DOI: 10.53660/CLM-1467-23G22C

T. Catappa confers an antioxidant effect to electrospun polyvinyl alcohol nanofibers.

T. Catappa confere um efeito antioxidante às nanofibras de álcool polivinílico eletrofiadas

Received: 2023-05-03 | Accepted: 2023-06-10 | Published: 2023-06-15

André Giarola Boscarato
ORCID: https://orcid.org/0000-0003-2281-8404
Universidade Paranaense - UNIPAR, Brasil
E-mail: andreboscarato@prof.unipar.br
Filipe Correa Pacheco
ORCID: https://orcid.org/0000-0002-1726-004X
Centro universitário Integrado, Brasil
E-mail: filipecorrea.vet@gmail.com
Douglas Cardoso Dragunski
ORCID: https://orcid.org/0000-0001-7870-097X
Universidade Estadual do Oeste do Paraná – UNIOESTE, Brasil
E-mail: dcdragunski@gmail.com
Guilherme Donadel
ORCID: https://orcid.org/0000-0001-7485-8016
Universidade Paranaense - UNIPAR, Brasil
E-mail: donadel425@gmail.com
Emerson Luiz Botelho Lourenço
ORCID: https://orcid.org/0000-0002-1798-7871
Universidade Paranaense - UNIPAR, Brasil
E-mail: emerson@prof.unipar.br
Ricardo de Melo Germano
ORCID: https://orcid.org/0000-0002-5925-1408
Universidade Paranaense - UNIPAR, Brasil
E-mail: germano@prof.unipar.br
Salviano Tramontini Bellettini
ORCID: https://orcid.org/0000-0002-0600-5836
Universidade Paranaense - UNIPAR, Brasil
E-mail: salviano@prof.unipar.br
Luiz Romulo Alberton
ORCID: https://orcid.org/0000-0002-5912-0467
Universidade Paranaense - UNIPAR, Brasil
E-mail: lralberton@gmail.com

ABSTRACT

In this study, the healing process of full-thickness skin wounds induced in Wistar rats (n = 75) was evaluated, divided into 5 treatments: negative control, PVA nanofiber film, and PVA nanofiber film added in concentrations of 3%, 6% and 10% with lyophilized extract of *T. catappa*. After euthanasia of 5 animals from each of the groups, at times 7, 14 and 21 days, samples were evaluated for the presence of oxidative stress markers reduced Glutathione (GSH) and levels of lipoperoxidation (LPO), and for the histological characteristics of tissues stained with hematoxylin-eosin and azan. The results showed that, in addition to

remaining on the surface of the wounds for an acceptable period, the nanofiber membrane was able to deliver the extract of *T. catappa* to the bed of the treated wounds and, despite not producing statistically relevant histological changes, it was observed significant antioxidant effect by reducing LPO and increasing GSH in a dose dependent manner. Thus, it is concluded that the PVA nanofiber film associated with the *T. catappa* extract has an antioxidant effect and can assist in wound healing.

Keywords: Antioxidant; Electrospinning; Healing; Medicinal plants; Skin.

RESUMO

Neste estudo, foi avaliado o processo de cicatrização de feridas cutâneas de espessura total induzidas em ratos Wistar (n = 75), divididas em 5 tratamentos: controle negativo, filme de nanofibra PVA e filme de nanofibra PVA adicionado nas concentrações de 3%, 6% e 10% com extrato liofilizado de *T. catappa*. Após a eutanásia de 5 animais de cada um dos grupos, nos tempos 7, 14 e 21 dias, as amostras foram avaliadas quanto aos marcadores de estresse oxidativo Glutationa reduzida (GSH) e níveis de lipoperoxidação (LPO), e quanto às características histológicas dos tecidos corado com hematoxilina-eosina e azan. Os resultados mostraram que, além de permanecer na superfície das feridas, a membrana de nanofibras foi capaz de entregar o extrato de *T. catappa* ao leito das feridas tratadas e, apesar de não produzir alterações histológicas estatisticamente relevantes, observou-se efeito antioxidante significativo ao reduzir o LPO e aumentar o GSH de maneira dose-dependente. Assim, conclui-se que o filme de nanofibras de PVA associado ao extrato de *T. catappa* possui efeito antioxidante e pode auxiliar na cicatrização de feridas.

Palavras-chave: Antioxidante; Eletrofiação; Cicatrizante; Plantas medicinais; Pele.

INTRODUCTION

The skin is a metabolically active organ, responsible for maintaining homeostasis, acting as an anatomical and physiological barrier between the organism and the environment (JORGENSEN *et al*., 2023). The interruption of the continuity of its physical barrier corresponds to a wound, which can be caused by external physical, chemical, thermal and biological factors (WILKINSON; HARDMAN, 2020). Skin tissue injury triggers a dynamic and complex process of distinct overlapping phases that is characterized by an initial vascular response, followed by the inflammatory phase (HOLLOWAY; HARDING, 2022), which in turn triggers the proliferative phase of tissue repair (MONIKA *et al*., 2021), and through angiogenesis, fibroplasia and re-epithelialization (GONZALEZ *et al*., 2016), it restores the integrity, strength and function of the skin (HARMAN; THEORET; VAN DE WALLE, 2021).

Films produced by electrospinning have been used as drug delivery devices for antimicrobial (SCHNEIDER *et al*.,2018) anti-inflammatory (GHOSAL *et al*., 2021), cells and growth factors (XUE *et al*., 2017; DOOSTMOHAMMADI; FOROOTANFAR; RAMAKRISHNA, 2020; STREETER *et al*., 2019), and compounds derived from medicinal plants (HADIZADEH *et al*., 2021), in target tissues such as the skin, seeking to accelerate the healing process (SHAHID *et al*.,2021; MOURO; FANGUEIRO; GOUVEIA, 2020). Polyvinyl alcohol (PVA) is a synthetic, biocompatible, non-toxic and hydrophilic polymer, used worldwide

in diverse biomedical applications (XIE *et al*., 2020), previously used in devices for the controlled release of bioactive substances derived from medicinal plants, with promising results in the healing of skin wounds (MOURO; FANGUEIRO; GOUVEIA, 2020; MAHMUD *et al*., 2020).

