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Carica papaya Latex Accelerates Wound Healing in Diabetic Wistar Rats

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Authors' contributions

This work was carried out in collaboration between both authors. Author RSA designed the study, supervised the experiment, wrote the protocol, performed the statistical analysis and wrote the final draft of the manuscript. Both authors RSA and KIO did the literature searches. Author KIO performed the experiment and managed the results. Both authors read and approved the final manuscript.

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ABSTRACT

Aim: The wound healing ability of *Carica papaya* latex in excisional wound of alloxan induced diabetic wistar rats was investigated.
Methodology: The excisional wounds of 24 diabetic rats in four equal groups were dressed respectively with latex (LD), gentamicin (GD), normal saline (ND) and propylene glycol (PD). A composite four non- diabetic (control) groups were also established (LC, GC, NC, and PC). All the eight groups had daily dressing till healed. Wound dimensions were taken at an interval of four days and obtained values were used to calculate wound contraction rates for specific period. Granulation tissue biopsies taken on day 4, 8 and 12 were processed for light microscopy. The healed scars were also processed for light microscopy.
Results: The latex control (LC) group had the shortest healing period (16 days) while the longest period of 32 days was recorded by the diabetic normal saline (ND) and diabetic propylene glycol (PD) groups. The differences in the healing duration amongst the groups were significant ($P < 0.001$).

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Comparison of highest and lowest wound contraction rates within the same period
The LC group had highest mean wound contraction rate of 21.56±7.04% on day 4 with the PD group being the lowest (6.45±2.32%). Similar pattern was observed on day 8; (LC: 55.71±4.87%) and (ND: 18.98±12.13%). For day 12, the results were LC: 84.86±5.11% and GD: 35.36±17.80%. All these paired values were of statistical significance.
Intra and intergroup comparisons of contraction rates
On day 4, LC had significantly higher contraction rate than LD (21.58±7.04; 11.06±2.82 respectively with P< 0.001). Also results for the same parameter on day 8 and day 12 were significant in LD vs GD (47.48±8.9 vs 21.06±5.83; P<0.001); LD vs ND (47.48±8.94 vs 18.98±12.13; P<0.001); LD vs PD (47.48±8.9 vs 23.12±5.77; P< 0.001) and GC vs GD (42.56±8.61 vs 21.06±5.83; P< 0.001).
Light microscopy of the granulation tissue from all the control groups showed more cellular infiltration than the respective diabetic group which might be due to hyperglycemia.
Conclusion: The study had been able to demonstrate that *Carica papaya* latex promotes wound healing in diabetic wistar rats. Possible mechanisms through which this was achieved include amelioration of the inhibitory effect of diabetes mellitus on wound healing, increased rapidity of wound contraction and reduction in the duration of healing.
From the results of this study, the latex of *Carica papaya* appeared superior to gentamicin, normal saline and propylene glycol as materials for wound management in diabetic wistar rats.
Latex is naturally occurring and its source (*Carica papaya*) is easily cultivated, readily available, affordable and cannot be adulterated.
It may thus be considered as an alternative to the synthetic materials in the current management of wounds in patients with diabetes mellitus.

Keywords: Carica papaya latex; wound healing; diabetic rats.

1. INTRODUCTION

A 2010 global estimate put the number of people between 20- 70 years of age afflicted by diabetes mellitus at over 284 million with a prevalence rate of 6.4%. These are expected to be 438.1 million and 7.7% respectively by 2030. Similar estimates for Nigeria are 2,819,000 and 3.9% for 2010 and 5,316,000 and 4.3% for 2030 [1].

Nearly all the body systems and organs are affected by uncontrolled diabetes and it could be a major source of agony to the patients and their relations and the care giver. Management of wounds in the setting of diabetes poses a serious challenge to the health care provider with not infrequent recourse to surgical options such as debridement, myocutaneous grafting and amputation of digits or limb [2,3].

The pathogenesis of delayed wound healing in diabetes involves impaired microcirculation, tissue hypoxemia, reduced collagen and fibronectin synthesis and impaired local immune defense [4].

Materials that constitute the main stay of wound management in our various health establishments are largely synthetic with somewhat restricted access and partial affordability by the patients. Also the use of fake and substandard materials and drugs poses a serious threat to the outcome of wound management.

The usage of plants (leaf, fruit, seed, stem, bark or root) for medicinal purposes has been with mankind from time immemorial. It could be in form of aqueous or alcoholic extracts.

