Original Article

Access this article online

Quick Response Code:

Website: jecajournal.com

DOI: 10.4103/jeca.jeca_6_19

Histological and morphometric assessment of cutaneous wound healing in streptozotocin-induced diabetic rats treated with n-hexane extract of *Leptadenia hastata*

Martha Orendu Oche Attah, Tonye Watson Jacks, Sani Hyelamada Garba, Helga B. Ishaya

Abstract:

CONTEXT: A wound is defined as a loss or breaking of cellular, anatomical, or functional continuity of living tissues. Diabetes may delay the process of wound healing leading to development of chronic wounds. Healing impairment of diabetic wounds presents serious clinical problems for both diabetic patients and physicians worldwide.

AIMS: This study aims to validate the use of *Leptadenia hastata* in the treatment of diabetic and nondiabetic wounds.

SETTINGS AND DESIGN: Diabetes mellitus was induced in twenty Albino rats using a single injection of streptozotocin (50 mg/kg i.p.). The rats were divided into four groups (III–VI) consisting of five rats each. In addition, ten nondiabetic rats were grouped into I and II.

SUBJECTS AND METHODS: Full-thickness excision wounds extending to the subcutaneous tissue were made on the mid-dorsal region, and rats in Group III–VI had their wounds treated with olive oil, 100 mg/kg of extract, 200 mg/kg of extract, and procaine penicillin, respectively. Rats in Groups I and II received olive oil and 200 mg/kg of extract, respectively, for 28 days. Wound areas were calculated, and histological sections of the wound area were analyzed.

STATISTICAL ANALYSIS USED: Data were statistically analyzed using GraphPad InStat software using one-way analysis of variance and expressed as mean ± standard error of mean and percentage followed by Bonferroni multiple comparisons test.

RESULTS: Analysis of wound area in all groups revealed that the extract promoted wound healing in the diabetic rats by significantly ($P < 0.05$) increasing the thickness of the epithelial layers and stimulated collagen synthesis.

CONCLUSIONS: The extract enhanced diabetic wound healing by reducing inflammation, increasing wound contraction and epithelialization.

Keywords:

Excision, *Leptadenia hastata,* re-epithelialization, topical application, wound healing

Department of Human Anatomy, Faculty of Basic Medical Colleges, University of Maiduguri, Maiduguri, Borno State, Nigeria

Address for correspondence:
Dr. Martha Orendu Oche Attah, Faculty of Basic Medical Sciences, Department of Human Anatomy, University of Maiduguri, Borno State, Nigeria.
E-mail: marthaorendua@gmail.com

Introduction

A wound is a breach formed in the normal continuum of the cellular and molecular structure of the body, thereby creating a disruption in the cellular, anatomic, and as well as in their functional continuity (Nasrabadi *et al.,* 2011). Wound healing is defined as the effort of adult

How to cite this article: Attah MO, Jacks TW, Garba SH, Ishaya HB. Histological and morphometric assessment of cutaneous wound healing in streptozotocin-induced diabetic rats treated with n-hexane extract of *Leptadenia hastata*. J Exp Clin Anat 2019;18(1):19-29.

tissues to restore normal tissue function and architecture after one of a variety of physical, mechanical, biological, or chemical insults (Davis, 2008). Wounding and wound healing occurs in all tissues and organs of the body. Although the process of healing is continuous, it is arbitrarily divided into several phases to aid understanding of the physiological processes that are taking place in the wound and surrounding tissue (Richardson, 2004). Healing is a complex process involving coordinated interactions between varied immunological and biological systems, and it involves a cascade of carefully and accurately regulated steps and events that correlate with the appearance of various cell types in the wound bed during distinct phases of the healing process (Glat and Longaker, 1997; Hunt *et al*., 2000; Attinger *et al.,* 2006; Broughton *et al.,* 2006). The various processes of acute tissue repair, which are triggered by tissue injury, may be united into a sequence of four time-dependent phases (Velnar *et al.,* 2009). These phases are coagulation and hemostasis, inflammation, wound proliferation, and wound remodeling. The process of wound healing in diabetic patients may well take longer than healing would take in nondiabetic patients, but the healing process is impaired rather than prevented (Falanga, 2005). A slow or nonhealing diabetic wound is prone to many complications which can delay the healing process, and delay has a significant negative effect on both patient and family. These complications include functional/anatomical limitations, including alteration in gait and difficulty in walking; infection including cellulitis, abscess, and osteomyelitis; gangrene and septicemia and possible malignant changes (Goodson and Hunt, 1977; Menke *et al.,* 2007). It has been discovered by several researchers that the inflammatory phase of wound healing is altered/impaired in the diabetic patient (Lioupis, 2005; Kidman, 2008). Kidman (2008) suggested that this could, in part, be due to the thickening of the blood vessels, therefore reducing the numbers and speed in which leukocytes reach the site of injury. Leukocytes play an important role in wound healing by ingesting foreign microorganisms, and the reduction in leukocyte numbers makes diabetic wounds prone to infection (Kidman, 2008). Proliferation phase, which follows the inflammatory phase, can also be compromised in the diabetic patient as the cytokine (or chemical messenger) profile of the wound bed can be altered (Loots *et al.,* 2002). Another complication in diabetic wound healing is decreased collagen concentration in the dermis (Nakao *et al.,* 2009). Collagen plays an important role in increasing wound strength. Healing impairment of diabetic wounds presents a serious clinical problem for both diabetic patients and physicians worldwide, hence the need for management of diabetic wounds adequately to prevent tissue death and subsequent amputation.

Leptadenia hastata (Pers.) Decne is a wild plant which belongs to the family *Asclepiadaceae* and is used as vegetable by many African populations and as medicine due to its nutritive and therapeutic properties (Maina *et al*., 2013). As a result of its nutritional and medicinal values, the plant has been used for centuries as remedies for human diseases including hypertension, catarrh and skin diseases (Thomas, 2012), treatment of wounds and stomach upset conditions in children (Aliero *et al.,* 2001; Tamboura *et al.,* 2005), prostate and rheumatism complaints (Thomas, 2012), rhinopharyngitis and polydipsia (Aliero and Wara, 2009). It has also been shown to have antimicrobial and anti-inflammatory properties (Thomas, 2012, Khan, *et al.,* 2014). Phytochemical analysis of the plant has revealed active constituents such as alkaloids, flavonoids, steroids, phenols, glycosides, resins, saponins, balsams, volatile oil, triterpenoids, and tannins (Abubakar *et al.,* 2014, Attah *et al.,* 2019). Wounds are caused by a number of factors, including cuts, abrasions, gunshots, crush injury, fire, heat, radiation, chemicals, and sunlight. Wounds differ according to the underlying causes, they can be open or closed wounds, and depending on how the wound heals, they are categorized into acute and chronic wounds (Mittal *et al*., 2013). Acute wounds result from cuts or surgical incisions; they heal within expected time frame (Alam *et al.,* 2011). However, if healing does not occur within the normal time frame, pain and swelling will be produced at the wound site by inflammatory mediators (Mittal *et al.,* 2013). Chronic wounds are wounds that have failed to heal within a normal time frame, and they, therefore, start to cause complications and their healing process becomes delayed. Sometimes, even after healing, they reoccur regularly (Alam *et al.,* 2011). Over the years, there has been increased demands for a wound healing therapy that is both readily available and effective in management of diabetic wounds and ulcers. This quest triggered the scientific investigation of *L. hastata* on topical diabetic and non-diabetic wounds, especially in areas with limited access to healthcare.

