Original Research Article

J. Funct. Mater. Biomol. 3(2) (2019) pp 342 – 347

SACRED HEART RESEARCH PUBLICATIONS

Journal of Functional Materials and Biomolecules

Journal homepage: www.shcpub.edu.in

ISSN: 2456-9429

Wound Healing Effect of Aqueous Extract of Trichome of *Borassus flabellifer* in Excision Wound Model - An *In vivo* Studies

A. Jerry[1], A. Poongothai [1*], V. Chandru[1], R. Mahaprabu[2], P. Pandikumar[3] and M.I. Niyas Ahamed[1]

Received on 10 December 2019, accepted on 15 December 2019,
Published online on 26 December 2019

Abstract

Borassus flabellifer L. (Arecaceae) is a palmyra tree used for biological activities. The present study was planned to evaluate the wound healing effect of *Borassus flabellifer*trichomes on excision wound model in Swiss Albino mice. The wound healing results of aqueous extract of 20% of *Borassus flabellifer*trichometreated-groups were significantly increased when compared with control povidone iodine and histopathological studies also revealed that theenhanced improvement when compared to untreated and control groups.The healing pattern of the wounds was evaluated by planimetric studies, macroscopic observations and histological studies. The results have shown faster healing patterns in the wounds treated with 20% of *Borassus flabellifer* trichomecompared to untreated controls. So, this study suggests that trichome of *Borassus flabellifer* trichomemay be a potential candidate as a wound dressing material and may be tried on the clinical wounds of animals before being applied on humans.

Keywords: *Borassus flabellifer*, Trichomes, Histopathology, Wound healing and Antibacterial activity.

1 Introduction

For the last two decades extensive work has been done to develop newer drugs from natural products because of the resistance to the existing drugs. Wounds represent a major global health challenge, which put much economic, financial and social stress on health institutions, care-givers, patients and their families. Wounds are defined as physical, chemical, or thermal injuries or insult that result in an opening or breaking in the integrity of the skin or the disruption of anatomical and functional integrity of living tissues. The use of medicinal plants in the management of acute and chronic wounds is common in most traditional medicine practices in the world. Based on this, many plants in the tropical and subtropical regions of the world have been screened for their wound-healing activity[1]. There are a lot of medicinal plants to be screened in the search for newer, efficacious and cost effective wound-healing agents.

The process proceed are inflammation, reepithelialization, tissue renovation, wound tightening and formation of granulation and formation of new blood vessels. All these happen in a timely manner and they are predictable. Disturbance in these process may lead to neither chronic wound like a venous ulcer nor keloid scar which said to be the over growth of scar tissue[2]. In the process of wound healing an inflammatory response occurs after the injury and the cells found below the dermis begin to increase the production of collagen the main structural protein of the skin[3].

The word 'Borassus' is a Greek word means the leathery covering of the fruit and '*flabellifer'*, which means fan-bearer. Palmyra palm tree belongs to the 'palme' family. The Palmyra tree is the official tree of Tamil Nadu state in India. In Tamil culture it is called karpaha, nungu, celestial tree and is highly respected by the people. It grows to a tallness of about 30 m, with a black stem and crown of leaves at the top; leaves would be around 0.9-1.5 m in diameter. It has unisexual flowers, large fruits, fan shaped palmate and hard horny spinescentserratures over the petiole edges. The life span of this tree is up to 100 years[4]. The countless constituents of *Borassus flabelifer*are gums, albuminoids, steroidal glycosides, fats, and carbohydrate like sucrose, spirostane type steroids like borassosides and dioscin are found in the plant.

The present study focused on the wound healing property of the trichome of the *Borassus flabellifer* through *in-vivo* method of excision wound model with histological evidence.To our knowledge, the trichome of *Borassus flabellifer* has not been studied previously as wound dressing material.

