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Evaluation of Wound Healing and Antibacterial Activities of Crude Ethanol Extract of Datural Metel Leaves on Wistar Rats

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ABSTRACT

A large number of plants are reported as used by folklore traditions for treatment of cuts, wounds, and burns, Datura metel is the notable one. Only a few scientific reports are confirming such claims. It is important to carry out studies to confirm such claims. The present investigation aimed to assess the in-vivo wound healing efficacy of Datura metal extract-based ointment as well as its anti-microbial potential which is a very important attribute of a potent wound healer using Wistar rats as case a case study. Phytochemical analysis of the crude extract revealed the presence of alkaloids, flavonoids, tannins, phenolic compounds, saponins, triterpenoids, and steroids. TLC spotting of the extract using ethylacetate/n-hexane (7:3) confirmed the presence of more than five compounds with Rf values between 0.1-0.8. The FT-IR of the extract showed the presence of O-H, N-H, C-H (SP2 AND SP3), and C-N signals among others. The results obtained for the extracts at different concentrations against Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli, Klebsiella pneumonia, Salmonella typhi, and Bacillus subtilis showed that the activity was concentration-dependent with zones of inhibition ranging from 10 to 28mm in diameter. There were significant differences in the mean percentage of wound healing between groups treated with the extract and sterile water. The group treated with 20% w/w extract-based ointment was found to have the best healing rate closely followed by 10% w/w. It was therefore concluded that the ethanol extract of Datura metel leaves has antibacterial and wound healing properties. This work was carried out in collaboration between the authors. Author FOO designed the study and performed the statistical analysis. Author TTO wrote the protocol and the first draft, author ABF managed the analysis of the study and the literature searches, while Author OM Balogun supervised the animal study.

Keywords: Datura metel, Wound healing, Antibacterial, Zone of inhibition, Alkaloids.

INTRODUCTION

Wounds are physical injuries resulting in an opening or usually a break of the skin that causes disturbance in the normal skin anatomy and function. Strodtbeck reported that wounds result in the loss of continuity of epithelium with or without the loss of underlying connective tissue [1]. Wounds represent a significant burden on the patients and healthcare professionals worldwide. They not only affect the physical and mental health of millions of patients but also impose a significant cost on them. Current estimates indicate that worldwide, nearly 6 million people suffer from chronic wounds [2]. Unhealed wounds constantly produce inflammatory mediators that produce pain and swelling at the wound site. Chronic wounds may even lead to multiple organ failures or the death of the patient [3]. Wounds are classified as open and closed based on the underlying cause of the wound. There are also acute and chronic wounds [4]. Wound healing is a complex process in which the skin or the affected organ repairs itself after injury. Within a few minutes after injury, platelets aggregate at the injury site to form a fibrin clot. This clot acts to control the active bleeding and to achieve hemostasis. The entire wound healing process that begins at the moment of injury can continue for even months or years [3]. There are three main phases of wound healing namely, inflammatory, proliferative, and