Carica papaya is a wildly cultivated plant with very minimal tendering and can be regarded as a domestic plant. Its fruit besides being a veritable source of vitamin C, has latex especially the unripe fruit. The fruits, leaves, latex and bark of *Carica papaya* have been elucidated to have various components that were claimed to subserve diverse health and medical functions [5]. Latex is a whitish juice obtained mostly from the skin of unripe *Carica papaya* fruit. It can also be described as a complex mixture of chemical compounds with potent proteolytic function [6]. Active ingredients of latex are papain, chemopapain, peptidase A and B and lysozymes [7-10]. Other elucidated contents of latex are papaya endopeptidases II and IV, omega endopeptidase, chinitases, protease inhibitors and linamares [11,12]. Other documented activities of *Carica papaya* are bactericidal [13], anti- inflammatory, [14,15], antioxidant [16], anti tumour activity [17] and management of cutaneous ulcers [18].

Though there are numerous reported studies on medicinal plants, those on the latex of C*arica papaya* in diabetic rat wound are very scarce. Thus the need to conduct a study in this area became very pertinent hence the justification of this study.

2. MATERIALS AND METHODS

2.1 Plant Materials

2.1.1 Latex collection

The *Carica papaya* trees were identified on a farm in Ajibode area of Ibadan, Oyo State, Nigeria.

The latex was obtained from unripe but almost matured fruits early in the morning in order to get good yield. This was done by making 3-4 vertical incisions with a depth of 2 mm using a stainless blade on the undetached fruits. Prior cleansing of the fruits with wet napkin was done.

2.1.2 Preparation of the dressing material

The collected latex was oven dried to produce an off white powder weighing 18 g. A gel of the dried latex was obtained by mixing it with propylene glycol and this was used as the wound dressing material.

2.2 Animals

Forty eight adult male albino wistar rats weighing 200-270 g were procured from the Central Animal House, University of Ibadan. They were preconditioned for two weeks under controlled environmental conditions of temperature, humidity and ensuring adequate aeration and illumination. They were fed with pelletized feed and water.

2.3 Design of the Experiment

The criteria for allotment into groups were induced diabetes mellitus and wound dressing material; consequently, eight groups of six animals each were created. The control subgroups were Latex Control (LC), Gentamycin Control (GC), Propylene glycol Control (PC) and Normal saline Control (NC); while the diabetic subgroups were Latex Diabetic (LD), Gentamycin Diabetic (GD), Propylene glycol Diabetic (PD) and Normal saline Diabetic (ND).

2.4 Induction of Diabetes Mellitus

The pre induction fasting blood sugar levels were estimated with single touch glucometer (ACCU-CHECK®, Roche Diagnostics, Germany) using the blood obtained from the tails of the rats. A value range of 55-75 mg/dl was obtained. Based on the outcome of a preliminary study carried out by us, a single dose of 100 mg/kg body weight of alloxan monohydrate dissolved in normal saline and administered intraperitoneally was used to induce diabetes mellitus. Post induction blood sugar levels above 200 mg/dl were considered diabetic.

2.5 Wound Creation and Management

Each rat was sedated with intramuscular ketamine hydrochloride (120 mg/kg). After initial cleansing with savlon antiseptic liquid, a 2 cm by 2 cm full thickness skin about 1.5 cm from the vertebral column was excised.

2.6 Wound Management and Data Collection

The latex gel, gentamicin ointment, normal saline and propylene glycol was used as wound dressing material for the respective group (LC,LD; GC,GD; NC,ND; PC,PD). The wounds were dressed daily till healed. Prior to change of dressing, wound size estimation was done by taking measurement along two perpendicular planes. This was repeated every four days. The obtained values were used to calculate wound contraction rates in percentages.

Granulation tissue was harvested from a member of each group on day 4, 8 and 12. These samples were processed for light microscopy using Heamatoxylin and Eosin (H&E) stain.

The processed granulation tissues were used for evaluation of wound healing in the groups in terms of cellularity, angiogenesis, fibroplasia and collagen synthesis. Animals that had granulation tissue excised on these days were removed from the study. The wounds of the other members of the groups were allowed to heal and the scars were in similar fashion processed for light microscopy.

2.7 Data Analysis and Processing

The numerical aspects of the results were analyzed with Statistical Package for the Social Sciences (SPSS) version 20 and expressed as percentages, means plus standard deviation of means (SD). The student t- test was used for inter group comparison and level of significance was set at $p<0.05$.

3. RESULTS

3.1 Weight Trend

The trend in the weight was downward in the diabetic groups and upward in the non-diabetic groups. The values of the mean weights were significantly lower in the diabetic groups when compared with the non-diabetic groups ($P< 0.05$) (see Table 1).

3.2 Duration of Healing

The latex control (LC) group had the shortest healing period (16 days) while the longest period of 32 days was recorded by the diabetic normal saline (ND) and diabetic propylene glycol (PD) groups. The differences in the healing duration amongst the groups were significant ($P< 0.001$).