Subjects and Methods

Experimental animals

A total of 30 albino rats weighing 135–190 g were used. The rats were obtained from the National Veterinary Research Institute Vom, Plateau State, Nigeria. They were kept in the Animal house of the Department of Human Anatomy, Faculty of Basic Medical Sciences, College of Medical Sciences, University of Maiduguri, Borno State for 2 weeks before the start of the experiment to acclimate to the new environment. The rats were weighed and maintained under controlled conditions of humidity of 50%–60%, temperature of 22°C ± 30C, 12 h light and 12 h dark as well as adequate ventilation. They were fed with

pelletized ECWA (Jos) feed and water *ad libitum*. The experimental procedures were conducted in accordance with of the institution. The animals were maintained in compliance with the principles and guidelines of the Research Ethics Committee of the Faculty of Basic Medical Science, University of Maiduguri, Nigeria with the reference number UM/HA/PGP 16.17–002.

Plant material and extraction

L. hastata was collected from a garden in the University of Maiduguri, Borno State, authenticated by a plant taxonomist, from the Department of Biological Sciences, Faculty of Science, University of Maiduguri. The leaves were harvested, washed, and shade dried for a period of 2 weeks and then ground to powder mechanically using a mortar and pestle. The powder was sieved to obtain the fine powder; it was then labeled and stored for use. Maceration technique as described by Azwanida (2015) was used for extraction in the current study. The leaf powder weighing 500 g was dissolved in 3 L of n-hexane in a 5 L stoppered container. Maceration involved soaking the plant which is allowed to stand at room temperature for a period of 3 days at the minimum with periodic agitation. The process softened and broke the plant's cell wall to release the soluble phytochemicals. After 3 days, the mixture was filtered using Whatman's filter paper. The resulting n-hexane filtrate was concentrated to dryness in vacuo using an evaporator, and the resulting powder was kept in an air-tight container and refrigerated. Olive oil was used as vehicle to dissolve the extract as it was insoluble in water.

Experimental design

The animals were divided into six groups:

- Group I: Nondiabetic rats. Wounds were treated with olive oil
- Group II: Nondiabetic rats; wounds were treated with 200 mg/kg of n-hexane extract of *L. hastata*
- Group III: Streptozotocin-induced diabetic rats; wounds were treated with olive oil
- Group IV: Streptozotocin-induced diabetic rats; wounds were treated with 100 mg/kg of n-hexane extract of *L. hastata*
- Group V: Streptozotocin-induced diabetic rats; wounds were treated with 200 mg/kg of n-hexane extract of *L. hastata*
- Group VI: Streptozotocin-induced diabetic rats; wounds were treated with procaine penicillin.

Experimental induction of diabetes in rats

Hyperglycemia was induced in overnight fasted albino Wistar rats by a single intraperitoneal injection of 50 mg/kg streptozotocin (Bristol-Sigma, Bristol Scientific Company, Missouri, United States of America) dissolved in 0.1M ice-cold sodium citrate buffer (pH = 4.5), immediately before use in a volume of 1 ml/kg body weight as described by Etuk (2010). Hyperglycemia was confirmed by the elevated plasma glucose levels determined in tail blood sample using a glucometer (Roche, Germany). Rats whose fasting blood glucose levels exceeded 250 mg/dl (13 mmol/dl) after 1 week were considered as diabetic and used for the study. Urinalysis was also carried out to confirm diabetes in all groups according to a method adopted by Houcine *et al*. (2011).

Wound creation

The rats in all groups were anesthetized by intraperitoneal injection of xylazine hydrochloride (10 mg/kg) and ketamine hydrochloride (25 mg/kg) to induce and sustain the subconscious state, respectively. This was according to a method carried out by Gal *et al*. (2008). The dorsal surface hair was then trimmed with a pair of scissors, and a depilatory cream (EU cream, United Trading and Manufacturing Ltd., Karachi, Pakistan) was used to remove the remaining hair. The shaved area was rinsed with 10% iodine solution (Gal *et al*., 2008; Oryan *et al*., 2010).

A paper template of 240 mm^2 surface area was placed on the mid-dorsum of the rats and the edges traced as boundary of the wound as shown in Figure 1a. Lignocaine (1 mg/kg) was used to numb the area, diclofenac (1 mg/kg) was used for analgesia, and procaine penicillin (50,000 IU/kg) was used as prophylaxis against infection. The template was removed, and an incision was made on the skin, extending to the fascia along the traced area. The skin was then removed using a pair of dissecting scissors and forceps [Figure 1]. The area around the wound was swabbed with antiseptic to ward off flies. The method adopted was as carried out by Karodi *et al*. (2008).

Measurement of rate of wound contraction

Photographs of the wounds from each group were taken on days 1, 3, 5, 10, 15, and 20, respectively, representing after wound creation, the end of inflammatory phase,

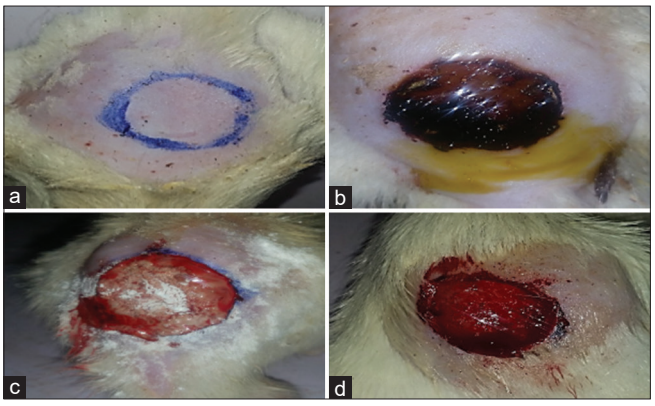

Figure 1: Traced region on shaved dorsal surface (a), the traced area is excised to create a wounded region which is treated with n-hexane extract of *Leptadenia hastata* (b), procaine penicillin powder (c), and olive oil (d)

during formation of granulation tissue, during re-epithelialization phases (early and late), and a few days before the day of sacrifice.

$$\text{Wound contraction } (\%) = \frac{\text{Initial wound size} - \text{Specific day woundsize} \times 100}{\text{Initial wound size}}$$

Tissue preparation

The rats were sacrificed at the end of the experimental study, and the skin tissue over the wounded region was removed and preserved in 10% formalin and processed through routine histological analysis and stained with hematoxylin and eosin to demonstrate general histological features and Masson's trichrome to demonstrate collagen fibers.