Terminalia catappa, popularly known as Indian almond, is one of 200 species of a total of 18 genera that comprise the family Combretaceae (MOGASHOA; MASOKO; ELOFF, 2019). Native to Southeast Asia (NUGROHO *et al*., 2019), it was introduced and naturalized worldwide by tropical and subtropical regions (RAVI, 2020). Hydrolysable tannins are the major compounds responsible for the therapeutic activities of this species; as a broad-spectrum antimicrobial effect (YADAV *et al*., 2021; EL-RAFIE; EL-RAFIE; ZAHRAN, 2017; DARMAWATI *et al*.,2022; BOSCARATO *et al*.,2021), antifungal (TERÇAS *et al*., 2017), antioxidant (YADAV *et al*., 2021; IHEAGWAM *et al*., 2022; DIVYA *et al*., 2019), antinociceptive and anti-inflammatory (RAVI, 2019) that arouse interest in the potential benefit to skin healing.

In this study, the objective was to evaluate the histological and oxidative stress parameters in different phases of the healing process of induced cutaneous wounds in Wistar rats, treated with functional films produced from the electrospinning of PVA solutions added with different concentrations of the lyophilized crude extract of the *T. catappa* leaves.

MATERIAL AND METHODS

Extract preparation

Mature green leaves were collected from adult trees of Terminalia catappa existing on campus II of Universidade Paranaense (UNIPAR; Umuarama, Parana, Brazil), at an elevation of 430m (23°45'52.4"S 53°16'20.5"W), in October 2018. Botanical identification was performed by comparison, and after drying in a forced circulation oven at 37ºC for 5 days, the material was pulverized and stored in paper bags. The hydroethanolic extract of *T. catappa* (EHTC) was prepared by macerating the plant material at room temperature for seven days, using 70% ethanol in a 9:1 ratio (Ethanol: pulverized leaves). Then it was filtered and concentrated at reduced pressure in a rotoevaporator, at a temperature not higher than 55ºC, being later lyophilized, obtaining 272g of crude extract (4.5% yield).

Production of nanofiber membranes of PVA/T. Catappa

The polymeric membranes were produced according to the methodology described by Boscarato et al. 2021. Initially, 8% (m/v) solutions of polyvinyl alcohol (PVA) (80,000 to 110,000g/mol and 98% hydrolysis - Sigma Aldrich®) were produced in distilled water and ethanol, at a ratio of 95:5 v/v, respectively. Initially, the PVA was dissolved in water under stirring in a water bath at a controlled temperature between 80 and 93°C. The 8% PVA viscoelastic solution obtained was directly subjected to the electrospinning process. After dissolving the

lyophilized extract of *T. catappa* in ethanol, the PVA solutions were added in proportions of 3%, 6% and 10% m/m (extract/polymer) respectively and homogenized in ultrasound for 15 minutes. After complete dissolution, each polymeric solution was placed in a 10mL syringe with a 10mm diameter plunger and subjected to the electrospinning process. A 25x0.8mm needle was used as an injection nozzle to promote the capillary action of the system. After placing the set in the infusion pump of the system, the connectors of the positive and negative poles of the voltage generator were positioned at the tip of the capillary needle, and to the collector target and grounded cable, respectively. The collecting apparatus was properly positioned at a distance of 15 cm. For the solution containing 3% of extract, a voltage of 15 kV was used, flow of 0.50 mL/h, while for the solutions with 6% and 10% of extract, the voltage was 19 kV and the flow of 0. 20 ml/h. After the end of the process, the polymeric films obtained were carefully removed from the collecting surface and stored in parchment paper envelopes protected from light and moisture. The geometric structure of the fibers created and their uniformity were observed by means of scanning electron microscopy (SEM). The samples were placed in double-sided carbon adhesive tape, which was fixed on a support. Subsequently, they were metallized with gold to a thickness of approximately 5 nm using a Denton Vacuum system for deposition of thin films and analyzed in a Tescan® Scanning Electron Microscope (Vega3).

Animals

Seventy-five male Wistar rats (Rattus novergicus) were used, with 80 days of age and average weight of 345g. The cages were kept in an environment with a controlled temperature of 22ºC ±2, alternating 12 hours of light-dark cycle and exhaust system. For adaptation purposes, the rats were given water and food (Purina Labina for rodents; Nestlé Purina Company) ad libitum for a period of seven days, housed in collective polypropylene cages with 5 animals per cage. Two days before the start of the experiment, the animals were housed individually. All procedures adopted in this experiment were approved by the Institutional Committee on Ethics in the Use of Animals (CEUA) of Universidade Paranaense, protocol No. 35559/2019, according to the rules of the National Council for the Control of Animal Experimentation (CONCEA).

