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RESEARCH NOTE

A preliminary study on the effects of an antibacterial steroidal saponin from *Borassus flabellifer* L. fruit, on wound healing

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Chronic wounds have become a great threat to the quality of the life of patients. Chronic wounds due to colonization by bacteria may also lead to chronic degenerative diseases. This situation is much more serious when bacteria develop resistance to known antibiotics. New antibiotics with totally different structures are potentially capable of overcoming such bacteria.

Flabelliferin B (F_B) is one such compound in palmyrah (*Borassus flabellifer* L.) fruit pulp reported in 1998[1]. F_B has a steroidal saponin structure with a molecular weight of 868, which was elucidated in 2002 as β-sitosterol with one glucosyl and two rhamnosyl groups (α 1, 2 and α 1, 4) attached to the OH at C-3 of the aglycone[2]. F_B has proven activity against bacterial species such as *Escherichia coli, Staphylococcus aureus, Staphylococcus epidermidis, Pseudomonas aeroginosa, Proteus rettigeri and Aciinetobacter calcoacetius* [1]. Flabelliferin B can also inhibit the activity of yeast. A naturally occurring fluorescent carotenoid is attached to the flabelliferin, which enhances the anti-microbial activity of this molecule against *Escherichia coli* [3]. Tests on wounds of rats, eye test on Wistar rats and New-Zealand white rabbits showed no allergenic or other toxic effects (Keerthi, *Unpublished data*).

A preliminary human trial was carried out to determine the efficacy of the local application made from F_B. Palmyrah fruits, which are known to be rich in F_B, were used in the preparation of new ointment. Pure F_B with the binder was extracted and stored in a freezer until use in experiments. A panel of human volunteers (n =7) was selected for a patch test by applying F_B on normal unbroken skin. Volunteers were given two plasters (1 × 1 cm^2) one soaked with 50 μL of 74 mg/mL of F_B in distilled water as test and another soaked with water for control. Plasters were placed on the skin of the bicep muscles and independent comments on sensation were elicited.

Commercially available white soft paraffin was obtained from the local pharmacy. F_B ointment (50 g of 2% F_B) was prepared by gradual mixing of separated compounds with white soft paraffin under hygienic conditions in a research laboratory. About 2-3 g prepared ointment was impregnated on 4 cm × 4 cm piece of gauze and covered by aluminum foil until use. The standard hospital treatments were used as controls.

An ethically approved prospective clinical trial was carried out in the Colombo South Teaching Hospital. Patients were selected from the Dermatology Clinic, the Ulcer Clinic and the Out-Patients Department. Patients with superficial ulcers without any subcutaneous tissue involvement were selected by physicians. Patients with ulcers due to diabetes, varicose veins and patients with other underlying pathology were not included in the study. Patients were not under any systemic antibiotic throughout the study as well as one-week prior to the start of experimental treatment. After detailed explanation, written consent was obtained from the patients. Patients were instructed to return to the hospital, immediately in case of any adverse effects. After careful observations by the physician, wound swabs were taken in to sterilized containers for microbiological assessments. Hypertonic saline was used to clean the wounds [4]. Many studies have reported the use of the reduction of the wound size to be a good indicator of healing [5]. Wound outline was

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taken on a transparency sheet and photographs were also obtained. Prepared gauze with the F_B preparation/control was placed on the wound and bandaged. The control patients were treated with normal hospital treatment, which was Povidone-iodine solution (Out-Patient Department), Povidone-iodine cream (Dermatology Clinic) a commercial cream containing Metronidazole (Ulcer Clinic) and Framycetin sulphate (Dermatology Clinic). Treatments were limited to six double blind cases in the Dermatology Clinic but due to logistic reasons double blinding could not be done in the Ulcer clinic and the Out-Patient Department. All relevant data including wound swabs, wound measurements and photographs were obtained repeatedly throughout the study period. Wound swabs and microbiological examinations were carried out under standard conditions. Except for admitted patients who were observed daily, others were observed at least twice a week.

