IMPACT: International Journal of Research in Applied, Natural and Social Sciences (IMPACT: IJRANSS)
ISSN(E): 2321-8851; ISSN(P): 2347-4580
Vol. 4, Issue 3, Mar 2016, 1-10

THE WOUND HEALING EFFECT OF POWDERED CARICA PAPAYA LEAVE

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ABSTRACT

Carica papaya has been investigated in treatments of ulcers and wounds especially in developing countries. This study was aimed at investigating the wound healing efficiency of powdered Carica papaya leave. Wistar rats were divided into 3 groups: Group 1(control): Treated with normal saline. Group 2 (control check): Treated with propylene glycol alone, Group 3 (experimental): Treated with powdered Carica papaya leave. Wound was inflicted and dressed with normal saline; propylene glycol and powdered Carica papaya leave respectively. The efficacy of treatment was assessed by the rate of wound closure, Wound contraction, fibroblast cell count and histology of granulation tissue. The result showed an insignificant difference in wound contraction ($P > 0.05$). Significant difference in wound closure was observed with group 3 been the fastest ($P < 0.05$). Fibroblast cell count showed statistical significant difference among the groups and across days ($P < 0.05$). Scar tissue also showed significant difference in fibroblast cell counts ($P < 0.05$). In conclusion, our study gave scientific background for the use powdered Carica papaya leave as a potential wound healing agent which is potent and faster in wound healing as against papaya extracts and normal saline among Wistar rats. It has also documented that propylene glycol has an enhancing therapeutic property in the wound healing process among Wistar rats.

KEYWORDS: Wound Healing, Carica Papaya, Fibroblast, Wound Contraction

INTRODUCTION

Wound healing is the body natural process of regenerating dermal and epidermal tissue (Stadelmann et al., [1998]; Iba et al., [2004]. A normal reaction to injury, a dynamic process with four continuous, overlapping events which overlap in time [Iba et al., [2004], and precisely programmed phases:-hemostasis, inflammation, proliferation, and remodeling (Peacock et al., [1984]; Mast [1992]). Components of plant (70%), mineral (20%) and animal origin10% are used for wounds and its management [Biswas et al., [2003] and it's fundamental to the practice of surgery [Bailey et al., [2000]. Propylene glycol is considered generally recognized as safe by the U.S. Food and Drug Administration, and it is used as a humectants (E1520) solvent, and preservative in food and for tobacco products, as well as being the major ingredient in the liquid used in electronic cigarettes. Wound contraction in rodents contributes about 80% - 90% of the total wound closure unlike in human that it contributes 25% - 50% (Theoret [2004].

Fibroblast later differentiates into myofibroblasts through which wound contraction is mediated to a great extent (a week after wounding), which in turn are attracted by fibronectin and growth factors to the wound edges (Junquera et al., [2010]. The actin in myofibroblasts usually contract resulting in the pulling of the wound edges together (Hinz, [2006]; Falanga, [2005]; Stadelmann et al., [1998]). Fibroblast begin entering wound 2-5 days after wounding as inflammatory phase is ending and their number peaks at 1-2 weeks post wounding (Larjava et al., [2002].

At the end of the first week fibroblast are the main cells in the wound and ends 2-4weeks after wounding [Stadelmann et al., [1998]. Oxygen is a major factor contributing to wound healing, it is important for cell metabolism, especially energy production by means of ATP, and is critical for nearly all wound-healing processes. It prevents wounds from infection, induces angiogenesis, increases keratinocyte differentiation, migration, and re-epithelialization, enhances fibroblast proliferation and collagen synthesis, and promotes wound contraction. (Bishop, [2008]; Rodriguez *et al*., [2008]). In addition, the level of superoxide production (a key factor for oxidative killing pathogens) by polymorphonuclear leukocytes is critically dependent on oxygen levels.