Experimental model and treatments

At the beginning of the experiment, the animals were randomly divided into 5 groups composed of 15 individuals each, corresponding to the control group (GC), polymer control group (GCP), polymer group 1 (GP1), polymer group 2 (GP2) and polymer group 3 (GP3). After 12h solid fasting, the animals were weighed and subjected to dissociative general anesthesia with xylazine and ketamine at a dosage determined by interspecific allometric extrapolation using the 10kg domestic dog as a model species (KOPROSKI; PACHALY, 2017). Trichotomy of the dorsal cervicothoracic region was performed and after positioning in the prone position, standard

surgical asepsis was performed with degerming polyvinyl pyrrolidone iodine (PVPI), 70% alcohol and topical PVPI. Then, a full-thickness circular lesion was produced on the skin using a dermatological punch measuring 8 mm in diameter, located in the midline and immediately cranial to the dorsal edge of the scapulae. After brief hemostasis by compression of sterile gauze, the treatment of each respective group was applied. The lesions in the GC group did not receive any type of dressing or intervention during the experimental period. The GCP group received the polyvinyl alcohol nanopolymer (PVA) and the GP1, GP2 and GP3 groups received the PVA nanopolymer associated with the *T. catappa* extract at concentrations of 3%, 6% and 10% (m:m), respectively. After the surgery, the animals received analgesia with tramadol hydrochloride and the anesthetic plan was reversed with yohimbine, both intramuscularly, at a dosage also determined by allometric extrapolation. A daily assessment of the animals was instituted according to the Grimace scale for a period of 72 hours after induction of the wounds and, when necessary, therapeutic rescue was carried out with the same analgesic drug, as recommended by the National Council for the Control of Animal Experimentation.

Euthanasia and sample collection

Sample collection was performed on days 7, 14 and 21 after induction of surgical wounds. At each of the three experimental times, 5 animals from each of the 5 groups were euthanized. Initially, sedation was performed with 1% acepromazine intramuscularly, at a dose determined by allometry. Immediately thereafter, euthanasia was performed by deepening the anesthetic plane with isoflurane in a saturation chamber. A full-thickness quadrangular skin fragment was excised from each animal, involving the initial lesion. This fragment was sectioned into two isometric parts, one for cryopreservation in liquid nitrogen, for analysis of oxidative markers, and another for fixation in 10% buffered formalin for 72 hours, later replaced by 70% alcohol for histological preparations.

Histological preparation of samples

The skin fragments already fixed and preserved were submitted to standard histological processing and, after the dehydration and diaphanization steps, the samples were embedded in paraffin blocks to obtain histological sections with 5 µm thickness. From each block corresponding to a sample, 2 slides were mounted, submitted to hematoxylin and eosin (HE) staining for qualitative morphological evaluation, and trichomic azan staining, to evaluate collagenization, which was classified in grades I (mild) II (moderate), III (intense) and IV (intense in remodeling).

Analysis of markers of oxidative injury in wounds

Wound samples were homogenized in potassium phosphate buffer (pH 6.5) at a 1:10 dilution (tissue: buffer) and centrifuged at 13,000 rpm for 20 minutes. Homogenization was carried out on ice and centrifugation was refrigerated at 4°C. From the supernatant, the levels of reduced glutathione (GSH) were analyzed based on the technique of Sedlak and Lindsay, (1968). and the levels of lipid peroxidation (LPO) by the FOX2 method, improved from the description by Jiang *et al*.1991.

Statistical analysis

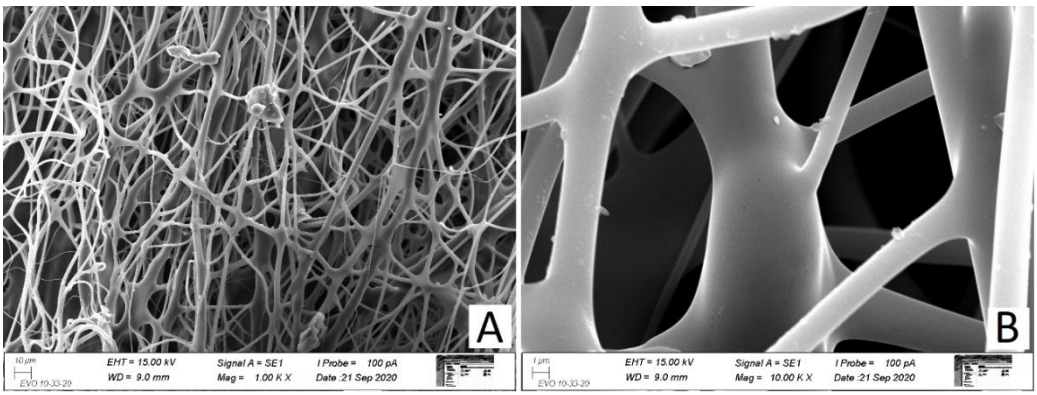
Descriptive statistics, generalized linear model (GLM) and one-way ANOVA were used, followed by the Newman-Keuls test with a significance level of 95% ($p < 0.05$), using the Graphpad Prism® version 5.0.

RESULTS AND DISCUSSION

In this experiment, full-thickness excisional wounds involving the epidermis, dermis, and subcutaneous tissue were created with the aid of an 8mm-diameter dermatological punch, a methodology often used in excisional models of healing by second intention (CHINAKA; EZEALISIJI; AKPOFURE, 2018; TABAKOGLU *et al*., 2016; FEKRAZAD; SOHRABI; FEKRAZAD, 2023; CHOI *et al*., 2021; HE *et al*., 2020). The PVA and PVA/Extract films produced by the electrospinning method showed good mechanical resistance to manipulation and excellent interaction with the surface of surgical wounds, making the use of auxiliary methods unnecessary for fixation on the wounds.

This characteristic can be attributed to the hygroscopic properties of PVA (MAHMUD *et al*., 2020) and also to the three-dimensional structure of the polymeric film which, when observed by SEM, showed uniform fibers with diameters below 1000nm (Fig. 1).

Figure 1- Scanning electron microscopy (SEM) at different magnifications. In A and B, electrospun PVA nanofibers at 1000x and 10000x magnification, respectively.

