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COMPARATIVE STUDY OF TOPICAL APPLICATION OF COCOS NUCIFERA VERSUS NORMAL SALINE SOLUTION IN INCISIONAL WOUND HEALING SPRAGUE DAWLEY RATS

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Abstract

Cocos nucifera has long been utilized as wound healing medium by folk healers in the Philippines yet there are quite a few studies to support efficacy and safety to substitute the standard wound healing therapy. This experimental study investigated the effect of Cocos nucifera application on incisional wound in Sprague Dawley rats versus Normal Saline Solution control group. After five (5) experimental days, result verified that application of Cocos nucifera and NSS are safe and effective wound healing agents as evidenced by improvement in size, edges, exudates, epithelization and pink/normal surrounding skin and nonappearance of wound hitches such as edema nor necrosis.

Despite the disparity between the two groups in terms of epithelization rate, the researchers believe that sodium or salt supplementation to Cocos nucifera topical preparation may enhance epithelization rate. Hence, this study highlighted that Cocos nucifera topical application is a, inexpensive, safe, effective and readily accessible substitute for standard NSS wound healing treatment.

Keywords: Cocos nucifera, Normal Saline Solution, Incisional Wounds, Wound Healing, Sodium, Sprague Dawley Rats.

Introduction

Topical application of domesticated Cocos nucifera endosperm oil in wounds has been widely used by traditional healers in the Bicol region and other nearby regions in the Philippines. Its' oil is highly revered for its healing properties and forms the basis for traditional cures and therapies. Being a natural food, rather than synthetic chemical, its' oil is believed to post no harmful side effects and is completely safe to use by folkloric healers. Despite the robust bio-nutritive constituents of Cocos nucifera that are helpful to promote wound healing, there is limited studies to back up its viability as substitute for standard wound healing agent.

Wound incidence rate in the Philippines is rising continuously without seasonality. Even though, a myriad of medical treatments is available to manage incisional wounds, these are usually inaccessible, unavailable and unaffordable most particularly to those living outback and below the poverty line. The passage of traditional medicine from generation to generation has become significant in our healthcare system. This became the foundation of Republic Act 8423 or Traditional Alternative Medicine Act, an act that aims to legalize, promote and advocate use of traditional medicine in the Philippines. This act has been in existence for almost three decades, however, there appears to be minimal scientific exploration of these natural healing remedies in the country [1].

Normal Saline Solution is physiologically safe and the preferred cleanser for most wounds in hospital setting [2]. Both Cocos nucifera and standard wound cleansing agent Normal Saline Solution

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contain sodium that maintains cellular hydration and nourishment necessary to promote wound healing. Hence, this research study intends to compare the effect of both interventions on incisional wound healing. This study will also yield as starting point for future studies to explore the underlying pharmacological mechanisms of action pertaining to these medicinal plants.

Research Design

The research study is a Quantitative Experimental posttest design. Ten (10) all female adults weighing over 250-300g Sprague Dawley rats purchased in a Research and Biotechnology Division at Quezon City. Five (5) rats were randomly assigned per group using lottery technique. The first group was assigned under Cocos nucifera experimental treatment while the second group was assigned as the control group under Normal Saline Solution (NSS0 treatment. Pre-experiment observation was done to establish baseline data. Interventions were given on 2nd to fifth day then post-test observation of the wound was done on the fifth day for all groups of rats. Quantitative numerical information was obtained using modified version Wound Measurement Guide and slightly modified Bates Jensen Wound Assessment Tool (BWAT) [3]. Eleven out of thirteen (11/13) parameters were adapted and 2 parameters (undermining and granulation tissue) were removed for it is applicable only for pressure sores.

Data Gathering Procedure

Step I: Pre-experiment phase

The researchers sought approval from the Institutional Animal Care and Use Committee and to Bureau of Animal Industry prior to implementation. The researchers then underwent IACUC certification on proper handling of laboratory rats. After that, the researchers adapted the facility's standard protocol. The researchers then purchased Sprague Dawley rats and conducted the experiment in a University Laboratory in Valenzuela City.