Carica papaya (Pawpaw) had been utilized for normal wound healing and antibacterial activities especially the leaf [(Giovannucci [1999]; Emeruwa, [1982]; Osato et al. [1993]), diabetic induced wounds [Iba et al., [2004]. Aqueous extract of Carica papaya leaves accelerates the progression of wound healing activity significantly, it prevents infection, reduces odour and possess antimicrobial activity (Mahmood et al. [2005]). The fresh leaves and extract of Carica papaya is used in treatment of burns, soft tissue wound and skin infection as its unripe fruit promotes granulation and healing especially skin and gastric ulcers (Hewitt et al., [2000]; Dawkins et al., [2003]; Chen et al., [1981]; Cho et al., [1984]). Antioxidant properties have been discovered and papaya leave contains Vitamin C in large quantity, involved in collage synthesis (Rimbach et al., [2000]). Anti-inflammatory (Gupta et al., [2000]), antifertility [(Udoh et al., [1999], antihypertensive properties (Eno et al., [2000], diuretic effects [Sripanidkulchai et al., [2001], urinary tract infections [Yusha'u et al., [2009] has been attributed to papaya leaves.

Propylene glycol is considered generally recognized as safe by the U.S. Food and Drug Administration, and it is used as a humectants (E1520) solvent, and preservative in food and for tobacco products, as well as being the major ingredient in the liquid used in electronic cigarettes. It is also used in pharmaceutical and personal care products as reported [Carl J. Sullivan (2005)]. Propylene glycol is a solvent in many pharmaceuticals especially for oral products, injectable and topical formulations, such as diazepam and lorazepam that are insoluble in water; it is use as a solvent in their clinical, injectable forms [Janusz et al., 1991].

MATERIALS AND METHOD

Animals

Female albino Wistar rats weighing 150-220 g were maintained the animal house of Department of Anatomy, University of Ibadan, in transparent plastic cage.

Experimental Design

The rats were randomly divided into three groups of 7 rats per group. All treatment was given topically. The contraction parameters and granulation tissues were taken on day 3, 6, 9 and scar tissue.

Wounds Evaluation/ Excision Wound Model

The animals were anesthetized with 1.5 ml of intravenous ketamine hydrochloride (120 mg/kg body weight). Wounds were created using the method of Mahmood et al. [2005]) with some slight modifications. The animals divided into three groups; Group 1 (control) received treatment with normal saline. Group 2 was treatment with propylene glycol and Group 3 was treated with Papaya powdered leave.

Measurement of Wound Area

A transparent sheet tracing the wound boundaries were used to measure the progressive wound area on every 3-days interval. The wound areas were recorded on a graph paper. Wound contraction was expressed as reduction in percentage of the original wound size. Wound healing efficacy was evaluated by the rate of wound sizes (closure), wound contraction, fibroblast cell count and histology of granulation tissue.

Ethical Approval

The authors here declare that the study was carried out with approval of the University of Ibadan Ethical Committee on Experimental Animal. Also the “Principles of laboratory animal care” as contained in the NIH publication No. 85-23, revised 1985 were duly observed by the authors.

Histological Analysis/Tissue Processing

Granulation tissue harvested on day 3, 6, and 9 together with the scar tissue were transferred to 10% formal saline to prevent autolysis and preserve the tissue for tissue processing. The tissues were then processed using standard method with Haematoxyline and Eosin stains. Fibroblast cell and blood vessel counts were obtained using a microscope and a graticle making counts at 5 different equidistant area of the granulation tissue on day 3, 6, 9 and on the scar tissue, at x400 in both control and experimental groups and slides were snapped with the aid of a camera.

Statistical Analysis

The mean of wound healing day, contraction rate, wound size and cell counts between the groups were determined from the results expressed in Mean± S.E.M using ONO-WAY ANOVA. The level of significance was taken at 95% confidence interval ($P< 0.05$)

RESULTS

Table 1: Wound Contraction Rate

Group	Days									
	0	3	6	9	12	15	18	21	24	27
GROUP 1	8.24	6.27	3.53	1.10	0.39	0.17	0.13	0.10	0.12	0.08
GROUP 2	8.59	4.96	3.09	0.72	0.32	0.16	0.08			
GROUP 3	8.73	5.27	2.44	0.40	0.13	0.12	0.04			

Group 1 was those treated with Normal Saline, Group 2 was treated with Propylene Glycol while Group 3 was treated with Powdered Papaya Leave.