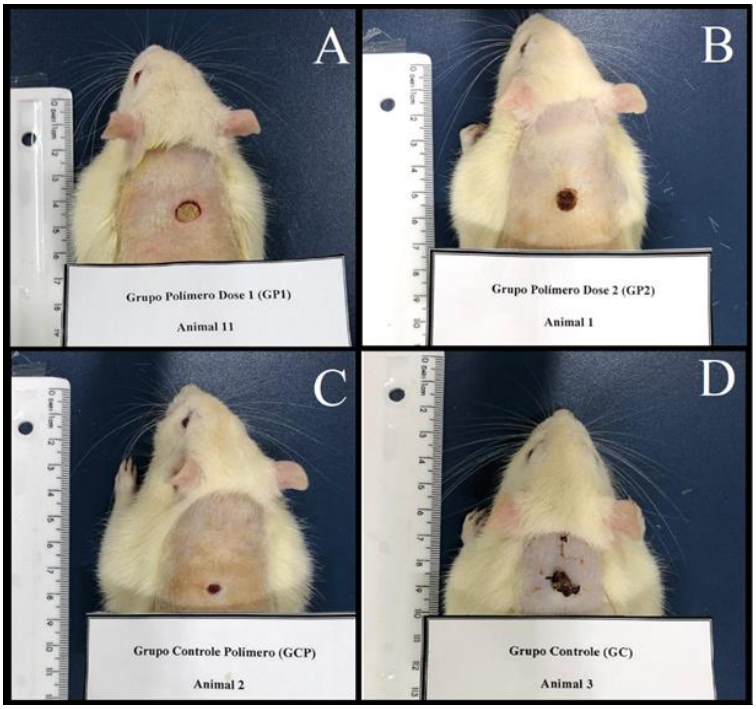


Source: Elaboration of the authors (2023).

This structural aspect makes the material more hygroscopic as it has a high surface/volume ratio and provides good interaction with moist surfaces as found in wounds (Fig. 2A). This interaction with the aqueous medium is necessary for the release of substances from the polymer matrix to occur. In previous studies, it was observed that PVA nanofibers were able to release the *T. catappa* extract from electrospun PVA polymers in a pH 5.8 buffer solution (BOSCARATO *et al*., 2021).

In the macroscopic evaluation, the treated groups did not present bleeding or crust formation (Fig. 2B), while in the control group, the formation of fibrino-hemorrhagic exudate crusts was observed (Fig 2D). Considering only the animals of the challenged groups that received polymeric film on the wounds (GCP, GP1, GP2 and GP3), there was a spontaneous fall of this in only 6.6% of the animals (4/60) until the 3rd day; 20% of the animals (12/60) up to the 7th day; 92.5% of the animals (37/40) up to the 10th day and in 100% of the animals (40/40) up to the 14th day. Thus, it is observed that most of the animals remained with the film until day 7 of the postoperative period, and that the spontaneous loss of the film occurred between days 7 and 10 (Fig.2C).

Figure 2- Macroscopic appearance of full-thickness skin wounds surgically induced with a 0.8mm punch in Wistar rats. A- PVA nanofiber membrane covering the wound in the immediate postoperative period. B- PVA nanofiber membrane covering the wound on the 7th postoperative day; C- Wound on the 7th postoperative day, after spontaneous fall of the PVA membrane. D- Control treatment wound (GC) with hemorrhagic crust observed on the seventh postoperative day.



Source: Elaboration of the authors (2023).

This feature has an advantage, as it allows a reduction in the frequency of dressing changes during wound care, decreasing pain (HE *et al*., 2020) and making it less traumatic and costly (YAZDANBAKHSH *et al*., 2018). In addition, this period coincides with the inflammatory phase, which begins after hemostasis and guarantees infiltration of neutrophils into the wound, predominant in the first 48 hours and which can last up to 7 days (WILKINSON; HARDMAN, 2020; BLAIR *et al*.,2020).

Thus, the nanofiber membrane can act in potential synergism with this healing phase, whose key objective is to prevent infection (HOLLOWAY; HARDING, 2022), both due to the antimicrobial effect of the *T. catappa* extract (DARMAWATI *et al*.,2022; BOSCARATO *et al*., 2021) and the formation of a physical barrier against microorganisms (MOURO; FANGUEIRO; GOUVEIA, 2020). However, none of the wounds among all groups showed macroscopic signs of infection, and no differences were observed between treatments in this regard. It is likely that the antimicrobial activity in vivo is better evidenced in wounds subjected to a greater microbiological challenge, as in models of chronic wounds (CHEN *et al*., 2021) or even, as proposed by Fathi *et al*. (2020), who induced methicillin-resistant S. aureus infection in skin lesions in rats, prior to treatment with a nanofiber membrane containing an antimicrobial.

At 14 days, no differences were observed in the evolution of the healing process between the groups, and the wounds still had a small unepithelialized central area. In the last experimental time, at 21 days, the epithelialization of the wounds was complete in the animals of all groups. However, the wounds that received the polymeric membrane, with or without extract, presented scars that were more uniform, flat and with a better-defined circular shape, coinciding with the initial shape of the lesion and the nanofiber film used as a dressing, while in the control group, the scars tended to a contour with irregular edges (Fig. 3). This characteristic corroborates with what was described by XIE *et al*. (2020), who in their results showed that polycaprolactone/gelatin nanofibers were able to decrease the synthesis and deposition of collagen type I/III and TGF-β1 and attenuate the formation of fibrosis in wounds, which provides better aesthetic conformation to the newly formed tissue.

Figure 3 – Macroscopic appearance of the lesions in the last sampling time at 21 days. There was complete re-epithelialization of full-thickness skin wounds induced in Wistar rats for all groups studied. A- Animal from the GCP group, uniform, flat scar with a better-defined circular shape, coincides with the initial shape of the lesion and the nanofiber film used as a dressing. B – Animal from the GC group, presence of tissue retraction and scar with irregular edges.