Morphometric analysis of the skin

The epidermal region in the skin sections were viewed under × 400 magnification, and each of the layers were measured in all groups using an ocular micrometer. The specimen preparations from the skin of each rat were subjected to morphometric analysis to determine as described by Omar, (2010) and Zaki, (2015). The following parameters were measured:-epidermal layer thickness, i.e., thickness of strata basale, granulosum, spinosum and corneum in µm, collagen fiber thickness (µm), and diameter of fibroblast.

Statistical analysis

Data were statistically analyzed using GraphPad InStat software Inc (version 3.75), California using one-way analysis of variance and expressed as mean ± standard error of mean and percentage followed by Bonferroni multiple comparisons test. $P < 0.05$ was considered to be statistically significant.

Results

Physical appearance of the wounds during the healing process

The wounds of the rats of all groups on the 1st day showed a bright red color corresponding to the inflammatory phase. The wound areas increased in all the animals from the 3rd to the 5th days leaving a wider wound area than was created [Figure 2]. This increase was significant ($P < 0.05$) in rats in Groups IV and V. After two successive daily applications of vehicles in the different groups (day 3), wound aspects differed across the different groups and vehicle used. In Group I (treated with olive oil), the wounds showed inflammatory and thick beading at the wound edges. This was also observed in Groups II, IV, and V. In the diabetic untreated group (Group III), there was no observed beading at the edge of the wounded area. There was a dark red area in the procaine treated wounds which had a dry surface (Group VI). This was more prominent by the 10th day [Figure 3a-f]. The diabetic untreated group exposed its granulation tissue last. The crusted tissue was more pronounced in the animals who had the extract applied (Groups II, IV, and V).

On the 5th day, the wounds in the normal control had a brown color characteristic of the presence of the crust with a narrowing of the wound surface area. In Group II, IV, and V, there was the formation of a dark red crust on the wound area. The group treated with procaine penicillin had a centralized clot surrounded with a pale region. The hard covering of the crusted scab tissue helped pull the wound edges together, and this facilitated wound contraction in the normal control and nondiabetic control groups, thus decreasing the wound areas in all groups from days 10 to 15 [Figure 4.].

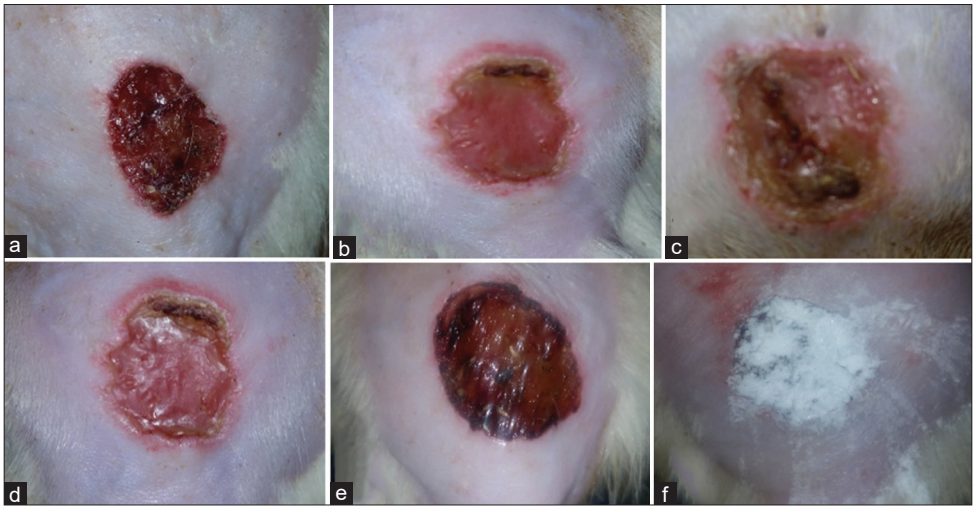

Figure 2: Wound areas on day 5 in experimental animals. Normal control (a), nondiabetic experimental (b), diabetic control (c), 100 mg/kg (d), 200 mg/kg (e), and procaine penicillin (f)

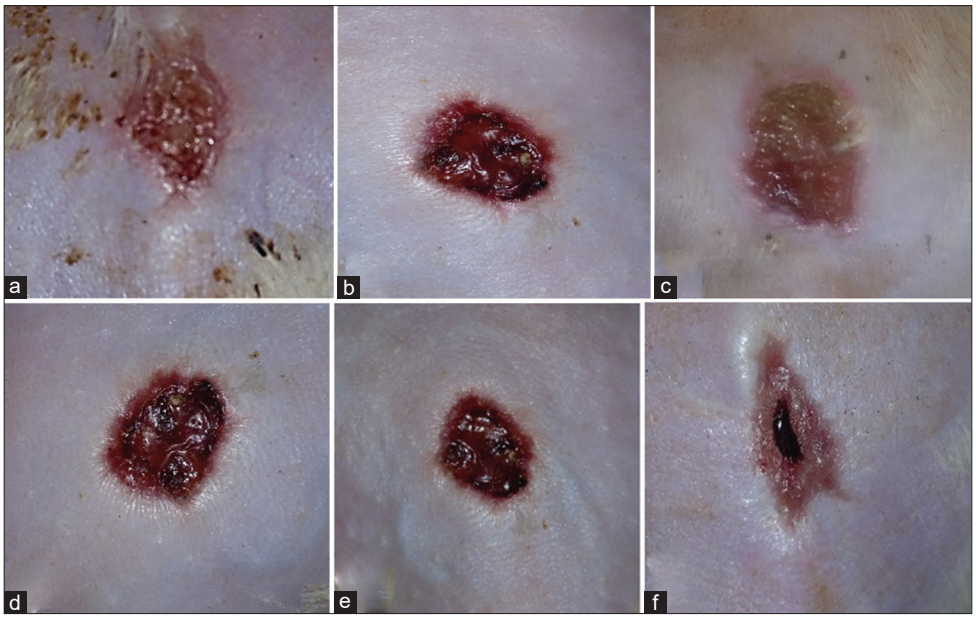

Figure 3: Wound areas on day 10 in experimental animals. Normal control (a), nondiabetic experimental (b), diabetic control (c), 100 mg/kg (d), 200 mg/kg (e), and procaine penicillin (f)

