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Promotion of wound healing in mice using a formulation of *Ficus sur* Forssk aqueous leaf extract

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Injuries from accidents are frequent occurrences. In most developing countries, patronage of alternative medicines to treat such injuries is common. *Ficus sur* Forssk (Moraceae) leaves are used for wound management in alternative medicine in Northern Nigeria. This study aimed at determining the effect of a formulation of the aqueous extract of *F. sur* on wound healing. A decoction of powdered leaves of *F. sur* was prepared using distilled water. Phytochemical test was carried out on the extract which was then formulated into cream preparation of 5, 10 and 20% concentrations. Skin irritation tests were conducted by topical application of the formulation by un-occluded procedure on mice. Effects of topical application of 5, 10 and 20% of the cream on wounds created using the excision wound and burn wound methods were evaluated. Phytochemical compounds including tannins, flavonoids, alkaloids and saponins were detected. The formulation did not produce any irritant reaction as noerythema or oedema was observed on shaved treated animal skins. The 10 and 20% formulation produced decrease in wound diameter created by excision and also promoted fibroblast proliferation along with enhancement of deposition of collagen and elastic fibers in the burn wound model. The 20% cream treated groups produced the shortest healing time when compared with the control. The formulation of the aqueous extract of *F. sur* (FEFS) accelerated wound healing and decreased the wound healing time which may be attributed to synergistic actions of the phyto-constituents of the extract.

Key words: Wounds, burns, skin irritation, herbal cream.

INTRODUCTION

The skin is the largest organ of the body and plays vital roles in thermoregulation, prevention of excessive water loss and also protects the body from external sources of injury (Kolarsick et al., 2011). Tears and breaks in the skins may compromise the integrity of the skin thereby affecting its ability to play this protective role. This defect

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makes the skin susceptible to complication such as compromised vascular status or infections thereby increasing morbidity and mortality risks to the animal (Stephen-Haynes, 2011).

Wounds are physical injuries that result in breaking of the skin or break in the epithelial integrity of the skin. This may be accompanied by disruption of the structure and function of underlying normal tissue, resulting from a contusion, hematoma, laceration, or an abrasion (Dan et al., 2018). Wounds are classified by different criteria, based on the nature of the wound, cause of injury, size, degree of severity or susceptibility to infections and time frame of healing (Velnar et al., 2009; Mohammed et al., 2013). Irrespective of the characteristics of the wound, the main aim of management is to encourage tissue repair and regeneration by promoting optimal environment for wound healing and prevention of complications that maybe caused by microbial infections and subsequently restore the integrity of the skin (Chamanga, 2016).

Ancient texts have documented various methods by which traditional medicine practitioners managed wounds and these methods incorporated plants in their procedures (Bhattacharya, 2012). The efficacy that these plants demonstrated in wound treatment has been attributed to their antiseptic, antimicrobial, analgesic, anti-inflammatory or haemostatic properties of the various constituents (Dan et al., 2018).

Ficus sur Forssk (Moraceae) commonly known as bush fig isa round-crowned deciduous tree which grows up to 25m and is widely distributed in the tropics. In traditional medicine, this plant has been used for conditions that include eye problem, pain, gonorrhea, cough, anti-emetic, sore throat, stomach pain, diarrhoea, oedema, infertility, peptic ulcers and wounds either as sap latex, decoction, infusion or as powdered leaves (PROTA, 2017). Several studies have been carried out on this plant that have shown its effects on the gastrointestinal tract (Akomas et al., 2018), anticonvulsant (Ishola et al., 2013), anti-microbial (Solomon-Wisdom et al., 2011), haematinic (Adebayo et al., 2017) and anti-ulcrogenic activities (Kunle et al., 1999). Despite its popular use in treating wounds, scientific basis for such use is limited. This study was designed to evaluate the effects of the aqueous extract of *F. sur* on experimentally created wounds.