Step II: Preparation of Cocos nucifera

The 9-12 months mature brown coconut was de-husked. Then endosperm was grated and squeezed then strained until half kilo of clean viscous slurry coconut milk was obtained. It was then set to undergo fermentation process for 2-3 hours in a vacuum covered beaker maintained in a room temperature. The set oil was then transferred in a sterile amber bottle using a micropipette.

Step III: Preparation Phase of Incisional Wound

Sprague Dawley rats were acclimated to the new animal house laboratory environment for 7 days to decrease their stress. After handwashing and donning, the researchers prepared the skin of laboratory rats by removing fur about 5 x 5 cm using a #40 blade in the surgical site. Laboratory rats are placed in surgical board and then restrained to prevent movement. Skin was cleansed 3 times using gauze with alcohol and saline solutions before applying betadine as a final antiseptic solution. Lidocaine chloride 2% diluted to 0.5% 7mg/kg total dose local anesthesia was injected subcutaneously to the site at 0.5cm apart. A 3 cm incision is created at the dorsum (thoraco-lumbar region) using a scalpel with size 15 blade and size 3 blade holder. Generally, the researchers evaluated presence or absence of different reflexes. Reflex that was observed are loss of optical facial winking reflex or stop of eye movement with presence of deep and regular breathing. Testing body reflexes was also evaluated by putting the tuberculin syringe on top of the dorsum then watching for presence of any movement. Monitoring of the color of the mucosa (white or cyanotic) to recognize any state of shock to the animal.

Step IV: Experiment Phase

Cocos nucifera (Experimental group A)

1. A medicine dropper was used to obtain solution of Cocos nucifera in an amber bottle, wherein 2 mL was applied per site
2. Use of linear strokes was used from clean to dirty area taking care not to transfer microorganisms from the periphery back to the site of incision.
3. After application of the area, the dropper was discarded and then another dropper was used for additional extract application.
4. The extract applied was extended over the incision site. Absorption of the extract was awaited prior to covering the incision site with sterile gauze then was secured with micropore adhesive tape
5. Transparent wound measuring guide was used in measuring the length and width in cm.
6. Incisional wound was scored and evaluated based on the BWAT tool
7. Photo documentation with adequate lighting was used to show size of incision as reference for progression of wound healing

Normal Saline Solution (NSS) (Control group B)

1. Two (2) cotton swabs soaked in normal saline solution was applied per site
2. Use of linear strokes was used from clean to dirty areas taking care not to transfer microorganisms from the periphery back to the site of incision.
3. After application of the area, the gauze was discarded.

4. The saline applied was extended over the incision site. Absorption of the extract was awaited prior to covering the incision site with sterile gauze then was secured with micropore adhesive tape
5. Transparent wound measuring guide was used in measuring the length and width in cm.
6. Incisional wound was scored and evaluated based on the BWAT tool
7. Photo documentation with adequate lighting was used to show size of incision as reference for progression of wound healing

Step V: Post-experiment Phase

All ten (10) rats were injected intraperitoneally with 0.5 ml of 70% ethanol and sacrificed using cervical dislocation. The researchers followed the standard protocol for disposal of dry instruments, sharps, and anatomical waste.

Results

Baseline data prior to experiment showed the both groups displayed homogenous
characteristics: wound size of 0.21-.50 sq.cm with shallow crater, rolled under thickened edges, bright red surrounding skin and less than 25% covered epithelization. Also, both groups do not have necrosis, induration, nor edema but they have scanty bloody exudate.
The experiment was done in a controlled animal surgical laboratory and aseptic technique was maintained all throughout the experiment. On the 5th day of experiment, observation findings revealed that twenty percent (20%) of the rats
under Cocos nucifera treatment group had demonstrated remarkable reduction in wound size of <0.15 sq.cm while another twenty percent (20%) of the rats had wound size of 0.21-50 sq. cm to < 0.16-20 sq. cm while majority (40%) maintained the baseline wound size of 0.21-0.50 sq. cm but the other 20% had an increased wound size of >51 sq. cm. While in the normal saline solution control showed that none (0%) had reached wound size reduction to ,0.15 sq. cm, twenty percent (20%) of the rats abridged wound size from 0.21-50 sq. cm to < 0.16-20 sq. cm while majority (60%) remained in the baseline wound size of 0.21-0.50 sq. cm but the other 20% had an increased wound size of >51 sq. cm.