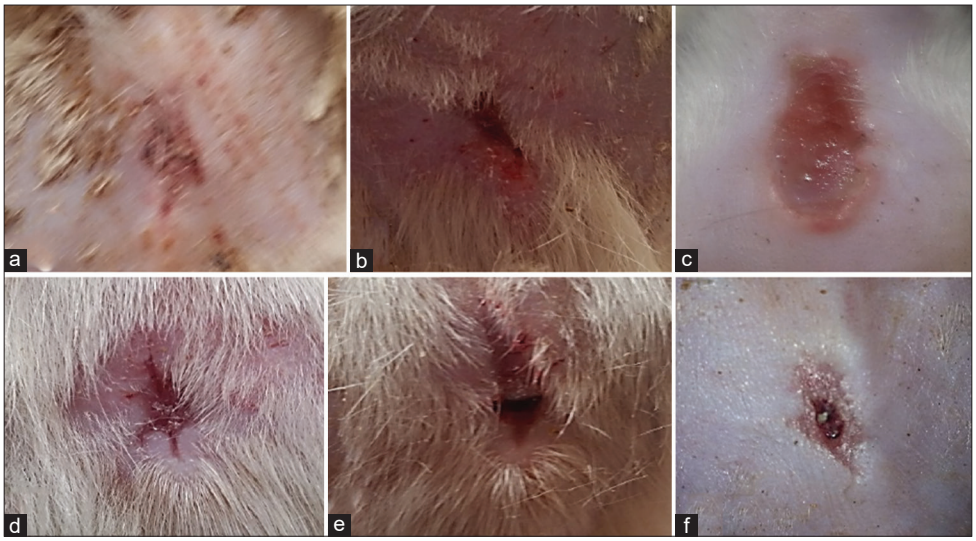

Figure 4: Wound areas on day 15 in experimental animals. Normal control (a), nondiabetic experimental (b), diabetic control (c), 100 mg/kg (d), 200 mg/kg (e), and procaine penicillin (f)

On day 10, some scabs formed in the nondiabetic rats (Groups I and II) and the 100 mg/kg and 200 mg/kg of extract treated rats (IV and V) began to raise at the edges, to reveal a pinkish color of granulation tissue underneath. In the wounds treated with procaine penicillin, the brown coloration continued to appear. The diabetic untreated group (Group III) still had a red color and moist appearance as well as a wider surface compared to the wound surfaces in other groups.

On day 16, the crust had fallen off in Group I and Group VI on day 17; the scab fell off from the nondiabetic control group. On the 18th and 19th days, the scab fell off the wound area in the Group IV and V, respectively. In Group III, the scab fell off on the 27th day, just before sacrifice. The wounded area had significantly ($P < 0.05$) decreased in size in the extract treated groups compared to the diabetic control group [Figure 5].

Quantitative assessment of wound healing

Wound healing activity was investigated in rats treated with olive oil (I and III), n-hexane extract of *L. hastata* (Groups II, IV, and V), and procaine penicillin for Group VI. The wound area in all groups in the 1st day was made following a template which was approximately 240 mm^2 wide. The wound area in animals in all groups was wider from the 3rd to 5th days [Table 1]. The wounds in the animals in Groups

II and IV were larger in size than all other groups. This expansion in wound area could be attributed to the action of underlying subcutaneous muscle, which led to an expanded wound area in all groups except the group treated with procaine penicillin powder (Group VI). Re-epithelialization was rapid in this group also as the granulation tissue was formed earlier (day 7) compared to other groups. There was also rapid wound closure in Groups I and II in the 10th to 15th days post injury to, and this could be attributed to normal wound healing processes in these groups as the rats in these groups were nondiabetic. Granulation tissue was laid fastest in the group treated with procaine penicillin and then later in the normal control and nondiabetic groups. The wound diameters in the diabetic animals that were treated with 100 mg/kg and 200 mg/kg of extract showed faster from days 5 to 10, showing that the extract may have an effect in early epithelialization stage of wound healing [Table 1].

Rate of wound contraction

The percentage change in the rate of wound contraction is presented in Table 2. Initially, there was an increase in wound diameter for rats in all groups which was significant in the extract treated groups (II, IV, and V) till day 10 where there was significant ($P < 0.05$) rate of

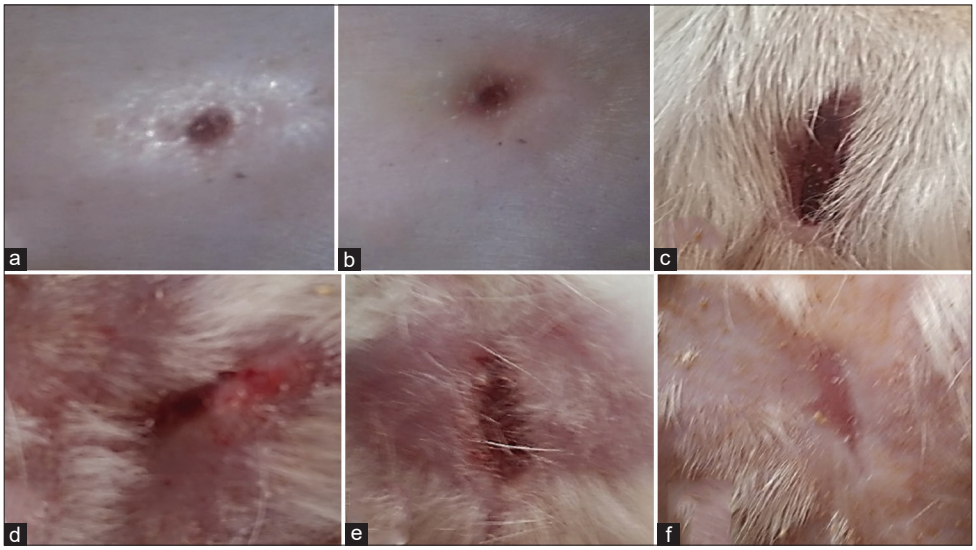

Figure 5: Wound areas on day 20 in experimental animals. Normal control (a), nondiabetic experimental (b), diabetic control (c), 100 mg/kg (d), 200 mg/kg (e), and procaine penicillin (f) wound areas

Table 1: Quantitative assessment of the healing process

Groups	Treatment	Days					
		1	3	5	10	15	20
I	-	240.0^{a}	258.0±7.3^{a}	291.8±2.1^{a}	243.8±11^{a}	89.7±8.6^{a}	38.8±1.9^{a}
II	Extract (200 mg/kg)	240.0^{a}	301.6±4.0^{b}	292.0±8.5^{a}	284.4±11^{a}	86.2±2.6^{a}	41.8±4.4^{a}
III	-	240.0^{a}	260.0±0.9^{a}	298.8±2.5^{a}	258.6±12^{b}	105.3±2.5^{b}	97.6±1.8^{b}
IV	Extract (100 mg/kg)	240.0^{a}	285.6±5.0^{b}	288.8±0.5^{a}	135.9±3.9^{b}	91.36±3.4^{a}	83.0±1.4^{b}
V	Extract (200 mg/kg)	240.0^{a}	328.8±5.5^{b}	284.8±4.2^{a}	143.4±4.6^{b}	89.2±7.6^{a}	73.6±3.2^{b}
VI	Insulin	240.0^{a}	233.2±3.6^{b}	280.4±6.9^{a}	212.2±3.0^{b}	121.9±14^{b}	96.5±2.9^{b}

The values are expressed as mean±SEM. Values in the same column with different superscript are significantly different at $P<0.05$. Values in the same column with the same superscript are not significant. The unit for the above measurements is mm^2. SEM - Standard error of mean