MATERIALS AND METHODS

Plant collection and extraction

The leaves of *F. sur* were collected by Mal I. Muazzam in the month of February 2016, around Suleja, Niger State, Nigeria. The plant was identified and authenticated by Mr. Lateef Akeem of the Department of Medicinal Plant Research and Traditional Medicine, National Institute for Pharmaceutical Research and Development (NIPRD), Idu, Abuja where a voucher specimen (NIPRD//H/6920) was prepared and deposited for future reference.

The leaves were air-dried until a constant weight was obtained under a shade. This was then pulverized to obtain a coarse powder using a pestle and mortar. 250 g of the dried powdered plant material was boiled in water for 20 min. It was then allowed to soak overnight. This was then filtered and the leaves pressed in the sieve to strain out as much liquid as possible. The filtrate was evaporated to dryness on a water bath set at 50°C to afford a dark greenish-brown solid (the extract) with a yield of 7.75±1.48%$^{w}/_{w}$. This was stored in a refrigerator in an air-tight container.

Phytochemical tests

Preliminary phytochemical tests were carried out on the plant following the methods described by Trease and Evans (1989) and Sofowora (1993). The extract was tested for the presence of alkaloids, tannins, saponins, flavonoids, carbohydrates, anthraquinones, sugars, volatile oils, terpenoid and steroidal compounds.

Preparation of oil and water ($^{o}/_{w}$) cream formulation

Cream containing extract of *F. sur* was prepared by o/w type cream formulation shown in Table 1. Ingredients of oil phase (A) were mixed together by melting in a beaker on constant stirring. Components of aqueous phase (B) were mixed together and warmed to about same temperature of oil phase. Aqueous phase was added to oil phase in aliquots on constant stirring. The *F. sur* extract was incorporated when the formulation began to solidify. The preservatives propyl paraben and methyl paraben were added after cooling to room temperature.

pH determination

A 10% suspension of the cream was made by dispersing in 45 ml of water in a 100-ml beaker. The pH of various suspensions was determined in triplicate by using Digital pH meter (Denver Instrument Model 215, NY USA). Results were taken in triplicate; thereafter, average was taken (Purushotham et al., 2010).

Animals

Wistar Albino mice (32 to 36 g) bred and maintained at the Animal Facility Centre of NIPRD were used in these studies. They were housed in standard polypropylene cages with saw dust as beddings, under ambient conditions. The animals were fed on standard rodent feed and had free access to clean drinking water *ad libitum*. The animals were handled according to the Institutional Animal Guidelines for Care and Use of Animals as recorded in the Standard Operating Procedure of the Department of Pharmacology and Toxicology, NIPRD (SOP No. 05:03:02).

Skin irritation test in mice

In this test, dorsal regions of mice (n=5) were carefully shaved and the area cleaned with 70% alcohol. The cream preparations (5, 10 and 20%) were applied once to un-occluded area of 1 × 2 cm. Mice were individually placed in cages to prevent licking of the test substance off their backs. The area was observed for inflammatory reaction on daily basis for 7 days after application. The cream base was used as control. The animal skins were observed for redness, oedema and erosion (Sekizawa et al., 1994).

Effect of cream on excision wound

Mice of both sexes were divided into 5 groups of 5 animals each.

Table 1. Composition of formulation of aqueous extract of *Ficus sur*.

Phase	Ingredients	Formulation (% $^{w}/_{w}$)		
		F1	F2	F3
A	Extract	5	10	20
	Strearic acid	10	10	10
	Emulsifying wax	10	10	10
	Propylene glycol	8	8	8
	Lanolin	2	2	2
	Liquid parafin	5	5	5
B	Propylparaben	0.01	0.01	0.01
	Methylparaben	0.02	0.02	0.02
	Purified water *qs*	100	100	100

The animals were anaesthetised with intraperitoneal injection of ketamine (10 mg/kg). The back hairs of the mice were depilated by shaving and the area cleaned with 70% alcohol. An area of diameter 7 mm was marked on the shaved backs and the entire thickness of the skin from the marked area was excised to obtain a wound. Groups 1 to 6 were topically treated as follows: group 1 was treated with the formulation base, while groups 2 to 4 were treated with the formulations prepared with the test extract at concentrations of 5, 10 and 20%, respectively. Group 5 was treated with povidone iodine. Mice were housed individually and treated once daily for 7 days starting from the day of wound creation. The wound diameter was measured at 3-day interval. The day of complete wound healing was also recorded for each animal.