remodeling phase. The inflammatory phase starts immediately after the injury that usually lasts between 24 and 48 hours and may persist for up to 2 weeks in some cases [5]. This phase launches the hemostatic mechanisms to immediately stop blood loss from the wound site. The phase is characterized by vasodilation and phagocytosis to produce inflammation at the wound site. The proliferative phase lasts up to 2 days to 3 weeks after the inflammatory phase [3]. This phase usually lasts for 3 weeks to 2 years. New collagen is formed in this phase. Tissue tensile strength is increased due to intermolecular cross-linking of collagen *via* vitamin C-dependent hydroxylation. The scar flattens and scar tissues become 80% as strong as the original tissue [3]. Many factors can adversely affect the normal biological process of wound healing. A thorough understanding of these factors and their influence on wound healing is essential for developing better therapeutic options for wound treatment [6]. It is reported that a serum albumin level of 3.5 gm/dl or more is necessary for proper healing, therefore a proper diet is very essential [7]. The presence of microorganisms such as *Staphylococcus aureus*, *Streptococcus pyogenes*, *Corynebacterium sp*, *Escherichia coli*, and *Pseudomonas aeruginosa* can adversely 3 affect the rate of wound healing [8]. Other factors are insufficient oxygen supply and tissue perfusion to the wound area, drugs, age, diabetes, and other disease conditions [9]. Traditional and folklore medicine play important roles in healthcare services around the globe. About three-quarters of the world's population rely on plants for health care [10,11]. A World Health Organization survey indicated that about 70%-80% of the world's population rely on non-conventional medicine, mainly of herbal sources, in their primary healthcare (WHO, 2007). Medicinal plants have an amazing array of thousands of phytochemicals that fights bacterial, fungi, insects, herbivores, and even animals. Some of these phytochemicals are also vitamins, nutrients, antioxidants, anti-carcinogens, and other compounds with medicinal values [12]. Man has always made use of various parts of plants in the treatment and prevention of various ailments [13]. Most of these herbal remedies have stood the test of time, particularly for the treatment of allergic, metabolic, and cardiovascular diseases [14]. Therefore, research into the effect of these local medicinal plants is expected to enhance their use against diseases caused by micro-organisms [15,16]. The biodiversity of tropical forest plants species, coupled with chemical diversity found within each plant leads to the conclusion that plants are the most valuable source of new bioactive chemical entities [17]. Datura belongs to the family Solanaceae. Species such as *Datura innoxia*, *Datura metel*, *Datura stramonium*, and *Datura wrightii* are cultivated as ornamental plants [18]. In China, it is known as 'Yangjinhua' and is used for the treatment of asthma, convulsion, pain, and rheumatism [19]. Ingestion of Datura manifests as a classic anticholinergic syndrome comprising central and peripheral signs and symptoms. Central toxic effects include confusion, agitation, anxiety, hallucination, seizures, and coma [20]. It is a strange ploy played by nature that a plant known almost exclusively for its toxicological effect finds its use in the medical field [21]. Datura metel is made by Zunis into a poultice to treat inflammation and bruises [22]. It has been known to be used in ethnoveterinary practices in Nepal and by the Gujjar community in India [23-24]. Munira and Munan identified the presence of saponins, flavonoids, phenols, alkaloids, glycosides, and steroids in aqueous extract of Datura metel while in the chloroform extract, steroids, flavonoids, triterpenoids were absent [25]. Bellila, et al reported that tannins were absent in the acetone extract [26]. Reports by Hossein, et al., agreed with the earlier report except for tannins which were not recorded in any of the extracts used [27]. A new antibacterial agent 5', 7' dimethyl 6'-hydroxyl 3', phenyl 3 α-amine β-yne sitosterol was isolated by Okwu and 4 Igara from Datural metel leaves [28]. The antibacterial activity of the plant was determined against gram-negative bacteria such as *P.aeruginosa*, *E.coli*, *K.pneumonia*, *S.typhi*, *Enterococcus faecalis*, *Vibrio sp*, by Kaushik and Goyal [29]. They concluded that the extract from the leaves is more potent and displayed better antibacterial activity as compared to the stems and roots. Methanol extract shows more inhibition while chloroform extract is found to be active against *Aspergillus niger*, *Aspergillus fumigatus*, and *Candida albicans* [30]. The anti-cancer investigation of methanol extract of the flowers of the plant by Pan, et al. indicated that the isolated compounds exhibited cytotoxic activities against cancer lines A549 (Lung), BGC-823 (Gastric), and K562 (Leukemia) with their IC 50 values ranging from 0.05 μM to 3.5 μM [19]. The antioxidant activity of the aqueous extract of the plant was determined by Munira and Munan [25]. He concluded that the activity was between 49.30%-23.82%. The wound healing properties were studied by Chah, et al. [31]. In their studies, they found that wound healing was increased due to enhanced epithelialization. The crude extract has an enhanced chemotactic effect which attracted inflammatory cells towards the wound site and cellular proliferation was observed by hematoxylin and eosin staining.

MATERIALS AND METHODS

Collection and preparation of plant materials

Fresh leaves of Datura metel were collected between March and April 2021 from Orogun, Ibadan, Oyo State, Nigeria. It was identified and authenticated at the Forestry Research Institute of Nigeria where voucher number FHI 110977 was issued. The leaves were washed with distilled water, air-dried, and completely powdered after which it was extracted with ethanol.

Thin Layer Chromatography (TLC)

Thin-layer chromatography was performed on pre-coated silica gel-G plates of size 20 cm × 20 cm (E. Merck, Darmstadt, Germany) for characterization of the extracts to determine the number of components present in the extract. The TLC plate was cut into the size of 6 cm by 1.5 cm. A pencil was used to draw a thin continuous line of about 0.5 cm from one end of the plate and the spotting was carried out with the aid of a thin capillary tube by touching the tube lightly on the baseline on the coated plate. The spots were allowed to dry before the development of the plates to prevent the dissolution of the samples in the chosen solvent.