Table 2: The rate of wound contraction

Groups	Treatment	Percentage change in wound diameter (%)				
		Day 3	Day 5	Day 10	Day 15	Day 20
I	-	−7.5±4.6^{b}	−21.5±6.5^{a}	−1.25±3.6^{a}	62.6±2.1^{a}	83.8±2.6^{a}
II	Extract (200 mg/kg)	−25.4±2.6^{a}	−21.6±4.3^{a}	−18.5±4.2^{a}	64.0±2.6^{a}	82.5±4.1^{a}
III	-	−8.5±6.63^{b}	−24.1±4.4^{a}	−7.4±4.5^{a}	56.0±3.4^{a}	59.3±4.8^{a}
IV	Extract (100 mg/kg)	−19.0±5.1^{a}	−20.0±3.6^{a}	43.8±3.2^{a}	61.9±2.1^{a}	65.0±6.6^{a}
V	Extract (200 mg/kg)	−36.6±6.3^{a}	−18.3±5.5^{a}	40.4±4.2^{b}	62.8±3.0^{a}	69.5±3.2^{a}
VI	Insulin	2.9±7.2^{b}	−16.6±6.6^{a}	11.6±4.6^{a}	49.2±2.6^{a}	59.8±4.4^{a}

The values are expressed as mean±SEM. Values in the same column with different superscript are significantly different at $P<0.05$. Values in the same column with the same superscript are not significant. SEM - Standard error of mean

closure in the extract treated rats. By day 15, there was a substantial increase in the rate of wound contraction in rats Groups II, IV, and V. However, on the 20th day, there was only a considerable decrease in wound size in all groups.

Histological observations in skin

Figures 6-9 are micrographs representing the epidermis and dermis of animals from the wounded area stained with hematoxylin and eosin to observe the general structure of the skin as well as Masson's trichrome to stain collagen fibers in the dermis. The slides of the rats from the diabetic control group [Figure 6c] revealed thinner cross-section compared to the other groups, and stratum corneum and stratum granulosum were thicker than observed in other groups. Stratum spinosum and basale were greatly reduced in this group. Group V also showed a greatly reduced epidermal layer [Figure 6e]. Group IV and VI exhibited normal histology of the epidermis [Figure 6d and f]. Figure 7a-f presented the micrograph of the dermis in all groups showing a dense deposition of collagen fibers which were arranged irregularly in all groups as part of the dense irregular connective tissue of the dermis. Fibroblast nuclei were also observed as flattened and dark staining structures.

The micrographs stained with Masson's trichrome [Figures 8 and 9] showed a deposition of collagen fiber in the dermis of all groups. The collagen fibers are stained blue in all groups whereas the cell nuclei of fibroblasts are darkly stained.

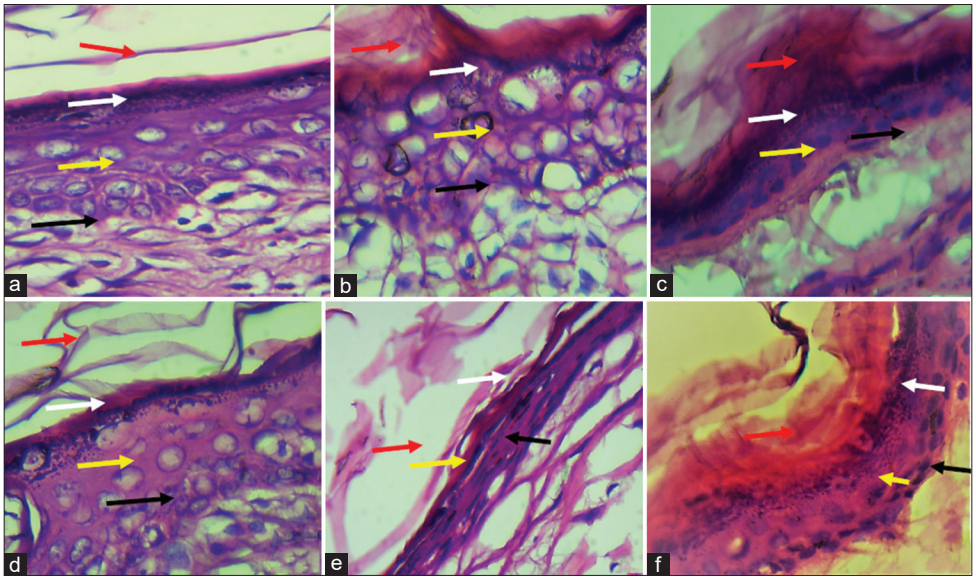

Figure 6: Photomicrographs of skin from wound areas in rats in all groups after the 28 days of treatment. Red arrow – stratum corneum, White arrow – stratum granulosum, yellow arrow – stratum spinosum, black arrow – stratum basale. Groups I (a), II (b), III (c), IV (d), V (e), and VI (f) (H and E, ×400)

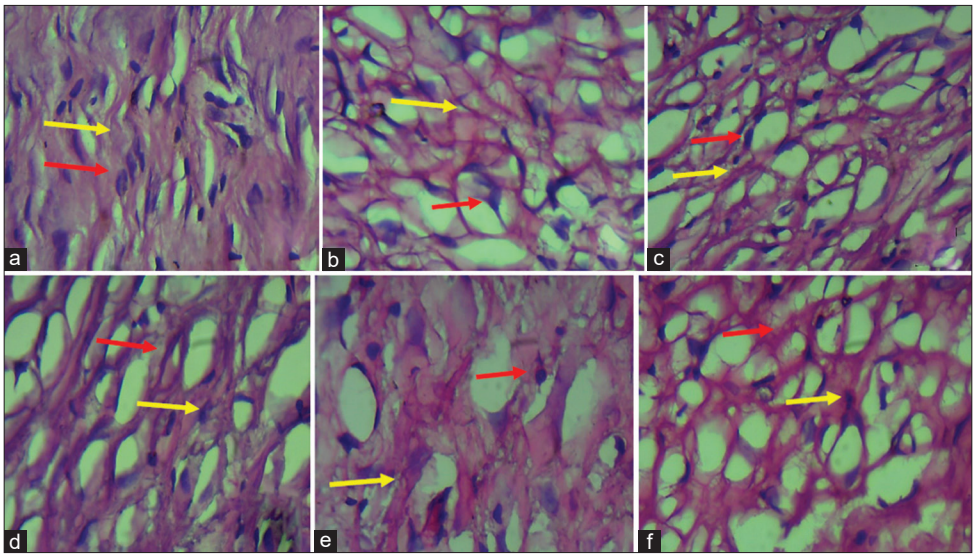

Figure 7: Photomicrographs of dermis of skin from wound areas in rats in all groups after the 28 days of treatment. Red arrow – nucleus of fibroblast, yellow arrow – collagen fiber. Groups I (a), II (b), III (c), IV (d), V (e), and VI (f) (H and E, ×400)