Effect on burn wound

An aluminium rod of 1 cm diameter was heated in boiling water and then placed for 20 s on the shaved back of mice while under anesthesia. The test formulations: cream base 5, 10 and 20% were then applied on the burn area. Application was repeated daily for 7 days after burn infliction. Silver sulphadiazine was used as control. On day 10, the animals were sacrificed by diethyl ether inhalation. The burn area was harvested and subjected to histopathological analysis. The skins were removed and fixed, dehydrated, embedded and cut for light microscopy. Three sections were made for each wound and stained with hematoxylin-eosin (HE, basic stain), Verhoeff-Van Gieson (VVG, for elastic fibre staining) and Haematoxylin/Van Gieson (HVG, collagen staining). Sections were evaluated by an independent observer.

Statistical analysis

Results were expressed as mean ± standard error of mean (SEM). Significance was determined using two-way analysis of variance (ANOVA) followed by Bonferroni's post hoc test. Results were regarded as significant at $P<0.05$. The software package Graph pad PRISM 6 was used to analyse the data.

RESULTS

Phytochemical analysis

The aqueous leaf extract of *F. sur* tested positive for the presence of tannins, flavonoids, alkaloids and saponins. The pH of the creams was determined as: F1 (5%), 5.86 ± 0.002; F2 (10%), 5.88 ± 0.010; and F3 (20%), 5.92 ± 0.012.

Skin irritation test

No swelling, reddening, and itching was noted during the observation period.

Effect on excision wound

Treatment with the formulation of the aqueous leaf extract of *F. sur* produced a concentration dependent decrease in wound area in treated mice. The number of days for wound healing is 18.20 ± 0.74 for the negative control. Extract treated groups produced 17.60 ± 0.60, 16.20 ± 0.86, and 15.60 ± 0.40 days, respectively while the povidone treated group had 15.4 ± 1.12 days. The reduction in wound diameter was significant for the 20% and povidone groups when compared with the control (Table 2).

Effect on burn wound

In the group treated with the base, there was regeneration of the epidermis with low amount of keratinocytes with proliferation and migration of fibroblast, adipocytes and the volume of collagen and elastic fibres were low. In the 5% treated group, the volume of keratinocytes in epidermis was low, likewise amounts of collagen and elastic fibres were low while presenting with active fibroblast and apocrine glands. Treatment with 10% cream showed low amount of keratinocytes in epidermis with activated fibroblast. There was slight increase in collagen fibres deposition but the volume of elastic fibres remained low. The 20% group shows

Table 2. Effect of cream formulation of the aqueous leaf extract of *Ficus sur* on excision wound model in mice.

Parameter		Control	F1 - 5%	F2 - 10%	F3 - 20%	Standard
Wound diameter (cm)	D3	7.31 ± 0.37	7.06 ± 0.36	6.18 ± 0.39	5.80 ± 0.26	6.10± 0.56
	D6	6.71 ± 0.54	6.26 ± 0.40	5.08 ± 0.53[a]	4.70 ± 0.31[b]	4.28± 0.26[c]
	D9	5.26 ± 0.79	4.70 ± 0.37	4.30 ± 0.48	4.30 ± 0.48	3.26 ± 0.30[b]
	D12	3.24 ± 0.71	3.08 ± 0.49	3.24 ± 0.25	2.30 ± 0.37	2.14 ± 0.12
	D15	1.84 ± 0.42	1.60 ± 0.49	1.40 ± 0.70	1.24 ± 0.34	1.18 ± 0.37
	D18	0.58 ± 0.25	0.28 ± 0.28	0.08 ± 0.08	0.00	0.00

Values are presented as mean ± SEM (n = 5), p<0.05 significant when compared with control. Two-way ANOVA followed by Bonferroni's post hoc test.