Development of spots

Several solvent mixtures were prepared for proper resolution of the components present in the ethanolic extracts each in 10 mL.

These included Hexane/ethyl acetate (4:1), Chloroform/methanol (7:3), Toluene/ethyl acetate (4:1), Petroleum ether/ethyl acetate (4:1), Petroleum ether/ethyl acetate (1:1), Petroleum ether/ethyl acetate (7:1), Hexane/ethyl acetate (4:6), Hexane/ethyl acetate (3:7) and Hexane/ethyl acetate (2:8).

The solvents were transferred into different development chambers, then shaken to ensure proper saturation of the atmosphere within the chamber and covered with glass lids. The lids were removed and the spotted plates were carefully placed in a diagonal position in such a way that the solvent levels were not above the baseline of the spots to avoid wash-off of the samples spotted. The development plates were removed from each chamber after the solvent had traveled about fifth-sixth of the plate and allowed to dry at room temperature. The spots were visualized in a desiccator using iodine as the derivatizing agent.

Spectroscopic analysis of the extract

Fourier Transform Infra-Red (FTIR) analysis of crude ethanol extract of the plant was carried out to determine the functional groups present in the extract.

Determination of the phytochemical constituents

The extract was evaluated for the presence of Tannins, flavonoids, saponins, and alkaloids using simple qualitative and quantitative methods of Trease and Evans and Sofowora [13, 32].

Preparation of ointments

The method of Okore, et al. was adopted in the preparation of three herbal ointments containing 10%, 20%, and 40% w/w of the extract in sterile soft white paraffin. The ointments were then aseptically transferred into sterile cream bottles and sealed [33].

Pathogen and preparation of inocula

The bacteria, *Escherichia coli* ATCC35219, used in this study were obtained from the Department of Pharmaceutical Microbiology, University of Ibadan. They were clinical isolates from patients, fully identified and maintained on nutrient agar slope at 4°C at the laboratory. Before use, the organisms were sub-cultured on sterile nutrient agar, incubated aerobically at 37°C for 24 hours. Colonies of each organism were homogenized in sterile Phosphate-Buffered Saline (PBS) and the turbidity was adjusted to correspond to 0.5 Mcfarland's turbidity standard (equivalent to 1 × 108 cfu/ml).

Screening for antibacterial activity

The extract screened for antibacterial activity against *Staphylococcus aureus* ATCC29213, *Pseudomonas aeruginosa* ATCC27353, *Klebsiella pneumonia* (clinical), *Escherichia coli* ATCC35219, *Salmonella typhi* (clinical), and *Bacillus subtilis* (clinical). Different concentrations of the extracts were screened for antibacterial activity using the agar well diffusion method as described by Adeniyi, et al. [34]. The concentrations of the extracts used were 100 mg/mL, 50 mg/mL, 25 mg/mL, 12.5 mg/mL, 6.25 mg/mL and 3.125 mg/Ml while ethanol and gentamicin (1×10^{-4}mg/mL) were used as negative and positive control respectively. Following incubation at 37°C for 24 hours, the Inhibition Zone Diameters (IZD) were measured using a meter rule. Each screening was carried out in triplicate and the mean IZD was recorded to the nearest millimeter.

Selection and grouping of animals Thirty female albino rats weighing between 85 g-140 g were used for this study. They were all obtained from the Central Animal House, College of Medicine, University of Ibadan and kept in the animal house where they were allowed to acclimatize for two weeks. They were fed with standard rat ration Growers mash (Top feed) and freshwater. The animals were divided into six groups of five animals per group. Groups D, E, and F were the test groups, C was placebo while A and B were negative and positive control groups respectively.

Preparation of animals for wound healing

The rats were anesthetized before the creation of the wounds, with 0.2 mL of intravenous ketamine hydrochloride (10 mg/kg body weight). The dorsal for the animal was shaved and the area of the wound to be created was outlined on the back of the animal with a permanent marker and transparent ruler. A full thickness of the excision wound 3.0 cm by 2.5 cm and 0.2 cm depth was created along the markings using toothed forceps, a surgical blade, and pointed scissors. The wound surface area was immediately measured using the transparent meter rule. *Escherichia coli* ATCC35219 standardized to contain approximately 1.3 × 108 cfu/mL (0.5 Macfarland) was used to contaminate the wound site of all the experimental animals shortly after the wound was created. The wound area of each animal was measured on the 1st, 4th, 7th, 13th, and 16th-day post wound creation. The percentage of wound healing on these days was determined. Physical changes in the wound surface were evaluated for each group of animals.