Source: Elaboration of the authors (2023).

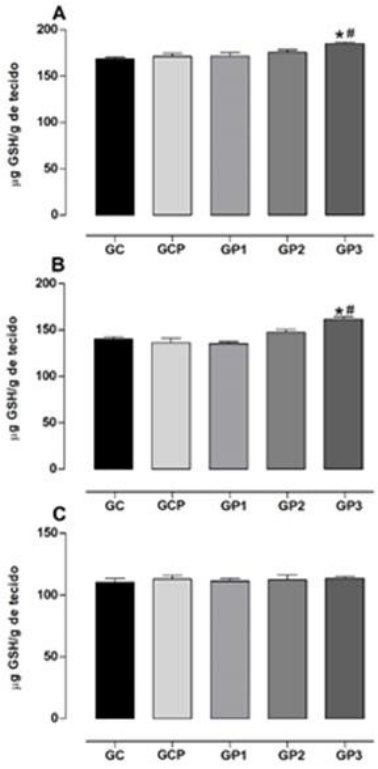
To determine the microscopic characteristics, qualitative analysis was performed on samples stained with azan regarding the degree of collagenization, and samples stained with hematoxylin-eosin (H.E.) regarding the presence of blood vessels in the healing area, as well as presence and characteristics re-epithelialization, presence of fibroblasts and mono- and polymorphonuclear cells. It was not possible to demonstrate in this study significant differences ($p>0.05$) between these parameters during the evolution of the healing process. The histological findings allowed the characterization of the different phases of the healing process at 7, 14 and 21 days, and are in agreement with the characterization described by Levenson *et al*. (1964) for wounds treated by second intention in rats.

Collagen synthesis, deposition and degradation are important events that occur throughout the skin repair process. This phase indicates the completion of tissue repair, and is characterized by a decrease in cell population and an increase in the organization of collagen in the granulation tissue that forms the scar (KOSYKH *et al*.,2023). The histological study of azan-stained wounds allowed us to observe the different phases of this aspect in the healing process. It was observed that throughout the experimental period, there was an increasing deposition and organization of collagen fibers, characterizing the three sampling periods. Although Mouro; Fangueiro; Gouveia, (2020) described better fibroplasia and consequent collagen deposition when using nanofiber membrane in dermal wounds, in this study there was no statistically significant difference ($p>0.05$) between the groups.

Nanofiber-based dressings have the ability to simulate the structural conformation of the extracellular matrix of the skin and, this characteristic is one of the responsible for improving cell migration and contribute to the healing of skin wounds. (HE *et al*., 2020). The parameters evaluated in this study during the evolution of tissue repair were not able to demonstrate significant differences that can be attributed to the morpho structural characteristics of the electrospun membranes. However, as reviewed by Zhang *et al*. (2021) and supporting the behavior previously evaluated in vitro (BOSCARATO *et al*., 2021), PVA nanofibers were able to carry and release the *T. catappa* extract into the wound bed in groups GP1, GP2 and GP3.

This behavior can be verified from the levels of glutathione (GSH) and lipid peroxidation (LPO), used in this experiment as tissue markers of oxidative stress. Figure 4 shows the levels of GSH in the wounds of the animals submitted to the different treatments. After 7 days of wound induction, an increase in tissue levels was observed in GP3 animals (185.0 ± 1.63 µg GSH/g of tissue) compared to the group of animals in the group that received only vehicle (168.7 ± 1.81 µg GSH/g tissue). The same increase in GSH was observed at time 14, with tissue GSH levels statistically higher in the GP3 group (161.7 ± 2.8 µg GSH/g tissue) compared to the GC group (140.3 ± 2.41 µg GSH/g tissue). After 21 days of wound induction, no statistically significant differences were observed in GSH levels between groups. The isolated use of the polymer (GCP) in wounds was not able to increase GSH levels at any experimental time.

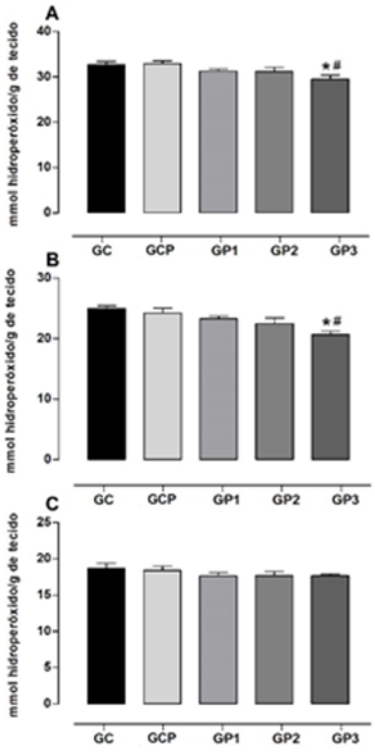
Figure 4 - Tissue levels of glutathione (GSH) at different experimental times. (A) 7 days; (B) 14 days and (C) 21 days after performing the excisional wound in Wistar rats. GC: negative control, without dressing; GCP: animals received polyvinyl alcohol nanopolymer (PVA) as a dressing; GP1: animals received PVA nanopolymer associated with 3% *T. catappa* extract; GP2: animals received PVA nanopolymer associated with 6% *T. catappa* extract; GP3: animals received PVA nanopolymer associated with 10% *T. catappa* extract. Data were statistically analyzed by one-way ANOVA, followed by the Newman-Keuls post-test. Values are expressed as mean ± E.P.M. *p<0.05 versus GC; #p<0.05 versus GCP.



Source: Elaboration of the authors (2023).