Progressive increase was observed in the wound contraction rate with group 3 been fastest though these differences were not statistically significant at $P>0.05$ as seen in Table 1.

Table 2: Mean Percentage Wound Contraction (%)

Group	Days										
	0	**3**	**6**	**9**	**12**	**15**	**18**	**21**	**24**	**27**	
GROUP 1	0	24.16	56.15	86.31	95.23	97.86	98.28	98.77	98.52	99.05	
GROUP 2	0	36.32	61.01	90.28	95.48	98.10	99.05				
GROUP 3	0	37.80	71.47	95.36	98.48	98.57	99.52				

Progressive increase was also observed in the mean percentage wound contraction which is as a result of the progress observed in the wound contraction rate with group 3 been fastest though these differences were not statistically significant at P>0.05 as seen in Table 2.

Table 3: Mean Group Duration of Wound Healing in Days (Mean ± SD)

	Group 1	Group 2	Group 3
Mean	25.00 ± 4.58	17.25 ± 3.30	15.33 ± 3.22*

An enhanced mean group duration of wound healing in days was observed with the experimental group 3 been the fastest, nevertheless a new finding was observed that group 2 (propylene glycol alone) which was the substance used as a scaffold tend to show a strong wound healing efficacy as the second fastest and this property was unaware to us as seen in Table 3. The difference found in the wound healing in days was statistically significant at P< 0.05.

Table 4: Mean Fibroblast Cell Counts (Mean ± SD)

Days			
	GROUP 1	**GROUP 2**	**GROUP 3**
DAY 3	4.40 ± 0.52	5.00 ± 1.14	6.40 ± 1.67*
DAY 6	11.20 ± 1.39*	9.40 ±1.51	10.20 ± 1.17
DAY 9	**20.20 ± 0.84**	**22.20 ± 1.23**	**23.50 ± 1.10***
SCAR TISSUE	7.80 ± 1.74	11.20 ± 1.19*	7.10 ± 0.89

A statistically significant difference in the mean fibroblast cell counts across the groups in the granulation tissue was observed on day 3 and 9 with group 3 having the greatest count at P< 0.05. Significant difference was also observed on day 6 with group 1 having the greatest count and on scar tissue with group 2 having the greatest fibroblast cell count at P< 0.05 as seen in Table 4. There was also significant difference across the days in mean fibroblast cell count reaching its peak on day 9 P< 0.05.

Inflammatory phase of the wounds were characterized with lot of neutrophils, macrophages. Fibroblast begin entering 2-5 days after wounding as inflammatory phase is ending with its peaks at 1-2 weeks post wounding. The fibroblast cell count reaching its peak on day 9 so much that at the end of the first week fibroblast are the main cells in the wound and ends 2-4weeks after wounding accounting for the reduction in cell count observed in the scar tissue. This however affected the rate of wound contraction with its peak from D_6 to D_{15}. Collagen fibers were also as seen in the figures 1-4.

Figure 1: Histology of granulation tissue of *carica papaya leaves* group1-3. **(a-c)** granulation tissue of day 3