Application of the ointment

Equal quantities of prepared ointment were applied topically to the wound area of experimental rats once a day for 16 days. The wound contraction was measured on the 4th, 7th, 10th, 13th, and 16th days post wound creation.

Histological examination of healed wound tissue

Specimen of skin from healed wounds from each group were taken and fixed in 10% buffered formalin solution for histopathological studies. Sections of the healed skin were made at a thickness of 5 μm and stained with hematoxylin and eosin (H and E), and assessed for histopathological changes. The microscopic slides were photographed. The rate of reepithelialization, collagenation, neovascularization (angiogenesis) and inflammatory cells were evaluated by a blind histopathologist.

RESULTS AND DISCUSSION

The percentage yield for the extraction process was 9.24%. The yield was higher than 4.84% reported by Okwu and Igara as well as 6.21% reported by Alabi, et al. [28,35]. The better yield recorded may be because the sample used was exhaustively extracted, geographical location of collection and time/season of collection of the fresh plant may also be another factor as well as the purity of the solvent used.

Thin-layer chromatography (Tlc) of datura metel crude extract

The TLC of Datura metel ethanol leaves extracts for a solvent combination of ethyl acetate: n-hexane (7:3) indicated five spots when the plate was put in the iodine tank. Ethyl acetate: n-hexane (7:3) gave the best resolution out of the several solvent combinations tried. The Rf values of spots in the ethyl acetate: n-hexane (7:3) combination were found to be 0.1, 0.3, 0.6, 0.7, and 0.8. This inferred that the crude extract contains more than five compounds.

Spectroscopic analysis of the extract

The spectrum of the Fourier Transform Infrared (FTIR) of crude ethanol extract of the plant is as shown in Figure 1. The IR spectrum of the crude extract shows major absorption bands at 3605.60 cm^{-1}, 3433.60 cm^{-1}, 2952.80 cm^{-1}, 2870.40 cm^{-1}, 2362.40 cm^{-1}, 2097.60 cm^{-1}, 1637.60 cm^{-1}, 1406.40 cm^{-1}, 1219.20 cm^{-1}, 1069.60 cm^{-1}, 1026 cm^{-1} and 635.60 cm^{-1}. The very broad absorption at 3605 cm^{-1} shows the presence of a hydroxyl (-OH) group which is not involved in hydrogen bonding. 3433 cm^{-1} shows the presence of secondary amine (N-H). The absorptions at 2952 cm^{-1} and 2870 cm^{-1} indicate the C-H stretching vibration of SP2 and SP3 CH groups. The strong absorption at 2362 cm-1 indicates the presence of the C≡N bond of aliphatic nitriles. Absorptions at 2097 cm^{-1} indicate C≡C. Absorption at 1637 cm^{-1} indicates the presence of non-conjugated C=C, N-H bending or O-H bending. 8 1406cm-1 indicates the In-plane C=C bending of aromatic compounds. 1219 cm^{-1} indicates C-N bending vibration of an amine. The absorption at 635cm^{-1} is more of C-Cl (stretching). The spectrum just shows the raw information about the functional groups in the compounds that can be isolated from the crude ethanol extract of Datura metel. To assign these signals and functional groups to a particular compound would require the isolation of various compounds in the crude extract.