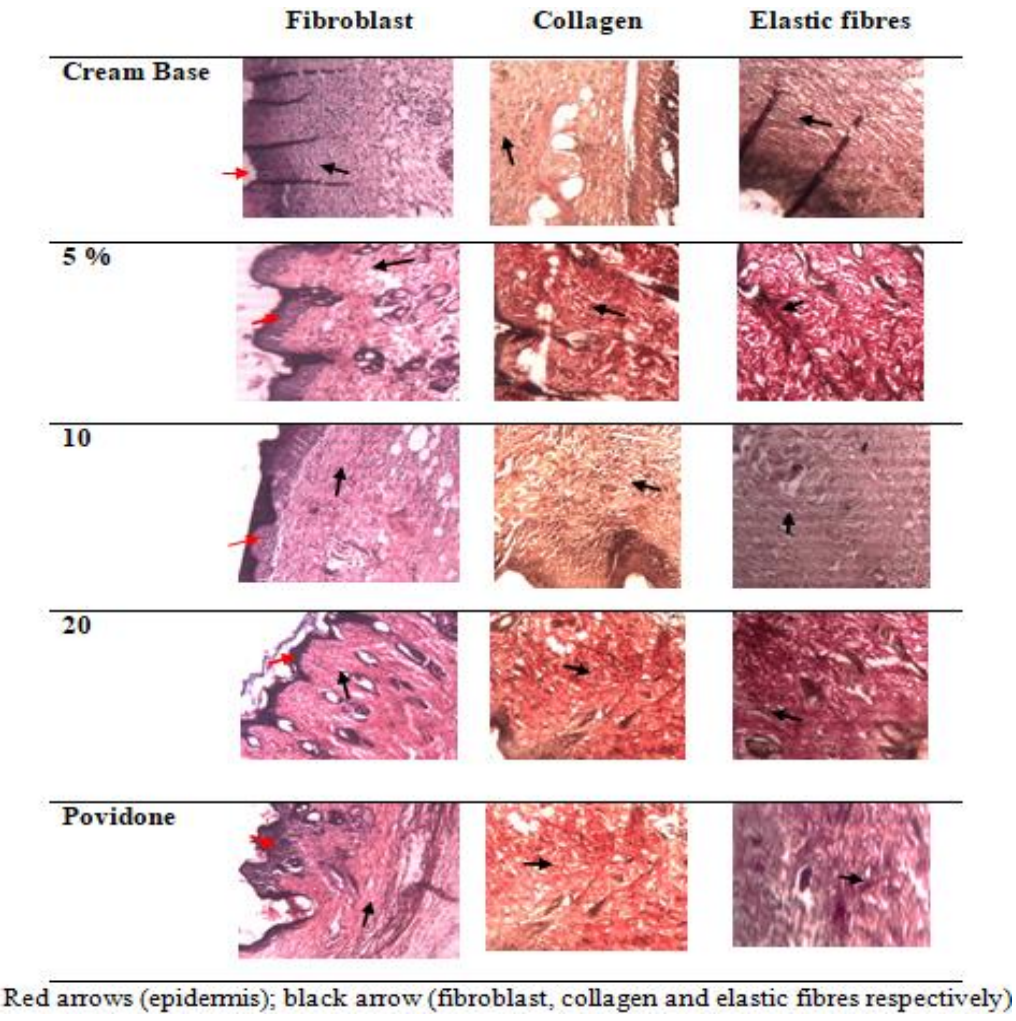

Figure 1. Effect of a formulation of the aqueous extract of *Ficus sur* on burn wound model in mice.

regenerated epidermis with keratinocyes and active fibroblast were detected with increased deposition of elastic fibres, higher volume, deposition and organization of collagen. The povidone treated groups presented with regenerated and formed epidermis and dermis. The amount of collagen and elastic fibres was increased (Figure 1).