2 Materials and Methods

2.1. Collection of Plant Material

Trichome of *Borassus flabellifer* was collected from Tirupattur Town, Tirupattur District, Tamil Nadu. It was

*Corresponding author: E-mail poongothai@shctpt.edu
[1]Department of Biochemistry, Sacred Heart College (Autonomous), Tirupattur, Tamil Nadu, India.
[2]Department of Veterinary Pathology, Madras Veterinary College, Chennai
[3]Division of Ethnopharmacology, Entomology Research Institute, Loyola College, Nungambakkam, Chennai.

authenticated by institutional botanist. The Samples were collected from young leaves of the trees. It was stored in dark until further use**.** Fig.1. shows the *Borassus flabellifer* and its parts.

2.2. Toxicity Study

The adverse effects of trichome of *Borassus flabellifer* were referred on first exposure for the behavior on anatomy and physiology to a single dose of aqueous extract of the sample.

Fig.1. *Borassus flabellifer* and its parts
A – Whole, Plant B – Tender Leaves and C – Trichome

2.3. Selection and Preparation of Animals

Six female Swiss albino mice that are young, non-pregnant were used. The average weight of the animals was 25g. The selected animals were marked for individual identification. Tail marking was used for the identification of animals. They were kept fasting for two hours before the test. The ethical number for this study is IAEC/ERI/LC/04/18.The committee allowed 30 mice for the study of wound healing with trichome of *Borassus flabellifer.*

2.4. Grouping of Animals

The Swiss albino mice of either sex were harvested from Tamil Nadu Veterinary and Animal Sciences University, Chennai. The mice were maintained on normal food and water. They were kept in an air conditioned room as per the requirement of Committee for the purpose of control and supervision of experiments on animals (CPCSEA). Animals were checked for weight loss and gain before and after the experimental wound. The mice were anaesthetized prior to and during the creation of wound for the experiment. They were closely watched for any infections and other kinds of negative signs.

2.5. Preparation of Extract for Toxicity Study

One gram of sample was weighed accurately and mashed with mixer grinder for power. The ground sample was filtered with flour filter. The powder obtained was boiled with 100 ml of distilled water for the aqueous extract. The boiled sample was filtered with Whatman No 1 filter paper. Later the extract obtained was concentrated to 2.0 ml. 0.23 ml of aqueous extract was given to each animal orally with syringe. Mortality was looked for, 2 hrs, 24 hours, 72 hours and 7 days after oral administration of the sample. Symptoms, signs and mode were looked for.

2.6. Creating Wound

General laboratory techniques recommended by committee for the purpose of control and supervision of experiments on animals were followed. The crude and aqueous extract of *Borsssus flabellifer* of 10% and 20% was prepared after weighing material required with sterile water. They were applied directly over the skin and the observations noted. It was followed till the study was completed on 15th day.

2.7. Excision Wound Model

Animals were anesthetized prior to and during creation of the wounds. The rats were inflicted with excision wounds as described by Morton and Malone[5]. The mice were divided in to four groups of six animals in each group. Fur on the back of the animals will be shaved using mechanical trimmer. A wound with 5 mm^2 diameter will be made using skin punch biopsy tool, under isoflurane anesthesia. After recovery from anesthesia, the animals will be given a subcutaneous injection of temgesic (0.05 mg/kg/day for three days). The wound on the first group of animals treated with sterile water and housed individually. This was considered as negative control group. The second group of animals was applied with Povidone Iodine, they were considered as positive control. The third group of animals was treated with 10% of aqueous crude extract of *Borassus flabellifer.* The last and fourth group of animals was treated with 20% crude of the same plant source.

Sterile waster was used to make the crude extract. The same application was followed minutely for 15 days from the day on which the wound is made. The wound contraction measured on days 0, 3, 5, 7, 9, 12, 15. The animals were euthanized on 15th day without causing anxiety, pain or distress with minimum time. It was carried out in a separate room without disturbing the other animals in the laboratory according to the rules and regulations of CPCSEA. The time of complete epithelialization, percentage of wound closure and period of epithelialization recorded. Scar tissue is taken for histological analysis.

2.8. Observations after Administering the Sample

The following were the observation after administering the sample orally. Table 1 shows the observations of toxicity test as follows,

2.9. Euthanizing of Animals

The animals to be euthanized were taken into a separate room which is free from environmental contaminants. Carbon monoxide was used to euthanize the animals. It was done based on the guidelines of CPCSEA. It was carried out quickly and painlessly with free from fear and anxiety for the animals. It was made sure that there would be minimum physiological and psychological disturbances. The scare was removed from the euthanized animals and preserved in the 10% formalin solution. It was used to preserve the animal skin before the histological purposes. Later the histological study was done.