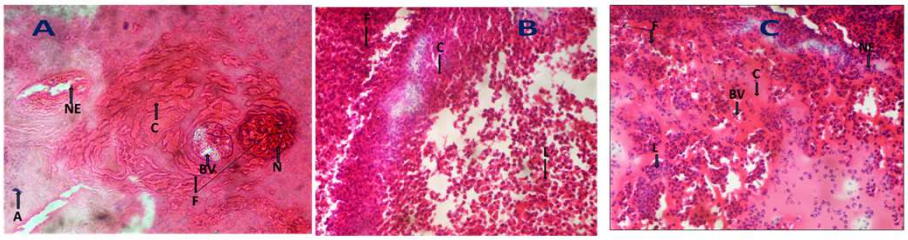

Figure 1: Histology of Granulation Tissue of *Carica Papaya Leaves* Group1-3. (a-c) Granulation Tissue of Day 3 Showing with Less Collagen (C) And More Macrophages, Little Fibroblast s(F), Neutrophils (Ne) Which Reflect a Recent History of Inflammation Triggered by the Injury, Collagen Fibers (C), Blood Vessels (Bv), Nerve Fibers (N) and Artifact (A). H & E X400.

Figure 2: Histology of Granulation Tissue of *Carica Papaya Leaves* Group1-3. Granulation Tissue of Day 6

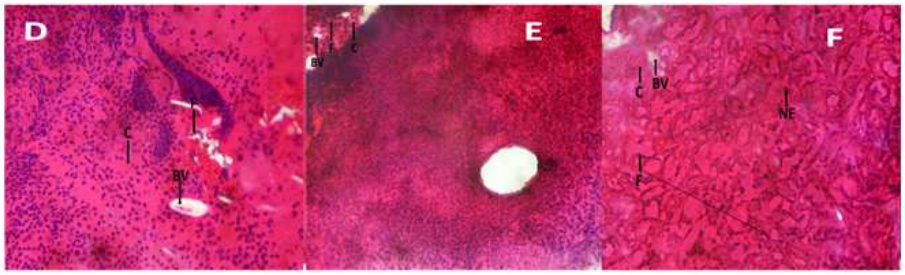

Figure 2: Histology of Granulation Tissue of *Carica Papaya Leaves* Group1-3. Granulation Tissue of Day 6 Showing Significant Collagenation, Fibroblasts H& E X400

Figure 3: histology of granulation tissue of *carica papaya leaves* group1-3. granulation tissue of day 9

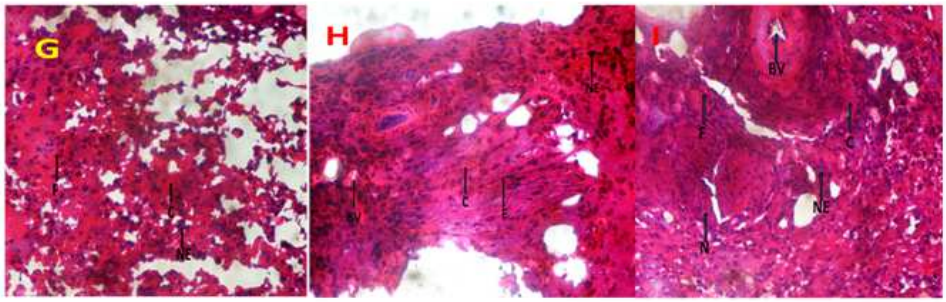

Figure 3: Histology of Granulation Tissue of *Carica Papaya Leaves* Group1-3. Granulation Tissue of Day 9 Showing Significant Collagenation, and Numerous Fibroblasts. H&E X400.

The histology of the scar tissue showed the presence of other cells present and the major division of the skin as well as the epidermal divisions of the skin. Scar tissues were not formed as seen specifically in figure 4.