4. DISCUSSION

4.1 Duration of Healing and Mechanism of Latex

Diabetes mellitus is a systemic metabolic disorder characterized by persistent elevated blood sugar (glucose) level. This hyperglycemia may result from insulin resistant (Type 1 or Juvenile variety) or insulin insufficiency (Type 2 or Adult onset variety). Both types have pan affectation of the human body and systems.

The severity of the induced hyperglycemia in the experimental groups (LD, GD, ND & PD) was considerable as evidenced by the very high blood sugar level and progressive weight loss (Table 1).

Wound healing is a multiphase physiological and anatomical response by an injured segment of the body to restore normalcy either by similar tissue (regeneration) or by fibrous tissue (repair). This healing process may be delayed or totally arrested by both local and systemic factors such as diabetes mellitus.

The non-diabetic latex treated group (LC) had the shortest healing period of 16 days as opposed to the diabetic normal saline treated group (ND) that recorded 32 days (Table 2). The observed difference between these groups was significant ($P<0.001$). The duration of healing for the diabetic latex treated group (LD) was in between those of LC and ND (20 days). From the foregoing, it could be reasonably deduced that though, diabetes mellitus (DM) was able to retard the healing process in both LD and ND; the latex was able to reduce the severity of this retardation. This assertation was further strengthened by comparison of the healing duration of LC with non-diabetic normal saline treated group (NC) (16 vs 28 days) which gave a difference of twelve days.

Amongst the diabetes groups, the LD has the shortest duration of healing (20days) while the ND and diabetic propylene glycol treated group (PD) had the longest duration of 32 days. Could this observation be suggestive of anti-diabetic property of latex or its ability to ameliorate the effect of diabetes mellitus on wound healing? Credence was further lent to this presumption by noting that the difference between the healing duration of LC and LD was four days and this pair was the earliest amongst the four pairs of the experiment.

Mortality was recorded in three of the diabetes group namely ND, GD and PD; but not in the LD, this may be suggestive of antidiabetic property of latex.

In a related study by Nyak et al. [19] it was demonstrated that topical application of aqueous extract of *Carica papaya* to excisional wounds in induced diabetic rats, promoted healing by increased contraction rate and collagen turn over. Wound contraction is dependent on the content of myofibroblasts; these cells have features of fibroblasts and smooth muscle. At any given period of the study, the latex groups (LC and LD) had the highest respective wound contraction rates (Table 2), it can thus be deduced that latex stimulates production of myofibroblasts.

During wound healing, collagen is laid down by fibroblasts whose precursors are the myofibroblasts. In this study collagen was first seen in the LC and LD (though to a lesser degree in the latter) (Plate 2), thus one of the mechanisms via which latex stimulates wound healing is by increased collagen synthesis.

The delayed collagen synthesis in other groups could be attributed to the documented inhibitory effect of diabetes on collagen synthesis [20,21].

A consideration of the highest and lowest mean values of contraction rates for specific period (Table 3) showed that not only were the LC values the highest for the respective period of day 4, day 8 and day 12; the differences were significant (LC vs PD; LC vs ND and LC vs GD) with $P< 0.001$. It can thus be implied that latex accelerates wound contraction.

Table 1. Mean fasting blood sugar level and mean group weight

Parameter	LC	LD	GC	GD	NC	ND	PC	PD
Pre induction sugar level (mg/dl)	73.66±2.03	53.30±6.15	64.00±4.06	55.66±6.25	66.33±4.25	67.00±3.72	72.00±1.08	66.00±4.01
Post induction sugar level (mg/dl)	NA	403.50±2.43	NA	370.83±6.10	NA	400.03±1.54	NA	362.00±5.27
Pre induction weight (g)	216±8.75	211±8.36	205±9.17	214±8.01	206±8.75	209±7.35	216±8.75	215±4.47
Post induction-14 days (g)	218±6.42	206±7.11	206±5.19	210±6.28	209±7.34	190±7.32	217±7.54	190±7.21
Post induction-28 days (g)	225±8.42	200±6.68	215±9.38	202±8.23	220±8.21	170±8.18	226±7.32	200±6.49

NA= Not applicable.

All the four diabetic groups had markedly elevated blood sugar level following induction

The post induction blood sugar levels were significantly lower than the pre induction values (P< 0.001)

Table 2. Interval mean values of wound contraction rates in percentage (%)

Group	Day 4	Day 8	Day 12	Day 16	Day 20	Day 24	Day 28	Day 32
LC	21.56±7.04	55.71±4.87	84.86±5.11	*	*	*	*	*
LD	11.01±2.82	47.48±8.94	74.85±10.93	97.53±2.38	*	*	*	*
GC	18.50±6.11	42.56±8.61	68.08±11.13	82.96±8.95	94.50±7.79	*	*	*
GD	11.95±3.76	21.06±5.83	35.36±17.80	69.13±20.73	81.36±20.22	84.68±16.56	*	*
NC	12.83±3.82	21.30±12.46	43.28±12.24	68.95±5.93	81.95±7.63	94.09±8.71	*	*
ND	9.78±4.37	18.98±12.13	42.40±19.89	57.83±13.75	67.64±12.19	75.29±9.19	87.03±8.31	*
PC	15.13±3.03	27.58±2.90	46.50±3.06	65.35±2.51	81.76±4.06	94.78±4.18	*	*
PD	6.45±2.32	23.12±5.77	39.05±6.17	50.18±5.72	62.67±5.45	75.44±6.23	88.75±6.14	*