2.10. Histology of the Skin

The healed scar tissues were isolated from each individual and fixed with 10% buffered formalin. 5µm of paraffin sections were cut on clean glass slides and stained with haematoxylin and eosin. The images were viewed under a light microscope and magnified images of the tissues structure were captured. Fig.2(a-g). shows the grouping of animals and their treatment

Table 1: Assessment of Toxicity

Observation	First Few Minutes	2 Hours	24 Hours	72 Hours	7 Days
Behavioral patterns	Rapid heart beat	Normal	Normal	Normal	Normal
Diarrhea	Not observed	Not observed	Not observed	Not observed	Not observed
Lethargy	Normal	Increased	Increased	Increased	Increased
Eyes	Normal	Normal	Normal	Normal	Normal
Salivation	Not observed	Not observed	Not observed	Not observed	Not observed
Skin and fur	Normal	Normal	Normal	Normal	Normal
Sleep	Normal	Normal	Normal	Normal	Normal
Coma	Not observed	Not observed	Not observed	Not observed	Not observed

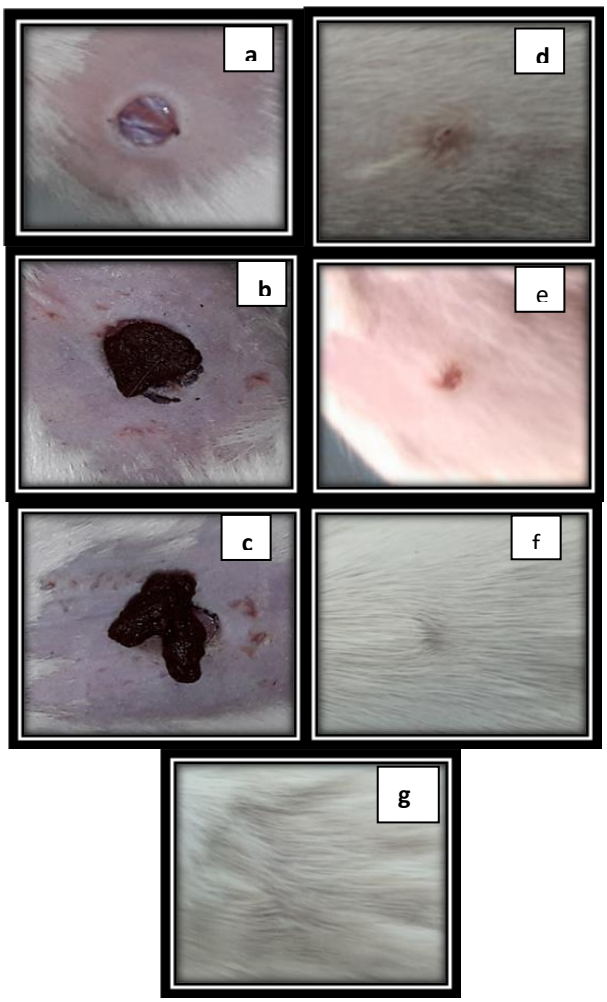


Fig.2. Grouping of animals and their treatment (a)Positive control (Povidone Iodine) on Day 1, (b)Treated wound with 10 % of trichome of *B.flabellifer* on Day, 1 (c) Treated wound with 20% of trichome of *B.flabellifer* on Day 1, (d)Healed wound on Day 15 – Left for Natural Healing, (e) Positive control (Povidone Iodine) on Day 15, (f) Treated wound with 10% of trichome of *B.flabellifer* on Day 15 and (g) Treated wound with 20 % of trichome of *B.flabellifer* on Day 15

3 Results and Discussion

During early wound healing, the vascular and lymphatic systems are of primary importance. Failure or delay of vascular regeneration decreases oxygen transport to the wound, which subsequently depresses the mobilization of excessive fluids from the wound site. The wound becomes edematous, leading to further damage, infection and eventually cell death. In wound healing, new blood vessels sprout up from platelets or macrophages to keep the wound open-ended. Hypoxia may be a stimulant to revascularization.