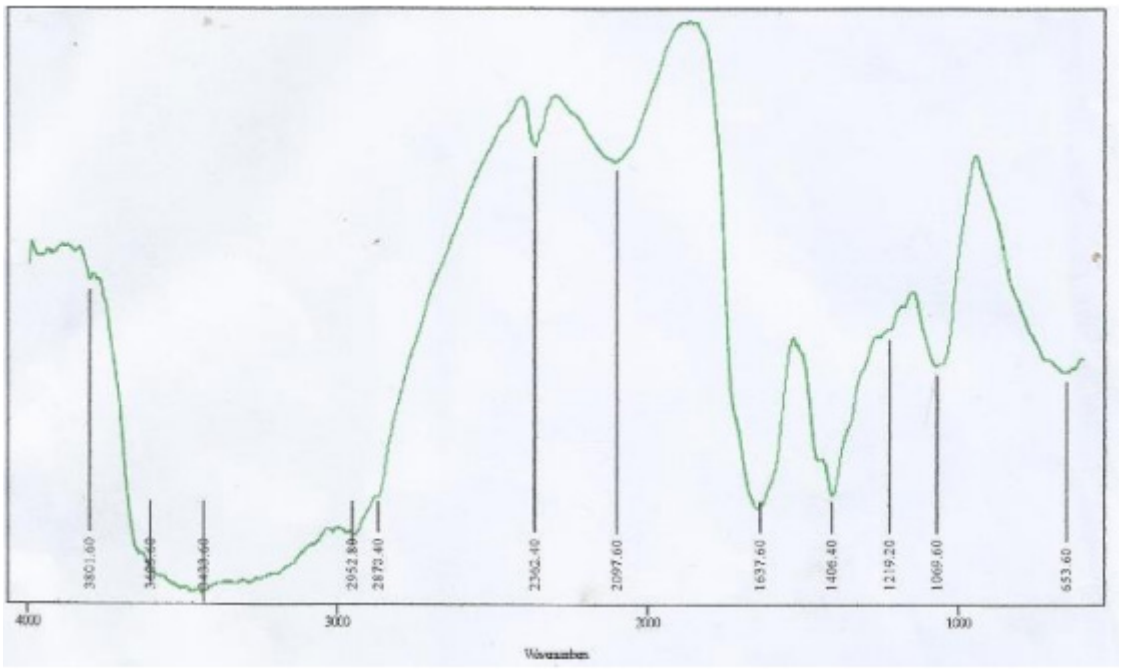

Figure 1. FT-IR Spectrum of datura metel leaves crude ethanol extract

Phytochemical analysis

The summary of results of phytochemical analysis on a crude ethanol extract of Datura metel leaves is as shown in Table 1. From the results, Datura metel leaves ethanol extract contained alkaloids, saponins, tannins, phenolics, terpenoids, flavonoids, terpenoids, and steroids while glycosides and reducing sugars were absent in the tested leaves.

Table 1: Result of phytochemical analysis

Compounds	Availability
Alkaloids	+
Saponins	+
Flavonoids	+
Terpenoids	+
Glycosides	-
Terpenoids and Steroids	+
Phenolic Compounds	+
Tannins	+
Reducing sugars	-

+ =Presence of the phytochemical E19E19
- = Absence of the phytochemical

Antibacterial activity of datura metel crude ethanol extract

The results of the antibacterial activity of the crude ethanol extracts of dried fresh Datura metel presented in Table 2 shows that the extract inhibited the growth of all the test organisms and had a mean inhibition zone diameter (IZD) ranging from 10 mm to 28 mm. The zone of inhibition of 100 mg/mL ethanol extract of Datura metel against bacteria was, *Staphylococcus aureus* (28 mm), *Pseudomonas aeruginosa* (14 mm), *Bacillus subtilis* (16 mm), *Salmonella typhi* (14 mm), *Escherichia coli* (26 mm), *Klebsiella pneumonia* (14 mm). At concentration less than 12.5 mg/mL, the extract had no activity against *Pseudomonas aeruginosa*, *Salmonella typhi*, and *Klebsiella pneumonia* but was able to inhibit the growth of these three organisms at 25 mg/mL and showed a dose-related increase in IZD when the 50 mg/mL and 100 mg/mL extract was used. This observation may lend credence to the antimicrobial properties of the extracts at the appropriate concentration. The ethanol, which served as the negative control and also as extract solvent had no activity against any of the test organisms while Gentamicin 1×10^{-4}mg/mL, the positive control inhibited the growth of all the test organisms with mean Inhibition Zone Diameter (IZD) between 36 mm and 40 mm. Gentamicin was found to be more potent than the extract for obvious reasons. Gentamicin is a pure synthetic antimicrobial drug while crude extract contains many compounds and impurities that contribute to the weight without any antimicrobial activity. The antimicrobial properties of this extract might be attributed to the secondary metabolites present in the extract. Okwu and Igara [28] in their study, isolated a new antibacterial agent 5′, 7′ dimethyl 6′- hydroxy 3′, phenyl 3α-amine β-yne sitosterol from Datura metal leaves. They reported that the compound displayed antibacterial activity against *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Proteus mirabilis*, *Salmonella typhi*, *Bacillus subtilis*, and *Klebsiella pneumonia* except for *Escherichia coli*. The activity of crude ethanol extract of Datura metel against *Escherichia coli* observed in this study may be due to other active compounds other than the one isolated by Okwu and Igara [28].