Figure 4: histology of scar tissue

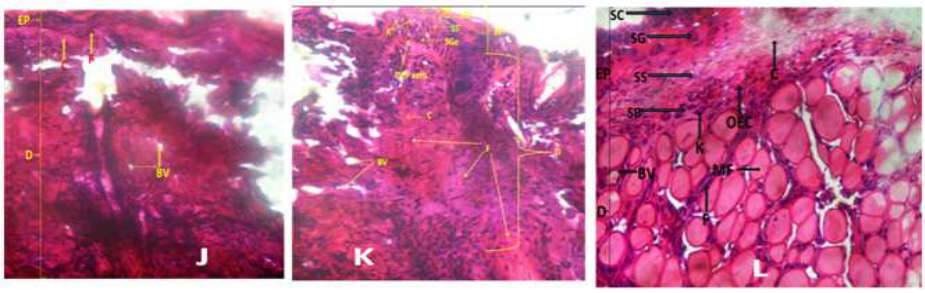

Figure 4: Histology of Scar Tissue Showing Layers of the Epidermis (Ep); Stratum Corneum (Sc), Stratum Granulosum (Sg), Stratum Spinosum(Ss), Stratum Basale (Sb),Also the Keratinocytes(k). Collagen Fibers(c), Lesser Fibroblasts (f) And Blood Vessels (Bv) And Nerve Fibers (n) Are Seen Within The Dermis (d). H&E X400

DISCUSSIONS

An enhanced wound contraction rate was observed which reflected in the percentage contraction, nevertheless this finding was not significant P>0.05.

Mean group duration of wound healing in days was observed in the experimental groups with group 3 been the fastest, however a new finding was observed that group 2 (propylene glycol) which was the substance used as a scaffold tend to show a strong wound healing efficacy as the second fastest and this property has not been. The differences in these mean wound healing day values among the groups was found to be statistically significant P< 0.05; this was in agreement with the work of Mahmood et al., (2005).

A statistically significant difference in the mean fibroblast cell counts across the groups in the granulation tissue was observed on day 3 and 9 with group 3 having the greatest count at P< 0.05. Significant difference was also observed on day 6 with group 1 having the greatest count and on scar tissue with group 2 having the greatest fibroblast cell count at P< 0.05. There was also significant difference across the days in mean fibroblast cell count reaching its peak on day 9 P< 0.05. This may be attributed to the enhanced wound contraction rate recorded between the groups. This assertion is based on the fact that wound contraction in rodents contributes about 80% - 90% of the total wound closure unlike in human that it contributes 25% - 50% (Theoret, 2004). Fibroblast begin entering 2-5 days after wounding as inflammatory phase is ending and their number peaks at 1-2 weeks post wounding (Larjava et al., [2002], this may be the reason why day 3 recorded the least count. Stadelmann et al., (1998) also reported that by the end of the first week fibroblast are the main cells in the wound and ends 2-4weeks after wounding accounting for the reduction in cell count observed in the scar tissue. In all groups the rate of wound contraction was at their peak from D_6 to D_{15}; thus, conforming to literature on wound contraction reaching its peak at 5 to 15 days post wounding in full thickness wound.

However the difference in wound contraction between the groups is most likely attributed to the fact that fibroblast, which later differentiates into myofibroblasts through which wound contraction is mediated to a great extent (Junquera et al., 1998), showed a significant difference between the groups (both the controls and the experimental) and across days. The fibroblast when stimulated by growth factors differentiate into myofibroblasts (a week after wounding), which in turn are attracted by fibronectin and growth factors to the wound edges. The actin in myofibroblasts usually contract resulting in the pulling of the wound edges together (Hinz, [2005]; Falanga, [2005]; Stadelmann et al., [1998]). Thus, any factor that facilitates fibroblast proliferation and growth factor excretion to wound sites could accelerate wound contraction.

Furthermore, no statistical difference was observed in the blood vessel counts in the granulation tissue of day 3, 6, 9 and the scar tissue in both controls and experimental groups, this may suggest that *Carica papaya* leave may not be a facilitator or promoter of angiogenesis. Increased angiogenesis requires a moist environment and angiogenesis occurs towards regions of low oxygen tension such that occlusive dressing may act as impairment to the process. Thus, the wound environment in the experimental group may not be adequately moist enough to bring about a statistically significant change in blood vessel count relative to the control group.

At the end of the experiment, there were no hypertrophic scars generated in wound dressed with both *Carica papaya* extracts and the controls.

CONCLUSIONS

This study has demonstrated that powdered *Carica papaya* leaves extract is a strong therapeutic agent in wound healing process significantly with respect to wound contraction, size and cell count. It has also documented that propylene glycol has an enhancing therapeutic property in the wound healing process.

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