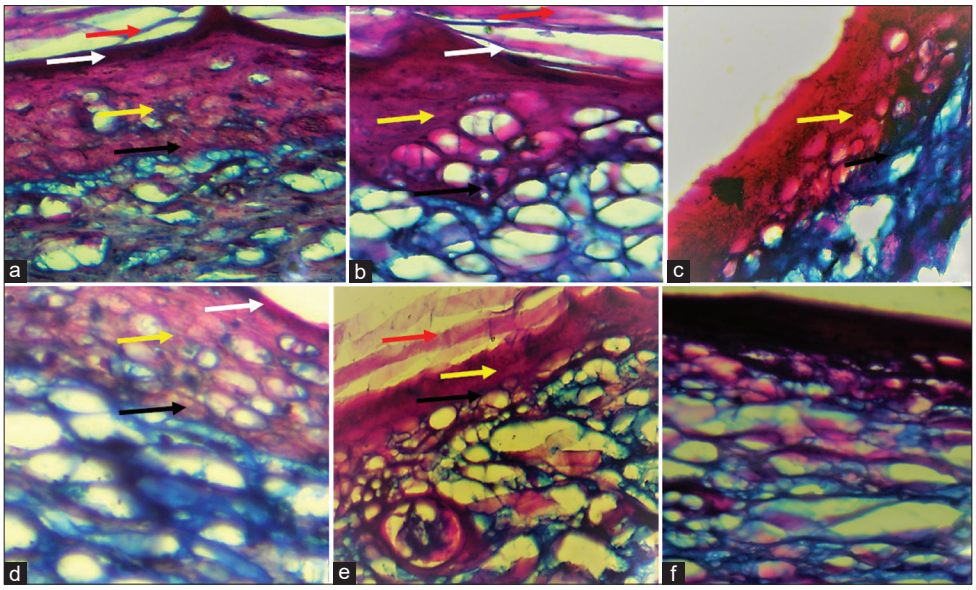

Figure 8: Photomicrographs of skin from wound areas in rats in all groups after the 28-day oral toxicity study. Red arrow – stratum corneum, white arrow – stratum granulosum, yellow arrow – stratum spinosum, black arrow – stratum basale. Groups I (a), II (b), III (c), IV (d), V (e), and VI (f) (MT, ×400)

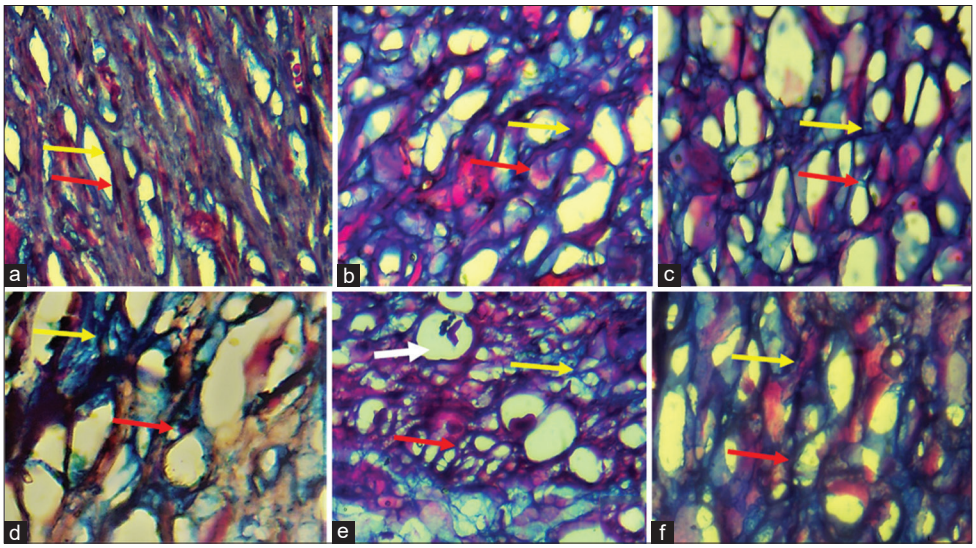

Figure 9: Photomicrographs of dermis of skin from wound areas in rats in all groups after the 28 days of treatment. Red arrow – nucleus of fibroblast, yellow arrow – collagen fiber, white arrow- blood vessel. Groups I (a), II (b), III (c), IV (d), V (e), and VI (f) (MT, ×400)

Morphometric Analysis of the skin

Table 3 represents the morphometric analysis of the skin in all groups. Group IV revealed a significantly thicker epidermal layer when compared to the other groups. The collagen fibers were also thickest in this group as well.

Discussion

Wound healing is a complex and dynamic process of restoring cellular structures and tissue layers in damaged tissue as closely as possible to its normal state. Wound contracture is a process that occurs throughout the healing process. The present study investigated the quantitative assessment of wound healing activity of n-hexane extract of *L. hastata* in diabetic and nondiabetic rats. There was also rapid wound closure in the normal control and nondiabetic control groups in the 10th to 15th days post injury. This could be attributed to normal wound healing processes in these groups as the rats in these groups were nondiabetic. Re-epithelialization was rapid in this group also as the granulation tissue was formed earlier (day 7) compared to other groups. It was observed that the extract was able to expedite ulcer wound healing in STZ-induced diabetic rats, and it showed an increased healing efficiency by reducing the time taken for the ulcer wounds to completely re-epithelize, and this may be due to the higher rate of infiltration of the active compound into the wound which

Table 3: The morphometric findings of the skin

Groups	Treatment	SB (μm)	SS (μm)	SG (μm)	SC (μm)	CF (μm)	FN (μm)
I		9.0±1.0^{a}	33.5±7.5^{a}	7.9±1.1^{a}	8.5±3.0^{a}	12.5±0.8^{a}	5.5±0.9^{a}
II	Extract (200 mg/kg)	2.5±0.0^{b}	9.0±0.6^{b}	5.5±0.9^{a}	10.5±2.0^{a}	14.5±1.6^{a}	5.5±0.9^{a}
III	-	2.5±0.0^{b}	12.0±3.9^{b}	6.0±1.0^{a}	16.0±3.9^{b}	7.0±0.9^{b}	6.5±1.0^{a}
IV	Extract (100 mg/kg)	14.0±2.2^{c}	76.5±14.7^{c}	19.0±3.1^{b}	26.0±4.2^{b}	11.0±2.9^{a}	6.2±0.5^{a}
V	Extract (200 mg/kg)	2.5±0.0^{b}	23.5±6.2^{a}	4.0±1.0^{a}	6.5±0.6^{a}	13.5±1.3^{a}	3.5±0.6^{b}
VI	Insulin	5.5±0.9^{b}	24.0±1.3^{a}	14.0±1.3^{b}	29.5±4.0^{b}	10.0±1.1^{a}	6.0±0.6^{a}

Data are presented as mean±SEM. The values are expressed as mean±SEM expressed (n=5). Values in the same column with different superscript are significantly different at P<0.05. Values in the same column with same superscript are not significant. SB - Stratum basale, SS - Stratum spinosum, SG - Stratum granulosum, SC - Stratum corneum, CF - Collagen fiber thickness, FN - Fibroblast nucleus, SEM - Standard error of mean

is in agreement with Abdennabi *et al*., (2016) and Lai *et al.* (2016) who determined that extract-treated diabetic wounds exhibited re-epithelialization, higher fibroblast proliferation, collagen synthesis, and angiogenesis as a result of the presence of active phytochemicals promoting wound healing and easy penetrability of the extract to the wound area.