Table 2: Mean inhibition zone diameter in millimeters induced by different concentrations of Datura metel extract

	Extract concentration in mg/ml							
Type Organism	100	50	25	12.5	6.25	3.125	Negative	Positive
Stapylococcus aureus ATCC29213	28	24	20	18	14	10	—	38
Pseudomonas aeruginosa ATCC27353	26	22	18	14	12	10	—	38
ATCC27353 (clinical)	16	14	12	10	—	—	—	40
Escherichia coli ATCC35218	14	12	10	—	—	—	—	38
Salmonella typhi (clinical)	14	12	10	—	—	—	—	36
***Bacillus subtilis* (clinical)**	14	12	10	—	—	—	—	36

Datura metel crude ethanol extracts and wound healing

The results of the wound healing property of the crude ethanol extracts of dried fresh Datura metel are presented in Table 3. There was a progressive daily decrease in the wound area throughout the experimental period in all the groups. In comparison, however, in the distilled water treated group (negative control), the decrease in wound area was not as drastic as the extract and antibacterial (Cicatrin®) treated groups. In the 10% w/w and 20%, w/w treated groups, a sharp remarkable decrease in wound area was observed between 1st and 7th day, having 71% and 78% wound healing respectively and comparatively showing the best wound healing activity than the other extract concentrations and Cicatrin® powder (Figure 2). As the number of the days increased, wound healing was observed for all the treated groups but only from the 10th day was a significant wound healing observed for distilled water treated group (recording 79% on the 10th day and 87% on the the13th day). This was probably due to the natural response to wounding as the body's immune system and blood clot factors set to play and bring about wound healing without the aid of any wound healing agent. On the 13th day post wounding over 99% and 96% wound healing had been recorded in the 20% w/w and 10% w/w extract ointment respectively and 97% in the Cicatrin® treated groups. The attainment of an almost equal rate of healing by all the groups from post-wounding days 10 to 13 is probably due to the excellent health of the experimental animals and their probably high immune responses. Again, the albino rats were young adults, and they continued to grow rapidly with a probably

high metabolic rate and this may have contributed to some of the above observations [36, 37]. Wound infection is probably the most common reason for impaired wound healing [3]. *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Bacillus subtilis*, *Salmonella typhi*, *Escherichia coli*, and *Klebsiella pneumonia* used in this research work are some important organisms causing wound infection [2]. As a result of this, every wound healing drug must have a very potent antimicrobial activity as exhibited by Datura metel extract. The rats did not exhibit any reaction to the plant extracts showing that their application caused no irritation or pain.

Table 3: Percentage wound healing in rats post infliction of excision wound

Group/Treatment	Percentage wound healing (mean ± SEM) days post wounding					
	Day 1	Day 4	Day 7	Day 10	Day 13	Day 16
A (No treatment)	0	43.57 ± 0.82	62.92 ± 2.09	79.73 ± 3.33	87.36 ± 3.93	94.31 ± 1.61
B (cicatrin)	0	56.81 ± 3.94	69.38 ± 4.92	88.78 ± 1.31	97.77 ± 1.00	99.95 ± 0.03
C (Placebo)	0	56.28 ± 4.43	65.12 ± 4.12	82.67 ± 2.32	89.47 ± 1.62	98.02 ± 0.84
D (10% w/w)	0	63.69 ± 2.67	71.96 ± 3.10	92.63 ± 2.25	95.96 ± 1.26	99.50 ± 0.20
E (20% w/w)	0	67.98 ± 4.23	78.35 ± 3.49	95.50 ± 1.21	99.22 ± 0.43	99.97 ± 0.02
F (40% w/w)	0	57.28 ± 3.66	66.12 ± 3.57	83.60 ± 4.21	91.78 ± 3.58	95.87 ± 2.40