The asterisks connote that the wounds had healed. Thus the Latex control (LC) was the first group to achieve complete healing by day 16 while the Diabetic Normal saline and Propylene glycol groups (ND & PD) were the last to heal (day 32)

4.2 Intra and Inter Group Comparisons of Wound Contraction Rates

It was only on day 4 that a significant difference (P< 0.01) was observed between the contraction rates of LC and LD. Values for day 8 and day 12 were not. Similar comparison in the other groups (GC vs GD, NC vs ND and PC vs PD) were insignificant. These results may suggest that latex is able to initiate wound contraction earlier than gentamycin, normal saline and propylene glycol.

The comparisons of the LD group with the other diabetic groups i.e. LD vs GD, LD vs ND and LD vs PD (Table 4) produced significant results on day 8 and day 12. Since diabetes is known to retard or ultimately impede wound healing this observation of the results becoming significant on day8 and day12 could infer that latex was able to ameliorate the inhibitory effect of diabetes on wound healing. Thus it can be concluded that latex is capable of exerting anti diabetic though it may not be immediate. Further study is needed to know if this protective effect is local or systemic. In a study by Juárez-Rojop et al. [22] it was reported that the aqueous extract of C*arica papaya* was able to significantly reduced blood glucose level and also increased insulin secretion in streptozotocin-induced diabetic rats.

4.3 Gentamycin and Latex as Wound Healing Materials

Gentamicin is an aminoglycoside sensitive against gram negative bacteria [23-25]. It is available in both parenteral and ointment formulations. The ointment is commonly used as wound dressing material.

Comparison of the wound contraction rates between LC and GC at specific period (day 4, 8 and 12) clearly showed GC lagging behind. Similarly, values for LD vs GD showed GD coming mostly second to LD. Comparison between GC and GD produced significant difference only on day 8 and day12 (with P< 0.001 and P< 0.01 respectively).

Table 3. Highest and lowest mean values of wound contraction rates for specific period

Period	Contraction rates (%)
Day 4	21.56±7.04 (LC)a***
	6.54±2.32 (PD)
Day 8	55.71±4.87 (LC)b***
	18.98±12.13 (ND)
Day 12	84.86±5.11 (LC)c***
	35.36±17.80 (GD)

*Comparisons between the highest and lowest mean contraction values on days 4,8 and 12 showed that the value for the LC group was significantly higher than that of the : (a) PD group on day 4 ; (b) ND group on day 8 and (c) GD on day 12. *** P< 0.001*

Table 4. Intra and intergroup comparisons of contraction rates

Compared groups	Day 4 mean values	Day 8 mean values	Day 12 mean values
LC vs LD	21.58±7.04^{a**}	55.71±4.87	84.86±5.11
	11.06±2.82	47.48±8.94	74.85±10.93
LD vs GD	11.06±2.82	47.48±8.94^{b}***	74.85±10.93^{b}**
	11.95±3.76	21.06±5.83	35.36±17.80
LD vs ND	11.06±2.82	47.48±8.94^{c}**	74.85±10.93^{c}**
	9.78±4.37	18.98±12.13	42.40±19.89
LD vs PD	11.06±2.82^{d}*	47.48±8.94^{d}***	74.85±10.93^{d}**
	6.45±2.32	23.12±5.77	39.05±6.17
GC vs GD	18.50±6.11^{e}*	42.56±8.61^{e}**	68.08±11.13^{e}**
	11.95±3.76	21.06±5.83	35.36±17.80
NC vs ND	12.83±3.82	21.30±12.46	43.28±12.24
	9.78±4.37	18.98±12.13	42.40±19.89

The significant values for intra and inter group comparisons of mean contraction rates were: (a) the Latex Control (LC) being higher than Latex Diabetic (LD) on day 4; (b, c) LD was higher than GD and ND on days 8 and 12; (d) LD also higher than PD on days 4, 8 and12; (e) GC was higher than GD on all the 3 days.
*P< 0.05, **P<0.01 and ***P <0.001