Recently many researchers have developed several wound dressing materials by fabricating and changing biomaterials. An ideal wound dressing should have several key attributes [6-7]. Only those dressing material with good mechanical properties can be applied on to wound properly. There is a possibility of investigating the wound healing in a reproducible, controlled environment using the animal models. The basic research is completed when animal wound repair with human wounds even though it is not a direct and true reflection of ours. But they are essential in finding out the variety of medicines for the

Days	Negative Control (in mm)	Positive Control (in mm)	10% ABF (in mm)	20% ABF (in mm)
Day 3	4.9	4.8	4.7	4.6
Day 5	4.2	3.7	3.8	3.7
Day 7	3.5	2.8	3.1	2.9
Day 9	2.4	2.2	2.1	2
Day 12	1.3	0.9	1.1	0.8
Day 15	0.09	0.06	0.08	0.06

wound healing. Moreover, the dynamism behind the wound healing is complex. The phenomenon must be understood with prerequisite knowledge for pathology. The complex process begins with inflammation, followed by proliferation of the epithelial tissue and origination of matrix compounds and ends with the regeneration of scar tissue marked with high amount of collagen matrix. Hence knowing the factors and understanding them for wound healing process and function is essential.

3.1. Acute Toxicity

The toxic effects of aqueous trichome extract of *B. flabellifer*on the appearance and over all behavior patterns of mice are noted. At the end of the toxic study no symptoms or mortality of any animals were observed, which lived for 7 days after administering the trichome extract at single dose level of 0.23 ml of average weight. The behavioral patterns of mice were observed from immediate time of administration of sample to Seven days. The trichome administered group of animals displayed the following: The heartbeats of the mice were rapid for first few minutes and it was alright with after below 30 minutes. Coma was not observed from any of the animals. Animals were not found with diarrhea or any other digestive disorders. The intake of food and water was normal for all the animals. First half an hour the animals seemed to be normal later they are found over energized and found increased movements without making any big fuss. The colour and ability of vision was normal. The salivation factor for the sample administered mice was not observed. The skin and fur were normal and animals slept well throughout the testing period.

3.2. Body Weight

Changes in the body mass are the indicators of adverse side effects, as the animals that survive shall not lose more than 10% of the initial body weight. Therefore it reveals the physiological status of the animals. The body weights of animals were calculated before and after the acute toxicity study.The average weight of the animals was 23 g before the test. The same groups of animals were weighed after the toxicity studies on 7th day found to be 22.6 g. Therefore, there is no significant reduction of body weight in the group of animals during the test.

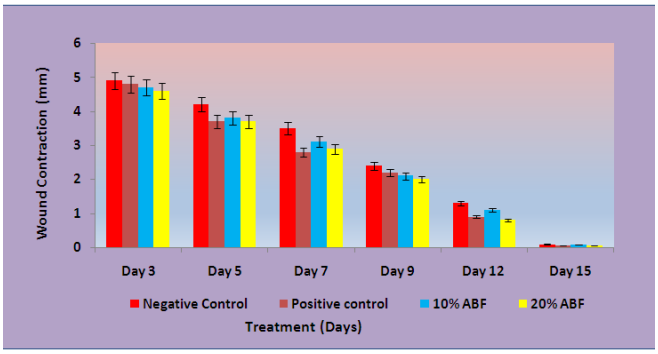
3.3. Wound Contraction

The wound contractions measured on days 0, 3, 5, 7, 9, 12 and 15 are given in the Fig.3. The wound contraction is the average of the healed wound of groups of animals. The Table 2 showed that the wound contraction of days and the measurement of wound healing in mm. It also shows the wound contraction as follows,

Table 2. Wound healing contraction effect of trichome

*Results are Average of Triplicate

The tabulated data of wound contraction reveals the gradual healing of the wound form 4.9 mm to 0.09 mm with negative control. The positive control with Povidone Iodine increases the speed of wound healing to 0.06 on 15th day. The 10% ABF shows significant healing effect with positive control and the 20% ABF depicts similar nature of wound healing with positive control. The same data is presented in Fig.3 where the wound healing is completed on 15th day without any mark of wounds in average. The bar chart also portrays the gradual healing of wounds of the animals. The red colour bar is for negative control, brown colour bar for the positive control, Blue colour is for the 10% ABF and Yellow colour bar chart for the 20% ABF. At the end the level of 20% ABF seems to be invisible on the 15th day. The wound contraction seems to be gradually increasing included. Fig.3. shows the wound contraction for the different groups are below.