Table 1: Incisional Wound Characteristics between Cocos nucifera and NSS on 5th day

Criteria		Cocos nucifera F (%)	NSS F (%)
Size	<0.15 sq. cm	1, 20%	0, 0%
	<0.16-0.20 sq. cm	1, 20%	1, 20%
	0.21-0.50 sq. cm	2, 40%	3, 60%
	>0.51 sq. cm	1, 20%	1, 20%
Depth	Shallow crater	3, 60%	5, 100%
	Deep crater	2, 40%	0, 0%
Edges	Attached	5,100%	5, 100%
Necrosis	No visible	5, 100%	5, 100%
Amount of Necrosis	None	5, 100%	5, 100%
Type of Exudate	None	3, 60%	5, 100%
	Serous	2, 40%	0, 0%
Amount of Exudate	None	3, 60%	5, 100%
	Scanty	2, 40%	0, 0%
Surrounding Skin Color	Pink or normal	5, 100%	5, 100%
Edema	None	5, 100%	5, 100%
Induration	<2cm	4, 80%	3, 60%
	2- 4 cm extending < 50%	1, 20%	2, 40%
Epithelization	75-,99% covered	0, 0%	1, 20%
	50-74% covered	1, 20%	3, 60%
	25-49% covered	3, 60%	1, 20%
	< 25% covered	1, 20%	0, 0%

Furthermore, fifth day findings revealed that all rats in the control group maintained wound depth of shallow crater. While forty percent (40%) of the rats treated with Cocos nucifera developed deep crater and the rest maintained shallow crater. All rats on both groups had attached wound edges,
normal pink surrounding skin without necrosis nor edema. All rats applied with normal saline solution had no exudate while forty percent (40%) rats in the experimental group developed scanty serous exudate.

Induration is the development of hardened tissue caused by consolidation of edema. Majority of the rats (80%) in the experimental group have <2cm induration while the rest had 2-4 cm extending less than 50% induration. On the other hand, majority of the rats (60%) in the control group had <2 cm

induration while the rest had 2-4 cm extending less than 50% induration.

On the other hand, epithelization is a sign of proliferative phase in wound healing where epithelial cells moves upwards to repair the wound. Study findings revealed that twenty percent (20%) of the rats applied with normal saline solution displayed maximal epithelization improvement of 75-,99% covered, majority (60%) had 50-74% covered epithelization from < 25% covered epithelization while the remaining twenty percent (20%) had 25-49% covered epithelization only. Most of the rats (60%) had demonstrated wound epithelization rate of 25-49% covered coverage from < 25%, only 20% had epithelization coverage of 50-74% while the remaining twenty percent (20%) had 25-49% covered epithelization.

Also, the study showed that application of Cocos nucifera and Normal Saline Solution on wound displayed no disparity in terms of size, depth, edges, type of exudate, amount of exudate and induration on day five (5). However, as displayed in table 2, on the fifth day of experiment application of Normal Saline Solution had superior epithelialization rate as compared to Cocos nucifera application in incision wounds.