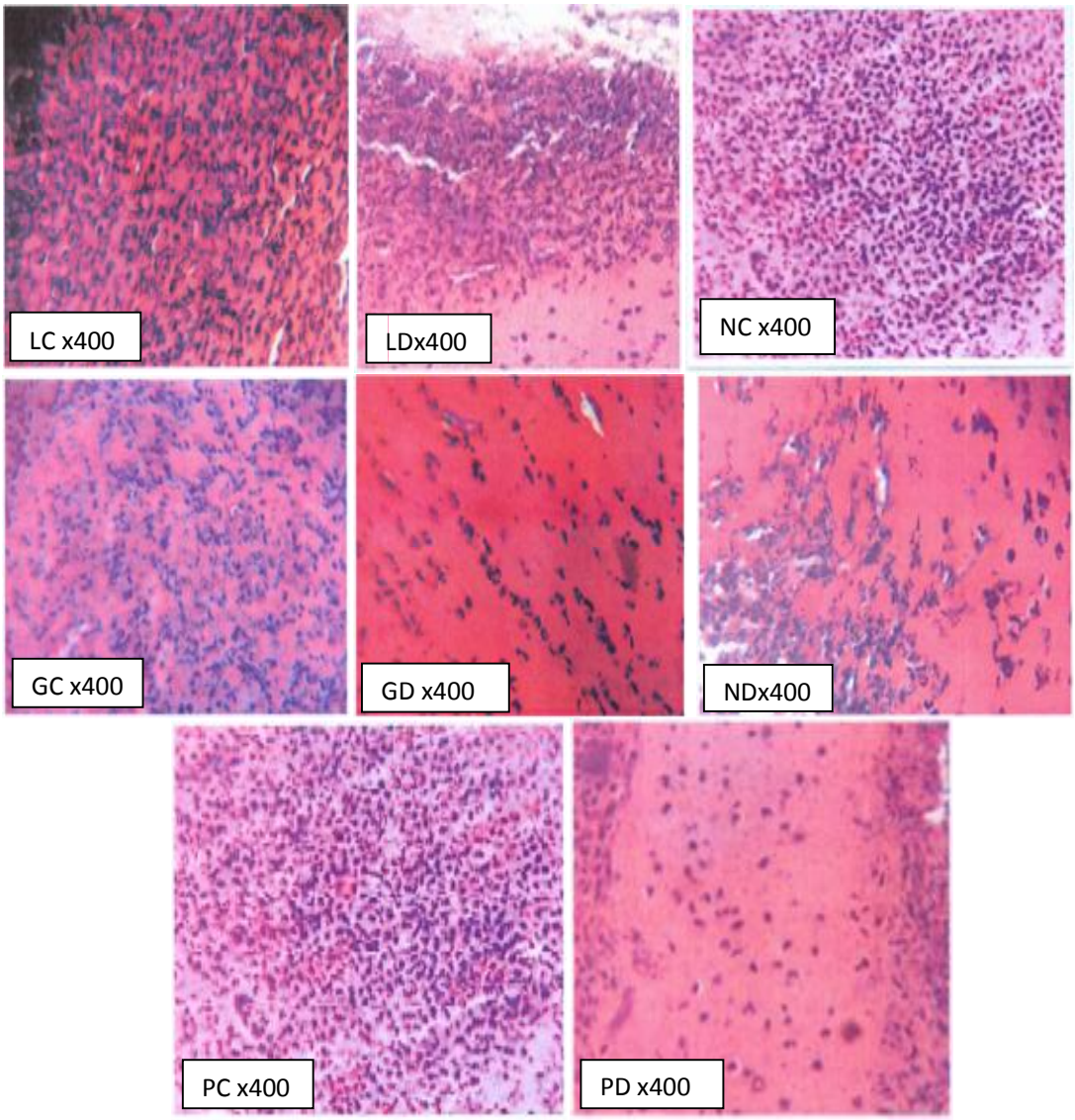

Plate 1. Granulation tissue at Day 4 H & E

LC (Latex control), LD (Latex diabetic), GC (Gentamycin control), GD (Gentamycin diabetic), NC (Normal saline control), ND (Normal saline diabetic), PC (Propylene glycol control) and PD (Propylene glycol diabetic)

Sections of the granulation tissue from all the control subgroups showed more cellular infiltration than the respective diabetic subgroup (see plate 1).

Section from the LC showed vascular endothelium (E), this is suggestive of angiogenesis. Fibroblasts and collagen are visible in the sections from GD and ND. Fibrillary structures suggestive of collagen are present in sections from LC, LD and PC (see plate 2).

Although, all the sections exhibited cellular infiltration but of lesser magnitude when compared with the day 4 sections.

Less intense cellular infiltration but increasing fibrillary structures were noted in all the groups on day 12 (see plate 3).