The reduction of the wound size was an effect of a reduction in the wound's inflammation phase. Triterpenes isolated from *L. hastata* latex have been known to possess anti-inflammatory activity (Nikiema *et al.*, 2001). Lupeol, lupeol acetate, and lupeol palmitate were found to be the main antiinflammatory constituents which have been known to reduce the inflammatory phase of wound healing and steroids have also been reported to be responsible for wound contraction and high rate of epithelialization; this is due to their astringent and antimicrobial activity (Mittal *et al.*, 2013).

Fibroblasts are responsible for synthesis of collagen fibers and areolar connective tissue. When they penetrate into the areolar connective tissue formed in the tissue regeneration, they cause connective tissue maturation and stimulation of newly built vessels in a process known as angiogenesis. The new blood vessels penetrate into the young granulation tissue, improving faster wound healing.

The collagen fibers in all the groups treated with the extract were well developed, and blood vessels were observed in the dermis of animals that were treated with a higher dose of the extract. This conforms to studies conducted by Feng *et al.*, (2018) who reported that substances rich in cardiac glycosides increased collagen synthesis when applied on an excision wound.

Wound contraction is an essential process in healing which leads to wound closure. Thus, visible appearances and measurements of wound contraction become reliable parameters in macroscopic evaluation for wound healing (Liu *et al.*, 2013). This study showed that the extract significantly stimulated the contraction of wounds as seen from the percentage of wound contraction. Re-epithelialization is important as it restores the integrity of the skin, making it less vulnerable to infection. The extract-treated animals showed a decreased time for epithelialization compared with the diabetic control group. Recent studies with other plant extracts have shown that phytochemical constituents such as cardiac glycosides (Liu *et al.*, 2013, Umaru *et al.*, 2018) and triterpenoids (Liu *et al.*, 2013, Putta *et al.*, 2016) are known to promote the wound-healing process mainly due to their astringent and antimicrobial properties.

Increased cellular proliferation observed in the histological analysis of the wound area may be due to the mitogenic activity of the plant extract, which might have significantly contributed to healing process. Early dermal and epidermal regeneration in treated rats also confirmed that the extract had a positive effect toward cellular proliferation, granular tissue formation, and epithelialization.

Enhanced healing activity has been attributed to increased collagen formation and angiogenesis as reported by Abdullah *et al*. (2010). Angiogenesis in granulation tissues also improves blood supplementation to the wound site, thus providing nutrients and oxygen essential for the healing (Davis, 2008).

Early dermal and epidermal regeneration occurred in rats treated topically with the extract which may be an indication of wound healing property of the extract. There was a marked deposition of collagen fibers in all groups, and in addition, the presence of blood vessels in the connective tissue matrix of the rats treated with 200 mg of extract suggesting that the extract may also play a role in promoting angiogenesis. This is similar to a report by Akcal *et al.*, 2015.

Topical application of the extract established complete epithelialization in the wounds of diabetic rats indicating that the extract may play a role in accelerating the formation of the epidermal layer; the dermis in treated animals also had significantly thicker collagen fibers indicating that the extract promoted collagen synthesis which is essential for wound strengthening and maturation.

CONCLUSION

The n-hexane extract of *Leptadenia hastata* showed considerable wound healing properties by accelerating diabetic wound healing by reducing inflammation, increasing wound contraction and epithelialization.

Acknowledgment

The authors appreciate Professor M.O. Attah, Dr D.S. Amaza, and Dr S.T. Balogun who devoted so much time, energy, untiring effort and whose guidance as well as helpful advice has helped throughout the course of the research. Much gratitude goes to all staff of Human Anatomy department, Dr. J.V. Zirahei, Dr. A. U. Ekanem, Mr. N.I. Dibal and Mrs. L.P.Mshelia for the tireless effort and support given to me during the course of the work. I acknowledge the helpful advice and support of as well as technical support given by the staff Histology and Gross Anatomy lab, Department of Human Anatomy, Biochemistry and Pharmacognosy lab, all of the University of Maiduguri for their support and assistance during this research work.

Financial support and sponsorship

Personally funded.

Conflicts of interest

There are no conflicts of interest.