*Results are presented as mean ± SD; statistically significant data are given as $p < 0.05$.

Fig. 3. The wound healing effect of trichome of *Borassus flabellifer* Control and Experimental Swiss albino mice

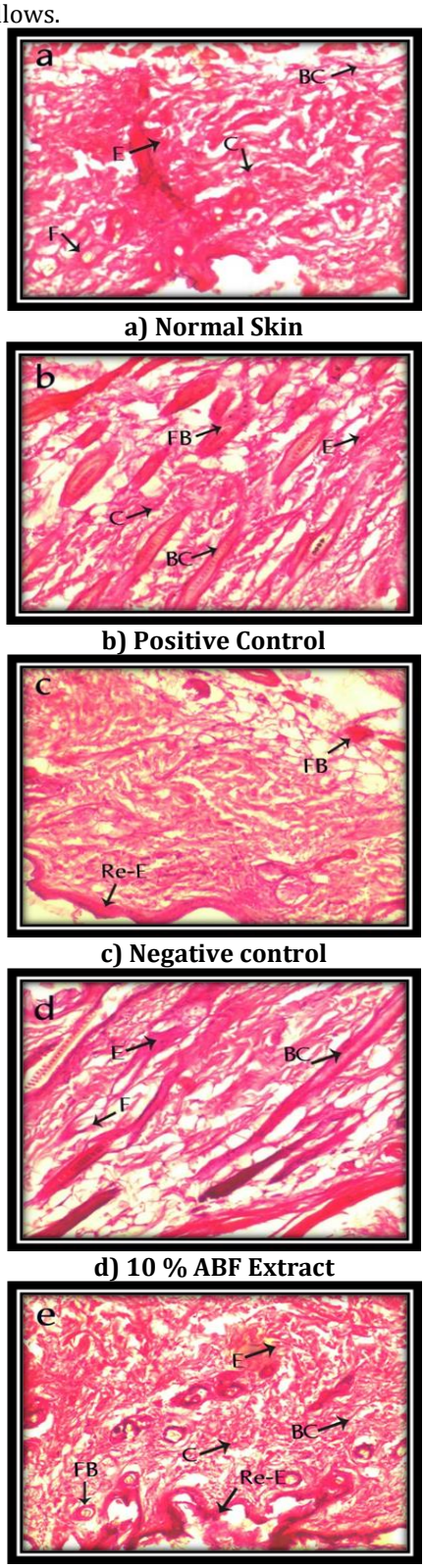
3.4. Histology

The fibroblast plays an important role on all phases of wound healing. Fibroblast plays either in inflammation, Proliferation,reepithelization and remodeling phase. However, it is mainly responsible for collagen deposition. Collagen is a key extracellular protein in the granulation tissue of healing wound and it's the vital component that ultimately plays an important role in wound strength and integrity of tissue matrix.

The results in Fig. 4. showed that the ABF promotes natural healing process and it could be efficiently stimulate the wound strength and increase the rate of epithelialization, tensile strength and Collagen viability around the wound area. ABF showed significant enhanced the epithelization. It may be due to the effect of ABF to enhanced collagen synthesis.