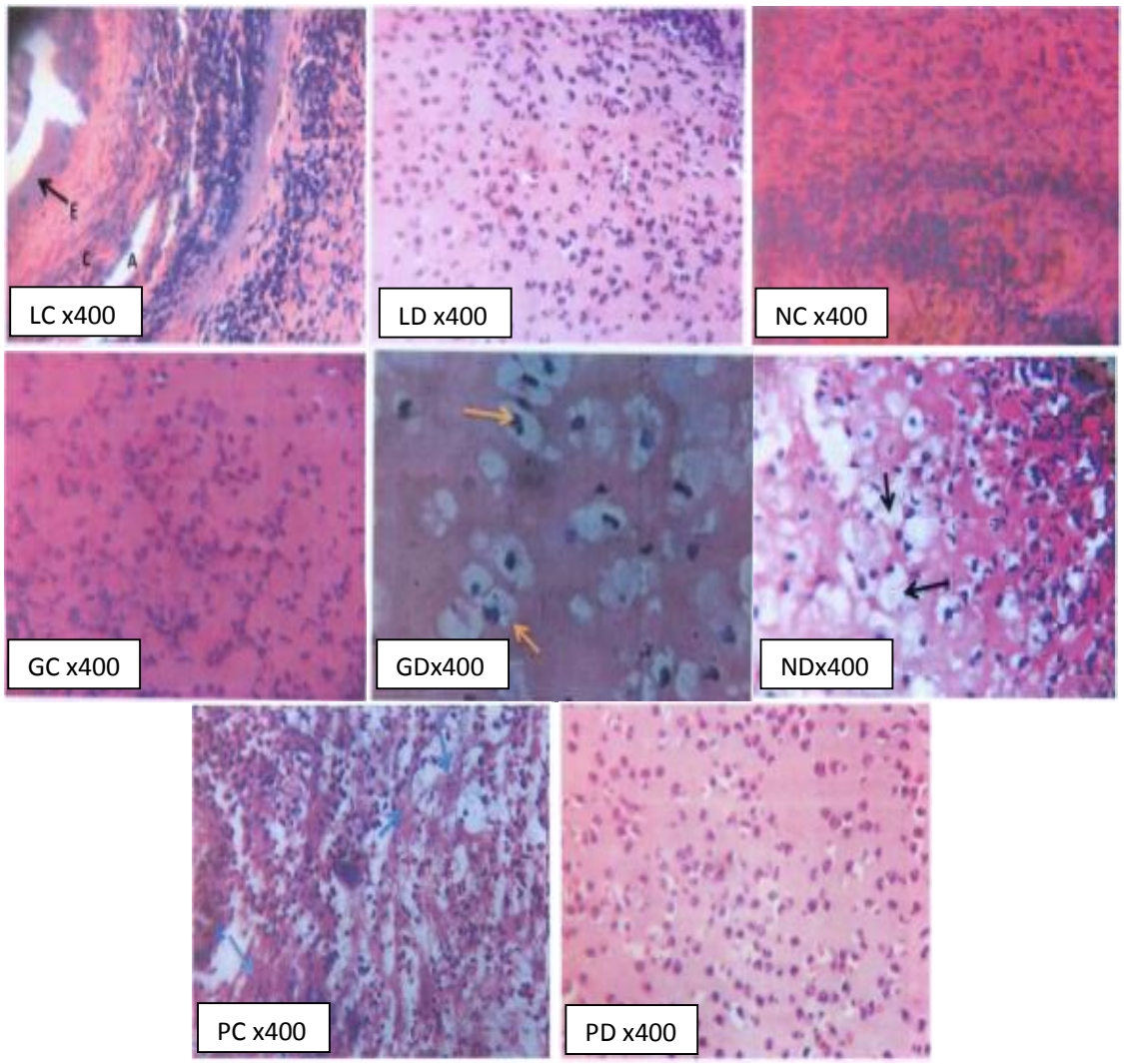

Plate 2. Granulation tissue at Day 8 (H&E)

LC (Latex control), LD (Latex diabetic), GC (Gentamycin control) GD (Gentamycin diabetic), NC (Normal saline control), ND (Normal saline diabetic), PC (Propylene glycol control) and PD (Propylene glycol diabetic)

The facts enumerated above showed that gentamicin like *Carica papaya* latex could ameliorate the negative effect of diabetes on wound healing in wistar rats but with lesser efficacy. Thus a deducible inference was latex could be a better wound dressing material to gentamicin ointment in the management of diabetic wound. This would confer a lot of benefits considering the readily availability and affordability of *Carica papaya* (source of latex) as opposed to gentamicin ointment which is synthetic and prone to adulteration.

In a related study in which hydrogel formulation of latex was used to dress induced burn wound in mice it produced superior result in terms of wound contraction and healing (epithelialization) time when matched against sulphadizine and chlorhexidine gluconate cream [26]. Also the Gomes et al. [27] study gave similar result.