DISCUSSION

This skin irritation test is applied for assessment of skin irritation potentials of chemicals for which detailed information on irritating potential may not be needed as in the case of pharmaceuticals or cosmetics agents which maybe directly applied to the skin: the test is useful for

prediction of potential dermal irritancy and toxicities of formulations that will come in contact with the skin as well as their ingredients (Sezikawa, 1992; Vinardell and Mitjans, 2018). In this test, none of the parameter used to assess skin irritation was detected implying that the aqueous extract of *F. sur* (FEFS) may not cause irritant reactions on application to the skin.

The human skin has a pH range of 4 to 6. This pH range is important for its role as a physical barrier because the acidic range is prohibitive to the growth of many invading bacteria; this pH is also necessary for the key enzymes involved in the synthesis and maintenance of competent skin barrier (Ali and Yoshipovitch, 2013). When wounds occur, the initial stage of healing begins; the processes of haemostasis set in with vasoconstriction and platelets aggregation resulting in the production of a fibrin clot to contain blood loss and also serve as first defense against microbial invasion and a provisional matrix for homing of inflammatory cells (Thirovort et al., 2015). At this stage, the wound pH is estimated to be about 7.4. Subsequently, when wound healing progresses pH changes and it would be restored to that of normal skin when the wound is healed (Sirkka et al, 2016). Initially, the wound undergoes acidosis, with increased lactic acid and oxygen in the wound decreasing the pH. Acidosis of the wound is required for the proliferation of fibroblasts, DNA cell synthesis, oxygenation, collagen formation, angiogenesis and macrophage activity as well as induction of keratinocytes differentiation (Bennison et al., 2007*).* The pH of the creams ranges from 5.86 ± 0.002 to 5.92 ± 0.012; this would regulate the pH of the wound and facilitate the processes of wound healing thus making the creams suitable for application for management of skin wounds (Gade et al., 2015).

Fibroblasts, keratinocytes and macrophages are some of the cell types that are mobilized in the inflammatory phase in the wound repair process. Inflammatory signals activate the proliferation and maturation of fibroblasts and keratinocytes, which is essential for wound healing. These cells act jointly to restore normal tissue homeostasis after wounding and contribute to the formation of collagen and promote cross-linking to form an extracellular matrix. Keratinocytes recruit, stimulate, and coordinate the actions of multiple cell types involved in healing and recapitulate the epidermal barrier layer of the skin (Wojtowicz et al., 2014). Fibroblast differentiates into myo-fibroblasts which are contractile cells that promote reduction of wound size, secretion of extracellular matrix proteins to facilitate wound closure (Li and Wang, 2011). Macrophages are critical to wound healing as they play a role in the production of factors that stimulate angiogenesis and fibroplasia; these cells play both tissue reparative and anti-inflammatory functions in wound healing (Koh and Dipietro, 2011). Macrophages, keratinocytes fibroblasts, endothelial cell and adipocytes have been shown to influence wound healing by generation of growth factors that promote cell proliferation, protein synthesis, influence extracellular matrix (ECM) content and cell remodeling (Koh and Dipietro, 2011).

Application of the cream prepared with the extract of *F. sur* promoted wound healing by reducing the wound diameter and facilitating wound closure in a faster rate as compared to the control. Histopathological examination revealed the presence of high amount of activated fibroblast, keratinocytes and elastic fibers. Deposition of collagen and regeneration of the epidermis were also observed. These activities probably played a role in the wound healing and facilitated wound healing and closure. FEFS probably promoted the migration of these cells in addition to enhancement of collagen synthesis and reorganization as well as extracellular matrix production and activation of fibroblasts.

Phytoconstituents such as flavonoids, tannins, saponins, and alkaloids detected in this plant extract have been shown to aid wound healing; this effect has been attributed to their antimicrobial, astringent and antioxidant properties (Agyare et al., 2014). In other experiments, *F. sur* has been shown to demonstrate antibacterial actions against *Staphylococcus aureus* and *Escherichia coli* (Ramde-Tiendrebeogo et al., 2012). These organisms have been implicated in many skin conditions and complications of wounds (Mohammed et al., 2013).

Conclusion

The results from this study revealed that the cream preparation of the FEFS demonstrated significant wound healing potentials.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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