During the wound healing process migration of fibroblast and epithelial cells to the wound site was observed in the treated group. The Fig.4. revealed that the histology of

normal and treated wound healing skin of Swiss albino mice as follows.



a) Normal Skin

b) Positive Control

c) Negative control

d) 10 % ABF Extract

e) 20 % ABF Extract

BE- Epithelial Cells, **FB -**Fibroblasts, **C-** Collagen, **BC-** Blood Capillaries, **Re –** Re- epithelization are marked with arrow mark

Fig. 4. Histology of Normal and Treated skin of Swiss albino mice

The regeneration of cell, re-epithelialization, and proliferation of fibroblasts are observed with the histology. It is the main activity for both for qualitative and quantitative healing of wounds. It shows the growth of tensile cells, distribution of fibers, fibroblasts and the distribution of cells within the tissue[8-9] reported that the bark of *Azadirachta indica* has wound healing activity using different extracts such as water, ethanol-water and ethanol. Water extract healed the wounds faster than the other extract and positive control. Povidone-Iodine ointment USP was taken as positive control.

The results of treated wound with 20% ABF shows a better result that of *Azadirachta indica* and *SilybummarianumAsteraceae.* The 20% ABF increased the level of collagen synthesis which leads to increased tensile strength in wound tissue, helping the process of healing. Enhanced activity naturally leads to angiogenesis. This makes the circulation higher, thus providing oxygen and nutrients, essential for further healing process strongly. Therefore wound healing activity showed that the tissue regeneration was much quicker in the ABF treated group compared to standard and control. The ointment of ABF showed significant wound healing activity in excision wound in Swiss albino mice.

4 Conclusions

It can be concluded that the study on wound healing effect of trichome of *Borassus flabellifer* states that the indigenous medicines are still relevant in the field of medicine. Treating of wounds with trichome is not poisonous. Trichome could be suggested for the fresh wound where the trichome stops forming pus and mark of wound after healing. The nature of the skin is as normal as a normal skin. Therefore these studiesstrongly recommended that the trichome has medicinal property of wound healing.

Acknowledgements

The authors are very much grateful to the Principal and Management of Sacred Heart College (Autonomous), Tirupattur, Tamil Nadu for having providing necessary facilities and for their encouragement. Special thanks to Rev. Dr. Savarimuthu Ignacimuthu, Former Director of ERI, Loyola College, Chennai for his gracious guidance.

References

[1] Y. Victor, and A. Barku, Wound Healing: Contributions from Medicinal Plants and Their Phytoconstituents, *Ann Res & Rev in Bio.* 26(2018) 1-14.

[2] Rupesh Thakur, Nitika Jain, RaghvendraPathak, and Sardul Singh Sandhu Practices in Wound Healing Studies of Plants, *Evidence-Based Complementary and Alternative Medicine,* 17 (2011) 438056.

[3] NaleshMalviya and Sanjay Jain, Wound healing activity of aqueous extract of radix paeoniae root, *ActaPoloniaePharmaceutica Drug Research*, 66(2009) 543 - 547.

[4] V.P. Gummadi, G.R. Battu, M.S. Diyya, and Kiran-Manda, A Review on Palmyra Palm *Borassus Flabellifer*, *Inter Jou Cur Pharm Res,* 8(2016) 17-20.

[5] J. J. P. Morton, and M.H. Malone, Evaluation of vulnerary activity by an open wound procedure in rats. *Arch Int. Pharmacodyn*. 196(1972) 117–26.

[6] M.I.N. Ahamed, and T.P. Sastry, An in vivo study on the wound healing activity of Cellulose-Chitosan composite incorporated with Silver nanoparticles *Int. J. of Research in Ayurveda and Pharmacy*, 2(2011) 1203-1209.

[7] M.I.N. Ahamed, S. Sankar, P.M. Kashif, S.K.H. Basha, Sastry TP, Evaluation of biomaterial containing regenerated cellulose and chitosan incorporated with silver nanoparticles. *Int. J. Biol. Macromol*. 72(2015) 680–686.

[8] F.A. Toppo, and R.S. Pawar, Appraisal on the wound healing activity of different extracts obtained from *Aeglemarmelos* and *Mucunapruriens* by *in vivo* experimental models, *Nigerian Journal of Clinical Practice*, 19(2016) 753 -760.

[9] K. Preeti Maan, Y. Singh, and Y. Narayan Prasad, Wound healing activity of *Azadirachta indica A. juss* stem bark in mice. *Phcog Mag,13, Suppl* S. 2(2017) 16-20.