References

1. Abdennabi R., Bardaab S., Mehdic M., Ratebd M.E., Raabe A., Alenezif F.N., *et al*. (2016). *Phoenix dactylifera* L. sap enhances wound healing in wistar rats: Phytochemical and histological assessment. Int J Biol Macromol 88:443-50.
2. Abdullah A.M., Ahmed A.K., Abu-Luhoom F.M., Muhanid M. (2010). Role of *Ficus deltoidea* extract in the enhancement of wound healing in experimental rats. Biomed Res 21 (3):241-5.
3. Abubakar S., Usman A.B., Ismaila I.Z., Aruwa G., Azizat S.G., Ogbadu G.H., *et al*. (2014). Nutritional and pharmacological potentials of *Leptadenia hastata* (pers.) Decne. Ethanolic leaves extract. J Food Nutr Res 2(1): 51-55.
4. Akcal A., Karsidag S., Yildiz K., Yesloglu N., Akif M.A., Kabukcuoglu F. (2015). The effect of locally applied procaine on wound healing. Arch Clin Exp Surg 4:41-5.
5. Alam G., Singh M.P., Singh A. (2011). Wound healing potential of some medicinal plants. Int J Pharm Sci Rev Res 9:136-45.
6. Aliero B.L., Umar M.A., Suberu H.A., Abubakar A. (2001). A Handbook of Common Plants in Northern Western Nigeria. 1st ed., Sokoto: Usmanu Danfodio University Press, p. 78.
7. Aliero A., Wara S.H. (2009). Validating the medicinal potential of *Leptadenia hastata*. Afr J Pharm Pharmacol 3 (6):335-8.
8. Attah M.O., Jacks T.W., Garba S.H., Mshelia H.E. (2019). Physico-Chemical and phytochemical screening of N-hexane extract of *Leptadenia hastata* leaves: A proposed herbal remedy in the treatment of diabetes mellitus. Int J Res Granthaalayah 7 (2):45-57.
9. Attinger C.E., Janis J.E., Steinberg J., Schwartz, J, Al-Attar A, Couch K. (2006). Clinical approach to wounds: Debridement and wound bed preparation including the use of dressings and wound-healing adjuvants. Plast Reconstr Surg 117 (7):72S-109S.
10. Azwanida N.N. (2015). A review on the extraction methods used in medicinal plant: Principles, strengths and limitations. Med Aromat Plants 4 (196):1-6.
11. Broughton G., Janis J.E., Attinger C.E. (2006). The basic science of wound healing. Plast Reconstr Surg 117 (7):12S-34S.
12. Davis P. (2008). The immunology of wound healing: The body as a battlefield. Wounds UK 4 (4):54-69.
13. Etuk E.U. (2010). Animals models for studying diabetes mellitus. Agric Biol J North Am 1 (2):130-4.
14. Falanga V. (2005). Wound healing and its impairment in the diabetic foot. Lancet 366:1736-43.
15. Feng X., Wang C., Xu Y., Turley J., Xie Z., Pierre S.V., Hao J. (2018). Topical digitoxigenin for wound healing: A feasibility study. Bioengineering 5 (21):1-11.
16. Gal P., Kilik R., Mokr B., Vidinsk T., Vasilenko S., Mozes, S. *et al*. (2008). Simple method of open skin wound healing model in corticosteroid-treated and diabetic rats: Standardization of semi-quantitative and quantitative histological assessments. Vet Med 53 (12):652-9.
17. Glat P.M., Longaker M.T. (1997). Wound healing. In: Aston S.J., Beasley R.W., Thorne C.H., editors. Grabb and Smith's Plastic Surgery. 5th ed. Lippincott–Raven, Philadelphia, 3-12.
18. Goodson W.H., Hunt T.K. (1977). Studies of wound healing in experimental diabetes mellitus. J Surg Res 22:1-227.
19. Houcine B., Rachid A., Rabah D., Farid L., Nabila B., Boufeldja T. (2011). Effect of saponosides crude extract isolated from *Citrullus colocynthis* (L.) seeds on blood glucose level in normal and streptozotocin induced diabetic rats. J Med Plants Res 5 (31):6864-8.
20. Hunt T.K., Hopf H., Hussain Z. (2000). Physiology of wound healing. Adv Skin Wound Care 13:6-11.
21. Karodi R., Jadhav M., Rub R., Bafna A. (2008). Evaluation of the wound healing activity of a crude extract of *Rubia cordifolia* L. (Indian madder) in mice. Int J Appl Res Natl Prod 2 (2):12-8.
22. Khan M.E., Anyam J.V., Penuel B.L. (2014). Comparison of the proximate composition and the anti- microbial properties of ethanol and aqueous extracts of the stem-bark of *Leptadenia hastata* (Rubiaceae). Int J Curr Res Chem Pharm Sci 1 (6):178-83.
23. Kidman K. (2008). Tissue repair and regeneration: The effects of diabetes on wound healing. Diabetic Foot J 11 (2):73-9.
24. Lai J.C., Lai H.Y., Rao N.K., Ng S.F. (2016). Treatment for diabetic ulcer wounds using a fern tannin optimized hydrogel formulation with antibacterial and anti-oxidative properties. J Ethnopharmacol 189:277-89.
25. Lioupis C. (2005). Effects of diabetes mellitus on wound healing: An update. J Wound Care 14 (2):84-6.
26. Liu H., Lin S., Xiao D., Zheng X., Gu Y., Guo S. (2013). Evaluation of the wound healing potential of resina draconis (*Dracaena cochinchinensis*) in animal models. Evid Based Complementary Altern Med 2013:1-10.
27. Loots M.A., Kenter S.B., Au F.L., Van Galen W.J., Middelkoop E., Bos J.D., *et al*. (2002). Fibroblasts derived from chronic diabetic ulcers differ in their response to stimulation with ECF, IGF-1, bFGF and PDVGF-AB compared to controls. Eur J Cell Biol 81:153-60.
28. Maina V.A., Garba A., Maurice N.A., Baraya Y.S., Owada A.H., Hambolu S.E., *et al*. (2013). Effect of dose rates on organs weight in *Leptadenia hastata* extract treated white albino rats. J Exp Biol Agric Sci 1 (1):26-31.
29. Mechesso A.F., Tadese A., Tesfaye A., Tamiru W., Eguale T. (2016). Experimental evaluation of wound healing activity of *Croton* macrostachyus in rat. Afr J Pharm Pharmacol 10 (39):832-8.
30. Menke N.B., Ward K.R., Witten T.M., Bonchev D.G., Diegelmann R.F. (2007). Impaired wound healing. Clin Dermatol 25:19-25.

31. Mittal A., Sardana S., Pandey A. (2013). Herbal boon for wounds. Int J Pharm Pharm Sci 5:1-12.
32. Nakao C., Angel M., Di Mateo S., Komesu M.C., (2009). Effects of topical tetracycline in wound healing on experimental diabetes in rats. Open Diabetes J 2:53-9.
33. Nasrabadi M.H., Ebrahimi M.T., Banadaki D., Kajousangi M.T., Zahedi F. (2011). Study of cutaneous wound healing in rats treated with *Lactobacillus plantarum* on days 1, 3, 7, 14 and 21. Afr J Pharm Pharmacol 5 (21):2395-401.
34. Nikiema J.B., Vanhaelen-Fastre R., Vanhaelen M., Fontaine J., Graef C.D., Heenen M. (2001). Effects of anti-inflammatory terpenes isolated from *Leptadenia hastata* latex on keratinocyte proliferation. Photother Resour 15 (1):131-4.
35. Nithyapriya S., Tangavelou A.C., Karthikeyan S. (2015). Wound healing activity of mallotus Philippensis in experimental animals. Int J Med Res Rev 3 (1):604-7.
36. Omar S.M. (2010). Effect of chromium picolinate on histological skin alterations of streptozotocin diabetic rats, a light and electron microscopic study. Egypt J Histol 33 (1):178-91.
37. Oryan A., Naeini A.T., Nikahval B., Gorjian E. (2010). Effect of aqueous extract of *Aloe vera* on experimental cutaneous wound healing in rat. Vet Arhiv 80:509-22.
38. Putta S., Yarla N.S., Surekha C., Aliev G., Divakara M.B., Santosh M.S., *et al*. (2016). Therapeutic potentials of triterpenes in diabetes and its associated complications. Curr Top Med Chem 16 (23):2532-42.
39. Richardson M. (2004). Acute wounds: An overview of the physiological healing process. Nurs Times 100:50-3.
40. Tamboura H.H., Bayala B., Lompo M., Guissou I.P., Sawadogo L. (2005). Ecological distribution, morphological characteristics and acute toxicity of aqueous extracts of *Holarrhena floribunda* (G. Don) durand and schinz, *Leptadenia hastata* (Pers.) decne and *Cassia sieberiana* (DC) used by veterinary healers in Burkina Faso. Afr J Tradit Complement Altern Med 2:13-24.
41. Thomas S. (2012). *Leptadenia hastata*: A review of its traditional uses and its pharmacologic activity. Med Chem 2 (7):148-50.
42. Umaru I.J., Badruddin F.A., Umaru H.A. (2018). Phytochemical, antifungal and antibacterial potential of *Leptadenia hastata* stem-bark extract. MOJ Toxicol 4 (4):263-8.
43. Velnar T., Bailey T., Smrkolj V. (2009). The wound healing process: An overview of the cellular and molecular mechanisms. J Int Med Res 37:1528-42.
44. Zaki S.M. (2015). Characteristics of the skin of the female albino rats in different ages: Histological, morphometric and electron microscopic study. J Cytol Histol S3:4.