Table 2: Cocos nucifera versus Normal Saline Solution Application on Incisional Wound

Areas	Computed t- value	Critical value	Level of Significance	Decision
Size	1.88	2.036	5%	Accept Ho
Depth	1.55	2.036	5%	Accept Ho
Edges	1.98	2.036	5%	Accept Ho
Type of Exudate	0.45	2.036	5%	Accept Ho
Amount of Exudate	0.14	2.036	5%	Accept Ho
Induration	0.75	2.036	5%	Accept Ho
Epithelialization	3.536	2.036	5%	Reject Ho

Discussion

Cocos nucifera endosperm oil have been widely employed in folkloric medicine for wound healing. This study verified that conform with findings of other In-vivo studies that Cocos nucifera is an effective treatment for wound healing as demonstrated by improvement in size, edges, exudate and epithelization rate [4]. While most studies available are limited to efficacy of Cocos nucifera as wound healing agent, this study provided a scientific basis that Cocos nucifera is almost comparable with the standard wound treatment using NSS. Granting that NSS demonstrated faster wound healing signs in terms of exudate and depth, the application of Cocos nucifera showed insignificant difference with NSS treatment. Further on the fifth day, all rats from both treatments demonstrated attached wound edges which indicated wound healing process has been established and edges are closing the wound [5]. This finding is also correlated morphologically with the evidence of superior reduction in wound size to <0.15 sq. cm compared with NSS group. This may be attributed to the high polyphenol content of Cocos nucifera which maintains normal levels of lipid parameters in tissues. Also, its serum content helps by trapping reactive oxygen species in aqueous components such as plasma and interstitial fluid [6]. Moreover, Cocos nucifera have adequate bio-nutritional constituents such as vitamin C, E, A and Zinc that promote wound healing activities [7].

Furthermore, findings support the research findings conducted in Manila, Philippines that Cocos nucifera intervention for incisional wound is considerably safe because of its natural properties [5]. Wound observation findings on the fifth day after being treated with Cocos nucifera have similar findings with use of NSS which demonstrated normal surrounding skin color and absence of wound complications such as edema nor necrosis. This finding supports the fact that moist environment has not been shown to increase the risk of infection, as compared to traditional dry therapies [8].

Cocos nucifera contains considerable amount of sodium. that consuming it could be a good ‘dietary salt’ substitute. This explains the presence of induration in the wound treated with both NSS and Cocos nucifera. The sodium attracts water that build around the edges of the wound that produces hardened appearance [9]. Similarly, presence of

scanty exudate is mainly water containing electrolytes, nutrients, proteins, inflammatory mediators, protein-digesting enzymes, such as matrix metalloproteinases (MMPs), growth factors and waste products, as well as cells such as neutrophils, macrophages and platelets. Clear, thin, watery plasma or serous exudate is normal during the inflammatory stage of wound healing [10]. Hence, the presence of exudate in experimental group is not a wound complication sign.

It is also congruent with the morphological observations that there is faster epithelization rate in NSS control group because it contains greater amount of sodium compared with Cocos nucifera. NSS contains just enough sodium to maintain wound moisture. A moist wound environment is scientifically proven to promote healing process by averting dehydration. Water is fundamental in transporting nutrients and oxygen to cells, thus keeping the cells alive and capable of regeneration. It also enhances angiogenesis, synthesis of collagen and breakdown of dead tissue and fibrin [10].

Conclusion

There are quite a few studies to support efficacy traditional herbal Cocos nucifera on wound healing and very limited literature that would claim its viability to substitute to standard wound treatments. Although these observations were only based on gross morphological evaluation, the study confirmed that application of Cocos nucifera and NSS are safe and effective wound healing agents. This is supported by remarkable improvement in size, edges, exudates, epithelization and pink/normal surrounding skin and nonappearance of wound hitches such as edema nor necrosis on the fifth day of experiment.

Even though there is disparity in the result of epithelization rate, this may be explained by lesser sodium content in Cocos nucifera as compared with NSS. That, if a safe dose of sodium or salt is supplemented to Cocos nucifera topical preparation, this may lead to better epithelization rate. Hence, this study highlighted that Cocos nucifera topical application is a viable, inexpensive, safe and effective substitute for standard NSS wound healing treatment.

Conflict of Interest

The authors declared no conflict of interests.

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