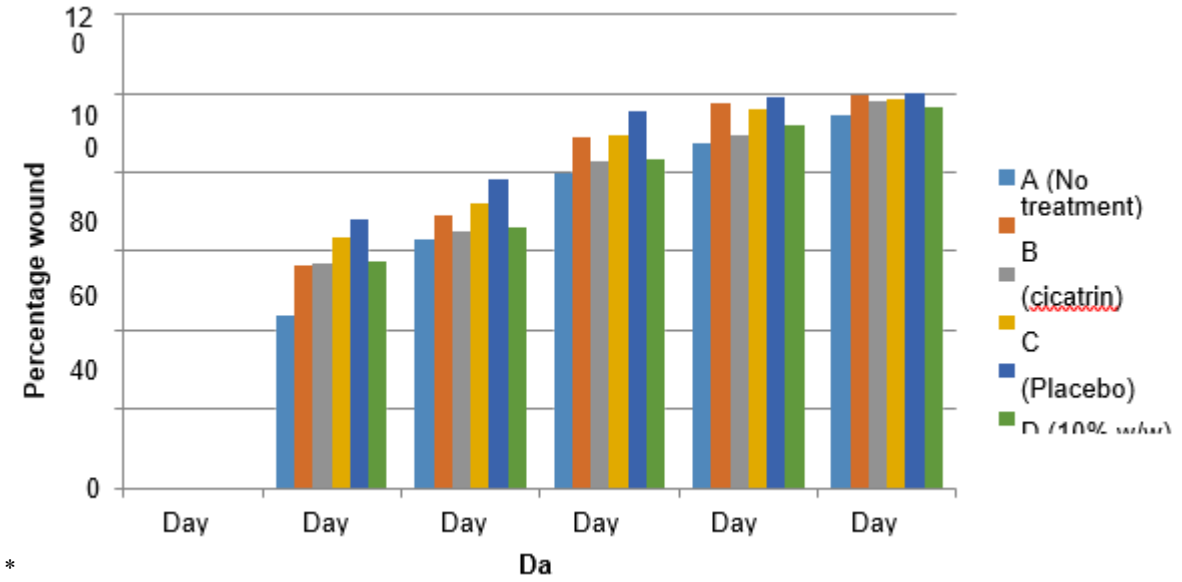

Figure 2. Effect of Datura metel extract-based ointment on wound contraction

Histological evaluation of healed wound tissues

Features of the result of the histological evaluation carried out on specimens of skin from healed wounds of each group indicated that in Group A (Negative Control), the epidermal layer is thin while the dermal area is highly dense and made up of mostly collagen fibers. Few hair follicles and sebaceous glands are seen (Figure 3 Group A). There is a severe erosion of the epidermal layer in Group B (Positive Control). Thick collagen fibers were also found in the dermis (Figure 3 Group B). In Group 0 to group 120 and from Day 1, Day 4, Day 7, Day 10, Day 13, and Day 16 Percentage wound Healing were Day A (No treatment), B (cicatrin), C (Placebo), D (10% w/w), E (20% w/w), F (40% w/w), and 12 C (Placebo), both epidermal and dermal compartments are seen. The dermal area is devoid of follicles and glands with highly dense dermal tissue (Figure 3 Group C). The epidermal layer in Group D (10% w/w) is normal and there is a moderate presence of glands and follicles in the dermal area. Fibrous tissue and collagen fibers are also present in the dermis (Figure 3 Group D). Group E (20% w/w) epidermal layer is normal and there is a moderate presence of glands and follicles in the dermal area. It was also observed that fibrous tissue and collagen fibers were present in the dermis (Figure 3 Group E). There is a thick epidermal layer and prominent hair follicles observed in Group F (40% w/w). The dermis is moderately congested and infiltrated by inflammatory cells (Figure 3 Group F). There was increased blood vessel formation and enhanced proliferation of cells as a result of treatment with Datura metel extract-based ointment. There was full thickness re-epithelialization, in which epidermis was normal and well organized, comparable to the normal adjacent skin which was not involved in the wound generation

and healing process. There was a full-thickness epidermal regeneration which covered completely the wound area.

Early dermal and epidermal regeneration in treated groups also confirmed that the extract had a positive effect on cellular proliferation, granular tissue formation, and epithelialization. The wound healing and repair were accelerated by applying Datura metel extract-based ointment, which was highlighted by the full thickness coverage of the wound area by an organized epidermis. The enhanced capacity of wound healing with the plant could be explained based on anti-inflammatory effects of the plant that are well documented in the literature. The Study showed an enhanced rate of wound contraction and a drastic reduction in healing time than the control, which might be due to enhanced epithelialisation.

