occupy much space, are easy to experimental manipulation, great resistance to infections and their anatomy is known. Furthermore, it allows standardization with regard to age, weight, gender and power, but his skin has an important difference in relation to human skin, which is the absence of defined boundaries between papillary and reticular dermis, which can distort results of experimental studies.

The punch surgical technique is precise, easily reproduced and standardized⁵.

The macroscopic evaluation which included the verification of wound contraction with reference to the measures marked on the day of surgery, corroborates the study done by Araujo LRR³. This

check was necessary, since the process of wound contraction is the fourth phase of the healing process and is the centripetal movement of the edges⁷. The fifth stage prior to wound contraction is the proliferation, responsible for "closing" of lesion itself and is divided into three sub-phases, which are the reepithelization, fibroplasy and angiogenesis. Finally, follows the remodeling phase of wound contraction and is the ultimate healing.

Histological evaluation with HE staining technique was used for assessment of the inflammatory process. Is regarded as the primary means of analysis when the object of study is the epithelial tissue. Through it can share basophilic differentiation, using hematoxylin and eosin acidophilus. This is because of the hematoxylin has attraction to basic components of the tissues, protein-rich amine radicals and nuclei, as well as rough endoplasmic reticulum. Eosin, in its turn, being acidophilus, bluish with acidic substances⁴. This study found a case with these cells within 1 week, both in control and plant group.

Another important finding was the presence of macrophages, responsible for the giant cell reaction⁷. Lymphocytes, by its lymphokines, have great influence on macrophages. These cells appear in the wound in about a week, and within this period, in this study, was found a case presenting this event in the control group and also a case in this same group in 2 weeks. In the group plan, there were two cases in subgroup 1 week, including the involvement of the epithelium that became ulcerated. There were two cases in the period of 2 weeks in the control group. However, in the group plant this property was not found during the time assessed.

Another technique used to evaluate the survey was immunohistochemistry, the antibody used for verification of neovascularization was the von Willebrand factor or factor VIII, which is a glycoprotein produced only by endothelial cells and megakaryocytes and, therefore, is routinely used to identify vessels in tissue sections. RFL Xavier¹², says this is a monoclonal antibody capable of reacting with endothelial cells displaying reactivity in granular pattern at the cytoplasm of positively stained cells.

Angiogenesis, essential for the supply of oxygen and nutrients for healing, had better outcome in the plant group in 1 week.

To verify collagen fibers formation, staining recommended was Masson's. Granulation tissue depends on the fibroblast cell, critical in the formation of the matrix. Far more than just collagen producer, the fibroblasts make elastin, fibronectin, glycosaminoglycan and proteases, responsible for the physiological remodeling and debridement. Within this analysis, the groups showed no significant differences between them.

CONCLUSION

Regarding to wound contraction, the group plant showed better results than the control. In relation to histology, inflammation was present in all groups, and, at the end, only the control group showed a greater number of inflammatory cases. With regard to vascular density, it was higher in the group plan in 1 week. Fibrosis did not differ between groups.

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