4.4 Granulation Tissue

When compared with respective control, the light microscopy of the day 4 (Plate 1) granulation tissue of the diabetic groups had considerable lesser cellular infiltration. An intra-diabetic group consideration of this observation showed that the LD had the largest cellular consideration.

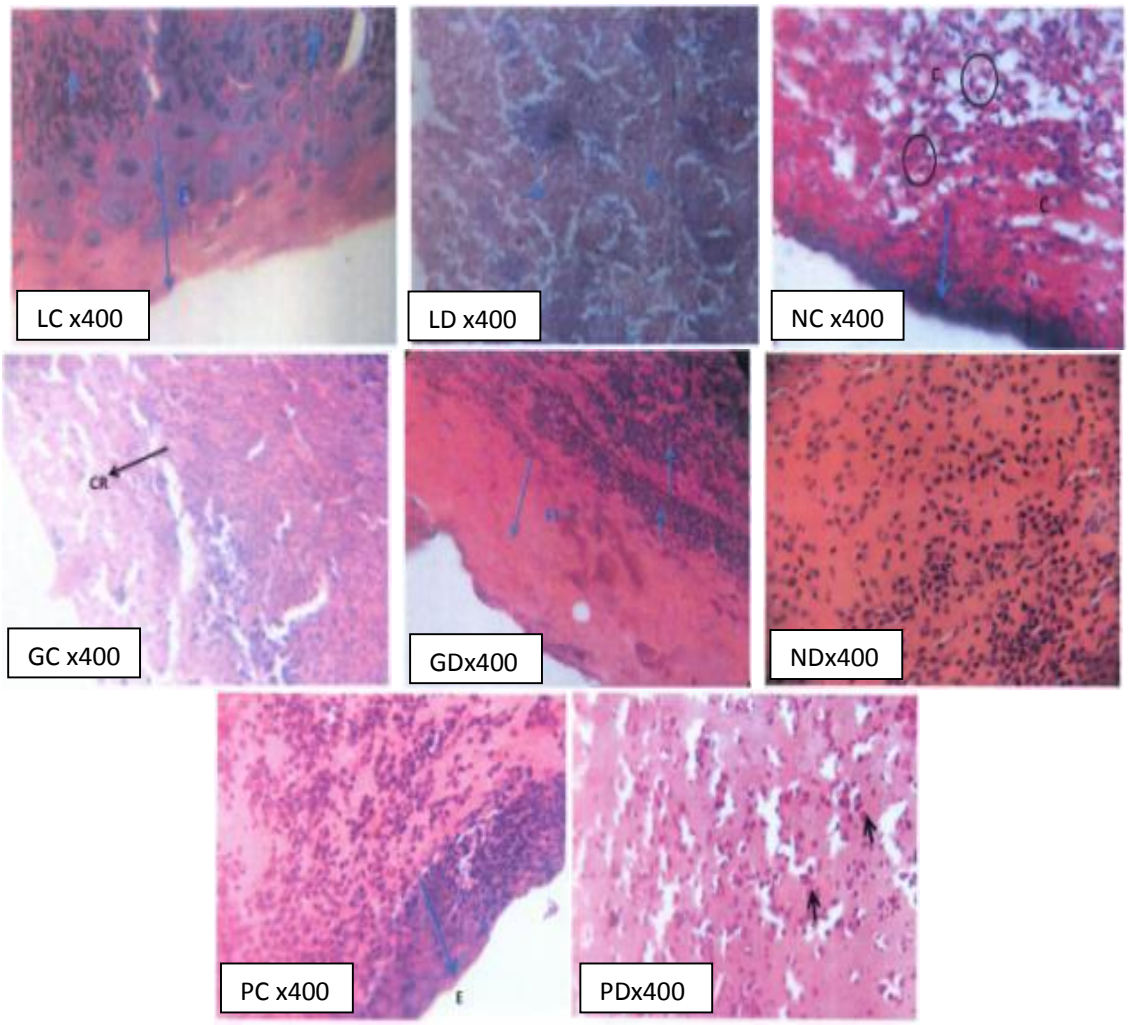

Plate 3. Granulation tissue on Day 12

LC (Latex control), LD (Latex diabetic), GC (Gentamycin control) GD (Gentamycin diabetic), NC (Normal saline control), ND (Normal saline diabetic), PC (Propylene glycol control) and PD (Propylene glycol diabetic).

Thus the initial inflammatory cellular infiltration of the wound healing process appeared delayed in all the diabetic groups, this delay was least in the LD group. This could further suggest the antidiabetic property of latex. Hyperglycemia had been reported to slow down neutrophil migration, chemotaxis, adherence and phagocytosis [28]. Since neutrophil is the main acute inflammatory leucocyte, the delayed cellular infiltration observed in the light microscopy of the granulation tissue of the diabetic groups was due to the inhibition of leukocyte function by hyperglycemia.