Regarding lipid peroxidation levels (Figure 5), 7 days after wound induction, a decrease in lipid peroxidation levels by 18% was observed in GP3 animals compared to GC group animals (32.69 ± 0.66 mmol hydroperoxide/g of fabric). At 14 days, the higher concentration of *T. catappa* associated with the polymer was also able to decrease tissue levels of LPO compared to the GC group (25.03 ± 0.41 mmol hydroperoxide/g tissue). After 21 days of wound induction, no statistically significant differences were observed in LPO levels between groups. The use of polymer alone (GCP) in wounds was not able to decrease LPO levels at any experimental time.

Figure 5 - Tissue levels of lipid peroxidation (LPO) at different experimental times. (A) 7 days; (B) 14 days and (C) 21 days after performing the excisional wound in Wistar rats. GC: negative control, without dressing; GCP: animals received polyvinyl alcohol nanopolymer (PVA) as a dressing; GP1: animals received PVA nanopolymer associated with 3% T. catappa extract; GP2: animals received PVA nanopolymer associated with 6% T. catappa extract; GP3: animals received PVA nanopolymer associated with 10% T. catappa extract. Data were statistically analyzed by one-way ANOVA, followed by the Newman-Keuls post-test. Values are expressed as mean ± E.P.M. *p<0.05 versus GC; #p<0.05 versus GP.



Source: Elaboration of the authors (2023).

Thus, it is possible to conclude that the association of 10% *T. catappa* with PVA nanopolymer provided the greatest increase in GSH levels, an important endogenous tripeptide, and decreased tissue lipid peroxidation rates, markers of cell membrane damage.

The evaluation of the qualitative chemical profile of the *T. catappa* extract used in this study has shown, among others, the presence of flavonoids such as quercetin, rutin, isoorientin, vitexin and isovitexin, as well as the presence of hydrolysable tannins such as gallic acid and ellagic acid, and also triterpenoid derivatives such as asian and ursolic acid (BOSCARATO *et al*., 2021; TERÇAS *et al*., 2017; VENKATALAKSHMI, P.; BRINDHA,2021; MININEL *et al*., 2014). These flavonoid and non-flavonoid polyphenols, abundant compounds derived from plant secondary metabolism, are related to the ability of medicinal plants to neutralize free radicals

(MOHAMMED *et al*.,2022), being attributed to them anti-inflammatory and antioxidant activities of *T. catappa* (YADAV *et al*., 2021; IHEAGWAM *et al*., 2022).

During the inflammatory phase, neutrophils and cytokines produce oxidizing substances, such as free radicals, for example, which steal electrons from molecules to stabilize their free valence, which can delay tissue repair. In response to the oxidative damage that occurs at the wound site, antioxidant substances can be used to stabilize free radicals, prevent damage to cell membranes, proteins and DNA, restoring the environment necessary for healing. Thus, the use of antioxidants can improve the healing process (KILIÇ; YEŞILOĞLU, 2013) especially in situations where oxidative stress is a limiting factor to the normal healing process, in conditions such as diabetes (BUCH; CHAI; GOLUCH, 2019) and/or infections (CANCHY *et al*.,2023).

Huang *et al*. (2018) demonstrated some antioxidant mechanisms exerted by *T. catappa* extract, such as the ability to neutralize hydroxyl radicals, 1,1-diphenyl-2-picrylhydrazyl, superoxide anion and hydrogen peroxide, in addition to ferrous chelation. It also showed that the extract inhibited the H2O2-induced mitogen-activated protein kinase signaling pathway, resulting in the inhibition of c-Jun, c-Fos, matrix metalloproteinase (MMP) -1, MMP-3, MMP-9, expression of cyclooxygenase-2 and also increased expression of hemeoxygenase-1 inhibited by H2O2. Free radical regulation through neutralization, complexation with transition metals, and inhibition of pro-oxidative enzymes are credited to phenolic compounds (RAJHI *et al*.,2022), present in abundance in this species.

CONCLUSION

The second intention healing model used proved to be adequate and provided sufficient tissue to carry out the proposed studies. The mechanical properties of the films allowed excellent interaction with the bed of experimentally induced excisional wounds, which may translate, in future applications, into a lower need for dressing changes in wounds, reducing tissue trauma and pain. The use of electrospun films associated with *T. catappa* extract at the different proposed concentrations did not produce significant histological changes. However, it was evident the antioxidant activity of the crude extract of *T. catappa* incorporated into the PVA nanofibers at a concentration of 10% (m:m) at 7 and 14 days. Thus, it is concluded that the incorporation of the crude extract of *T. catappa* to PVA in the proportion of 10% confers an antioxidant effect on films produced by electrospinning, with potential application in regenerative medicine for the treatment of wounds under conditions of oxidative stress challenge.

REFERENCES

BLAIR, Michael J. *et al*. Skin structure–function relationships and the wound healing response to intrinsic aging. **Advances in wound care**, v. 9, n. 3, p. 127-143, 2020.

BOSCARATO, Andre. G. *et al.* Phytochemical characterization and antimicrobial activity of terminalia catappa and its inclusion in the preparation of pva electrospun film with potential healing effect. **World Journal of Pharmacy and Pharmaceutical Sciences**. 2021. v.10 n.4. p.1672-1691.

BUCH, Pranali J.; CHAI, Yunrong; GOLUCH, Edgar D. Treating polymicrobial infections in chronic diabetic wounds. **Clinical microbiology reviews**, v. 32, n. 2, p. e00091-18, 2019.

CANCHY, Ludivine *et al.* Wound healing and microbiome, an unexpected relationship. **Journal of the European Academy of Dermatology and Venereology**, v. 37, p. 7-15, 2023.

CHEN, Xiaofeng *et al.* A novel chronic wound biofilm model sustaining coexistence of Pseudomonas aeruginosa and Staphylococcus aureus suitable for testing of antibiofilm effect of antimicrobial solutions and wound dressings. **Wound Repair and Regeneration**, v. 29, n. 5, p. 820-829, 2021.