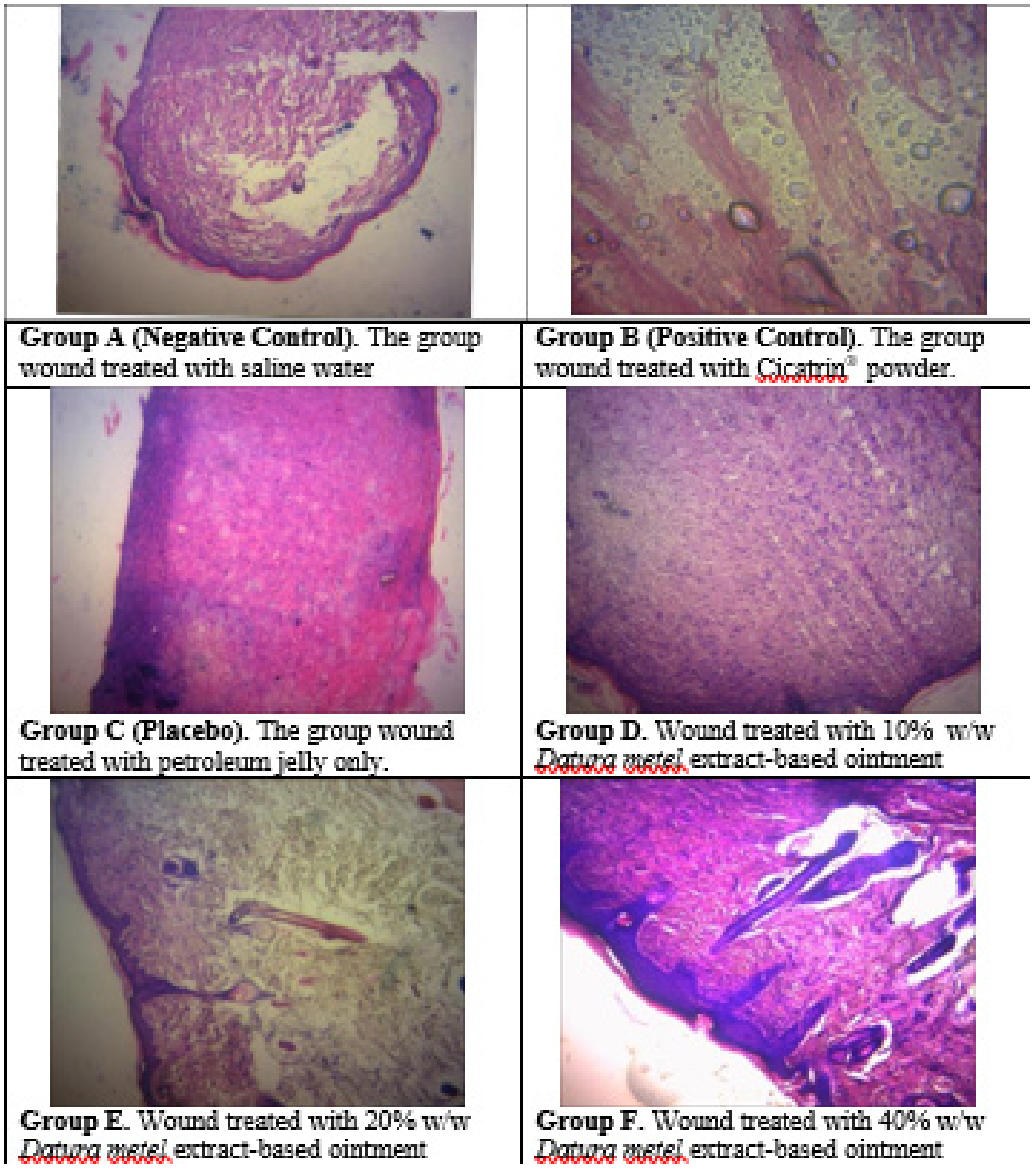

Figure 3. (A-F): Histological Evaluation of healed wound tissues

CONCLUSION

The enhanced capacity of wound healing with Datura metel extract and its anti-inflammatory and antioxidant effects have been well documented in the literature by Maheshwari. Based on the results obtained in the present investigation, it is possible to conclude that the ointment of the ethanol extract of Datura metel has significant wound healing activity. The antioxidant and anti-inflammatory activities of flavonoids in ethanol extract are believed to be one of the important mechanisms in wound healing. The presence of tannin is thought to improve the regeneration and organization of the new tissue thereby hastening the wound healing process. Several phytoconstituents like alkaloids and saponins are known to promote the wound healing process due to their antioxidant and antimicrobial activities. The herbal extracts may prevent infection that leads to a high risk of sepsis thereby preventing the prolongation of the inflammatory phase. The wound healing potential observed may be attributed to individual 14 or

the cumulative effect of phytoconstituents present and this provides scientific evidence to the ethnomedicinal use of Datura metel.

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