The histologic sections of the granulation tissues from all the groups on day 8 and day 12 showed progressive leveling of the cellular infiltration (Plates 2 & 3). Also, sections of the scar from all the groups showed epidermal appendages (Plate 4). These statements may present an erroneous impression that diabetes mellitus (DM) is self-limiting (reversible), it should be borne in mind that in this scenario, diabetes was induced, however, in humans the metabolic disorder (DM) is sequel to pancreatic destruction and malfunction.

Other studies that entailed use of *Carica papaya* extracts as wound dressing material in rats reported shorter duration of healing and increased wound contraction rates [29,30].

At this stage, epidermal and dermal structures are visible in all the groups (see plate 1).

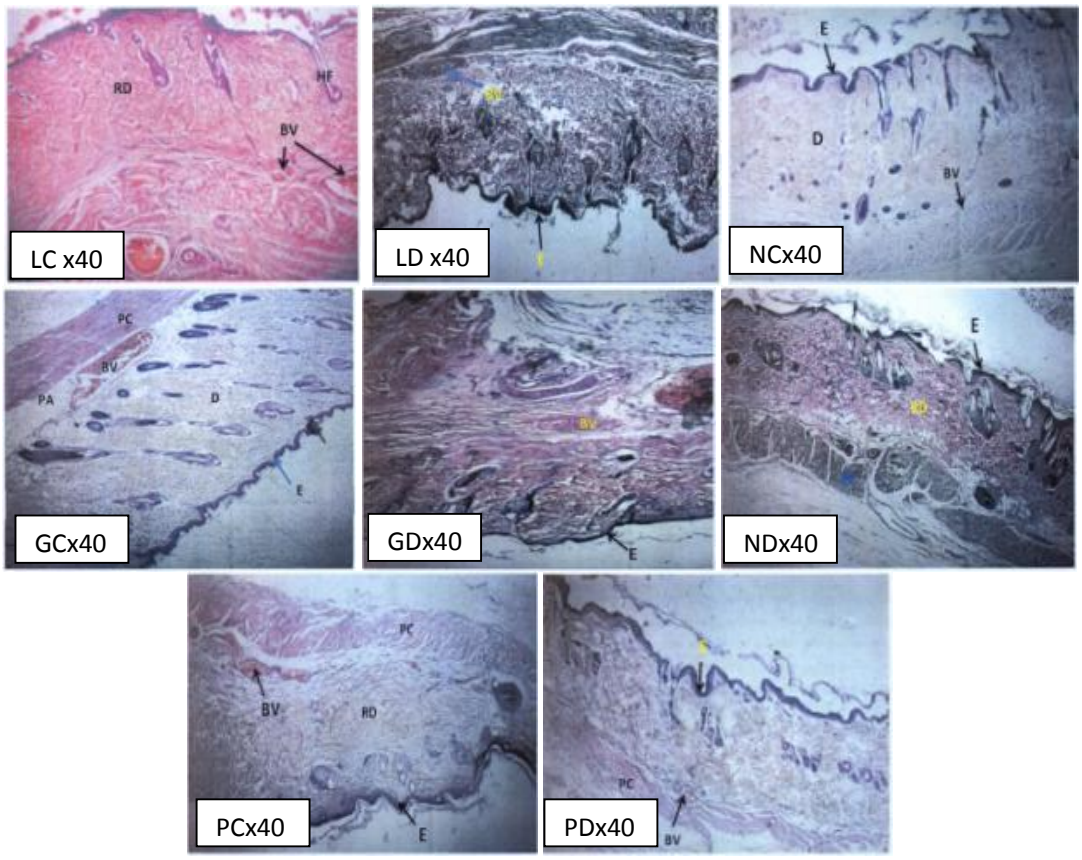

Plate 4. Sections from the wound scars (H & E)

LC (Latex control), LD (Latex diabetic), GC (Gentamycin control) GD (Gentamycin diabetic), NC (Normal saline control), ND (Normal saline diabetic), PC (Propylene glycol control) and PD (Propylene glycol diabetic)

5. CONCLUSION

The study had been able to demonstrate that *Carica papaya* latex promotes wound healing in diabetic wistar rats. Possible mechanisms through which this is achieved include amelioration of the inhibitory effect of diabetes mellitus on wound healing, increased rapidity of wound contraction and reduction in the duration of healing.

From the results of this study, the latex of *Carica papaya* appeared superior to gentamicin, normal saline and propylene glycol as materials for wound management in diabetic wistar rats.

Latex is naturally occurring and its source (*Carica papaya*) is easily cultivated, readily available, affordable and cannot be adulterated.

It may thus be considered as an alternative to the synthetic materials in the current management of wounds in patients with diabetes mellitus.

CONSENT

This is not relevant to this study.

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.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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