CHINAKA, Chioma Nnennaya; EZEALISIJI, Kenneth Maduabuchi; AKPOFURE, Rukevwe Elohor. Phyto-chemical Characterization of the Leaf extracts of Terminalia catappa L.(Combretaceae) Using Ultra violet-Visible, Fourier transform infrared and Gas chromatography-Mass Spectroscopic techniques. **Journal of Pharmacognosy and Phytochemistry**, v. 7, n. 1, p. 2017-2023, 2018.

CHOI, Minji *et al.* Fibroblast cell derived extracellular matrix containing electrospun scaffold as a hybrid biomaterial to promote in vitro endothelial cell expansion and functionalization. **Materials Science and Engineering: C**, v. 120, p. 111659, 2021.

DARMAWATI, Sri *et al.* Antibacterial Activity of Ketapang (Terminalia cattapa L.) Leaf Extract Against Staphylococcus aureus and Pseudomonas aeruginosa Isolates of Diabetic Wounds. In: **First International Conference on Medical Technology (ICoMTech 2021)**. Atlantis Press, 2022. p. 93-101.

DIVYA, Natarajan *et al.* Phytotherapeutic efficacy of the medicinal plant Terminalia catappa L. **Saudi journal of biological sciences**, v. 26, n. 5, p. 985-988, 2019.

DOOSTMOHAMMADI, Mohsen; FOROOTANFAR, Hamid; RAMAKRISHNA, Seeram. Regenerative medicine and drug delivery: Progress via electrospun biomaterials. **Materials Science and Engineering: C**, v. 109, p. 110521, 2020.

EL-RAFIE, H. M.; EL-RAFIE, M. H.; ZAHRAN, M. K. Anti-Inflammatory and Antibacterial Activities of Nanosilver-Treated Cotton Fabric Prepared From Ethanolic Extracts of Three Terminalia SpecieS. **Egyptian Journal of Chemistry**, v. 60, n. Conference Issue (The 8th International Conference of The Textile Research Division (ICTRD 2017), National Research Centre, Cairo 12622, Egypt.), p. 129-142, 2017.

FATHI, Heba A. *et al.* Electrospun vancomycin-loaded nanofibers for management of methicillin-resistant Staphylococcus aureus-induced skin infections. **International Journal of Pharmaceutics**, v. 586, p. 119620, 2020.

FEKRAZAD, Sepehr; SOHRABI, Mona; FEKRAZAD, Reza. Angiogenetic and anti-inflammatory effects of photobiomodulation on bone regeneration in rat: A histopathological, immunohistochemical, and molecular analysis. **Journal of Photochemistry and Photobiology B: Biology**, v. 238, p. 112599, 2023.

GHOSAL, Kajal *et al*. Novel drug delivery systems based on triaxial electrospinning based nanofibers. **Reactive and Functional Polymers**, v. 163, p. 104895, 2021.

GONZALEZ, Ana Cristina de Oliveira *et al*. Wound healing-A literature review. **Anais brasileiros de dermatologia**, v. 91, p. 614-620, 2016.

HADIZADEH, Mohadeseh *et al*. A bifunctional electrospun nanocomposite wound dressing containing surfactin and curcumin: in vitro and in vivo studies. **Materials Science and Engineering: C**, v. 129, p. 112362, 2021.

HARMAN, Rebecca M.; THEORET, Christine L.; VAN DE WALLE, Gerlinde R. The horse as a model for the study of cutaneous wound healing. **Advances in Wound Care**, v. 10, n. 7, p. 381-399, 2021.

HE, Ronghan *et al*. Efficacy of a synthetic biomimetic skin substitute of PLLA/gelatin nanofiber membrane in facilitating chronic cutaneous wound healing. **Materials Technology**, v. 35, n. 13-14, p. 872-880, 2020.

HOLLOWAY, Samantha; HARDING, Keith G. Wound dressings. **Surgery (Oxford)**, v. 40, n. 1, p. 25-32, 2022.

HUANG, Ya-Han *et al*. Protective effects and mechanisms of Terminalia catappa L. methenolic extract on hydrogen-peroxide-induced oxidative stress in human skin fibroblasts. **BMC complementary and alternative medicine**, v. 18, n. 1, p. 1-9, 2018.

IHEAGWAM, Franklyn Nonso *et al*. Terminalia catappa aqueous leaf extract reverses insulin resistance, improves glucose transport and activates PI3K/AKT signalling in high fat/streptozotocin-induced diabetic rats. **Scientific Reports**, v. 12, n. 1, p. 10711, 2022.

JIANG, Zhen-Yue; WOOLLARD, Alison CS; WOLFF, Simon P. Lipid hydroperoxide measurement by oxidation of Fe2+ in the presence of xylenol orange. Comparison with the TBA assay and an iodometric method. **Lipids**, v. 26, n. 10, p. 853-856, 1991.

JORGENSEN, Adam M. *et al*. Advances in Skin Tissue Engineering and Regenerative Medicine. **Journal of Burn Care & Research**, v. 44, n. Supplement_1, p. S33-S41, 2023.

KILIÇ, Ismail; YEŞILOĞLU, Yeşim. Spectroscopic studies on the antioxidant activity of p-coumaric acid. **Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy**, v. 115, p. 719-724, 2013.

KOPROSKI, Letícia; PACHALY, José R. Contenção farmacológica do rato-do-bambu (Dactylomys dactylinus Desmarest, 1817) e relato de ocorrência da espécie na região Sul do Brasil. **Pesquisa Veterinária Brasileira**, v. 37, p. 608-612, 2017.