Patch tests carried out confirmed that there are no adverse effects from application of F_B on normal healthy human skin. Results obtained from the human trial are given in Table 1. Wound healing rate is expressed as the percentage reduction of the wound area compared to initial state per week.

Table 1: Effect of F_B on wound healing in humans

Patient No n/n'	% Wound healing rate/week* Test	Control
1/1'	58.2 (B)	31.8 (B)
2/2'	26.6 (B)	14.5 (B)
3/3'	12.2	-3.1 (B)
4/4'	16.7	36.2 (B)
5/5'	1.4	16.2
6/6'	27.2	13.9
-/7'	-	12.7
-/8'	-	18.3
Average	23.7	17.5

(B) - Conducted as double blind trials.
* - Wound healing rate is given as the percentage reduction of the wound size, compared to its' initial size.
n/n' - Patient No for Test/Patient No for Control. Test and controls are from different patients.

Mainly *Staphylococcus*, *Pseudomonas* species and Gram +ve *bacilli* were found in the infected wounds. F_B (4 mg/mL) completely inhibited mixed bacterial cultures in liquid medium under laboratory conditions except for one case, which resulted in slight turbidity. This indicated the inhibition of bacterial growth by F_B. However, there were no remarkable differences between the colony counts of the same wound throughout the study.

The prepared F_B ointment resulted in wound healing without any adverse effects. This compound is a steroidal saponin containing a vital carbohydrate structural moiety for anti-bacterial activity. The attached fluorescent compound (phytoene and/or phytofluene) can alter the characteristics of its complex[3]. Though there is no supporting scientific data, it is presumed that the binder alters membrane permeability. Prepared ointment was impregnated on a piece of gauze and covered by an aluminum foil to avoid any possible destruction of attached fluorescent binder. The above treatment can act in three different ways on the wound. Primarily this can act as a cleanser. This can clean the surface of the wound and the surrounding area to facilitate the wound healing process. After diffusion into the wound, due to the anti bacterial activity it can inhibit or kill harmful pathogens non-specifically. Inhibition of the mixed cultures obtained from the test wound confirmed the activity of F_B on wound bacteria non-specifically. A wound contains many micro-environments, and infected pathogens also vary according to these micro-environments. Therefore, swabs obtained may not represent the entire pathogen population of the wounds.

It was observed that F_B can act as cleansing agent as well as a wound debridement agent. This debridement is similar to the action of proteases or collagenases, which cleans the wound by breakdown of necrotic tissues present in the wound. These three factors act in concert to give wound healing. Having all these effects together in one molecule increases the potential of using this compound in the treatment of wounds.

This study can only be treated as a pilot study as there would be some variation of the ulcers in different individuals. Further the immunological response of different individuals would vary. Therefore, fully controlled pilot trials are not possible. Before implementation a population study should be carried out using a large number of tests and controls.

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References

1. Nikawala J.K., Wijeyaratne S.C., Jansz E.R. & Abeysekara A.M. (1998). Flabelliferins, steroidal saponin from palmyrah fruit (*Borassus flabellifer*) pulp II. Preliminary studies on the effect on yeast and bacteria. *Journal of the National Science Council of Sri Lanka* **26**: 141- 50.
2. Ariyasena D.D., Jansz E.R., Jansson P.E. & Baeckstrom P. (2002). Structural elucidation of the antimicrobial flabelliferin from Palmyrah (*Borassus flabellifer* L.) fruit pulp.*Chemistry in Sri Lanka* **19**: 13-14.
3. Uluwaduge I., Keerthi A.A.P., Senadheera S.N. & Jansz E.R. (2005). Studies on the natural hydrophobic binders of flabelliferins and their effect of some bioactivities. *Journal of the National Science Foundation of Sri Lanka* **33**: 187-191.
4 Tierney L.M., McPhee S.J. & Papadakis M.A. (2004). *Current Medical Diagnosis & Treatment.* 43rd Edition, Lange Medical Books / McGraw-Hill.
5. Gilman T. (2004). Wound outcomes: the utility of surface measures. *Lower Extremity Wounds* **3**:125-132.