KOSYKH, Anastasiya V.; TERESHINA, Maria B.; GURSKAYA, Nadya G. Potential Role of AGR2 for Mammalian Skin Wound Healing. **International Journal of Molecular Sciences**, v. 24, n. 9, p. 7895, 2023.

LEVENSON, S. M. *et al*. Some studies of wound healing: experimental methods, effect of ascorbic acid and effect of deuterium oxide. **Journal of Trauma and Acute Care Surgery**, v. 4, n. 5, p. 543-566, 1964.

LUO, Hua *et al*. Paracrine effects of adipose-derived stem cells in cutaneous wound healing in streptozotocin-induced diabetic rats. **Journal of Wound Care**, v. 31, n. Sup3, p. S29-S38, 2022.

MAHMUD, Md Musavvir *et al*. Controlled release of curcumin from electrospun fiber mats with antibacterial activity. **Journal of Drug Delivery Science and Technology**, v. 55, p. 101386, 2020.

MININEL, Francisco José *et al*. Characterization and quantification of compounds in the hydroalcoholic extract of the leaves from Terminalia catappa Linn.(Combretaceae) and their mutagenic activity. **Evidence-Based Complementary and Alternative Medicine**, v. 2014, 2014.

MOGASHOA, M. M.; MASOKO, P.; ELOFF, J. N. Different Combretum molle (Combretaceae) leaf extracts contain several different antifungal and antibacterial compounds. **South African Journal of Botany**, v. 126, p. 322-327, 2019.

MOHAMMED, Hamdoon A. *et al*. Bio-evaluation of the wound healing activity of Artemisia judaica L. as part of the plant's use in traditional medicine; Phytochemical, antioxidant, anti-inflammatory, and antibiofilm properties of the plant's essential oils. **Antioxidants**, v. 11, n. 2, p. 332, 2022.

MONIKA, Prakash *et al*. Myofibroblast progeny in wound biology and wound healing studies. **Wound Repair and Regeneration**, v. 29, n. 4, p. 531-547, 2021.

MOURO, Cláudia; FANGUEIRO, Raul; GOUVEIA, Isabel C. Preparation and characterization of electrospun double-layered nanocomposites membranes as a carrier for Centella asiatica (L.). **Polymers**, v. 12, n. 11, p. 2653, 2020.

NUGROHO, Rudy Agung *et al*. Wound healing potency of Terminalia catappa in mice (Mus musculus). **EurAsian Journal of BioSciences**, v. 13, n. 2, p. 2337-2342, 2019.
RAJHI, Imene *et al*. Assessment of dehulling effect on volatiles, phenolic compounds and antioxidant activities of faba bean seeds and flours. **South African Journal of Botany**, v. 147, p. 741-753, 2022.

RAVI, Lokesh. A review on medicinal potential of Terminalia catappa. **International Journal of Green Pharmacy (IJGP)**, v. 14, n. 03, 2020.

SCHNEIDER, Rodrigo *et al*. Biocompatible electrospun nanofibers containing cloxacillin: Antibacterial activity and effect of pH on the release profile. **Reactive and Functional Polymers**, v. 132, p. 26-35, 2018.

SEDLAK, Jozef; LINDSAY, Raymond H. Estimation of total, protein-bound, and nonprotein sulfhydryl groups in tissue with Ellman's reagent. **Analytical biochemistry**, v. 25, p. 192-205, 1968.
SHAHID, Md Abdus *et al*. Antibacterial wound dressing electrospun nanofibrous material from polyvinyl alcohol, honey and Curcumin longa extract. **Journal of Industrial Textiles**, v. 51, n. 3, p. 455-469, 2021.

STREETER, Benjamin W. *et al*. Electrospun nanofiber-based patches for the delivery of cardiac progenitor cells. **ACS applied materials & interfaces**, v. 11, n. 20, p. 18242-18253, 2019.

TABAKOGLU, Hasim O. *et al*. Assessment of circular wound healing in rats after exposure to 808-nm laser pulses during specific healing phases. **Lasers in surgery and medicine**, v. 48, n. 4, p. 409-415, 2016.

TERÇAS, Analucia G. *et al*. Phytochemical characterization of Terminalia catappa linn. Extracts and their antifungal activities against candida spp. **Frontiers in Microbiology**, v. 8, p. 595, 2017.

VENKATALAKSHMI, P.; BRINDHA, P. In vivo Immunomodulatory Potential of Aqueous Extract of Different Parts of Terminalia catappa L. **Indian Journal of Pharmaceutical Sciences**, v. 83, n. 3, p. 584-593, 2021.

WILKINSON, Holly N.; HARDMAN, Matthew J. Wound healing: Cellular mechanisms and pathological outcomes. **Open biology**, v. 10, n. 9, p. 200223, 2020.

XIE, Xianrui *et al*. Electrospinning nanofiber scaffolds for soft and hard tissue regeneration. **Journal of Materials Science & Technology**, v. 59, p. 243-261, 2020.
XUE, Jiajia *et al*. Electrospun nanofibers: new concepts, materials, and applications. **Accounts of chemical research**, v. 50, n. 8, p. 1976-1987, 2017.

YADAV, Sarita *et al*. Terminalia catappa Linn.: A TREASURY OF PHARMACOLOGICAL BENEFITS. **UTTAR PRADESH JOURNAL OF ZOOLOGY**, p. 1386-1396, 2021.

YAZDANBAKHSH, M. F. *et al*. The effect of impregnated alpha-cellulose nanofibers with ciprofloxacin hydrochloride on staphylococcus aureus in vitro and healing process of wound in rat. **Regenerative Engineering and Translational Medicine**, v. 4, p. 247-256, 2018.

ZHANG, Xi *et al*. Nanoengineered electrospun fibers and their biomedical applications: a review. **Nanocomposites**, v. 7, n. 1, p. 1-34, 2021.