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### **RESEARCH PAPER**

# COMPARATIVE STUDY ON THE EFFECTS OF METHANOLIC EXTRACT OF NEWBOULDIA LEAVIS STEM BARK AND HONEY ON ULCERATED SOFT TISSUE INJURY OF WISTAR RAT.

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\*10mokpo K, I., 10riaghan E.A., 1Esekie O.T. and 1Isalar E.L.

<sup>1</sup>Institute of Health Technology, University of Benin Teaching Hospital, Benin City, Nigeria. \*Corresponding Author:komokpo@yahoo.com

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## ABSTRACT

This study investigates the effect of methanolic extract of *newbouldia laevis* stem bark on ulcerated soft tissue injuries of Wistar rats. Sixteen Wistar rats (weighing between 250g-300g) were involved. They were divided into 4 groups (A-D) and received feed mash and water *ad libitum*. Group A served as control, while B, C and D served as test whereby lesions were induced. For 2-weeks, group B had lesions on subcutaneous tissue without treatment, group C had lesions and was treated with *newbouldia laevis* extract, while group D had lesion treated with honey. The results showed no significant difference (p>0.05) between the weights of the test animals and control. Excised tissues obtained from the wound site after histological processing revealed *newbouldia laevis* stem bark's methanolic extract to be most efficacious in treatment of soft tissue injuries compared to honey with the latter reconfirming potentiality for wound healing when compared to that of the untreated wound. Our findings suggest therefore that, *newbouldia laevis* extract can be recommended for the treatment of soft tissue injuries. However, further work needs to be done to ascertain the active components of *newbouldia laevis* and the mechanism of action that can explain its wound healing potentials.

Keywords: Tissue injury, Honey, Newbouldia laevis, Herbal extract.

#### INTRODUCTION

The use of plants, herbs and spices as medicine is an ancient medical practice is common to all societies especially in Africa and other developing national societies. For example, honey for thousands of years, has been known for its medicinal values (Krishna, 2004) such as its healing properties sequel to its antimicrobial properties in treating wounds, infection and diseases (Dynodude, 2009; Moore et al, 2001; Khan et al, 2007; Cooper et al 2008 and Idu et al., 2009).

In fact, long before the discovery of bacteria, honey was considered to be the oldest in wound dressing (Olaitan et al., 2007) as it dates back to ancient medical writings of Egypt, Greece and parts of India (Lusby et al., 2002; Robson et al., 2009). Its use on skin wounds has been documented, including its use on skin grafts, trauma wounds, necrotizing fasciitis, pilonidal sinuses, pressure ulcers, lacerations, burns, surgical wounds, herpetic lesions, atopic dermatitis, animal bites, and rheumatoid ulcers (Pieper, 2009).

Despite these facts on honey however, burn management in developing countries is still riddled with difficulties as these patients face a higher morbidity than mortality because of a large uncovered surface getting infected, necessitating long periods of dressings, leading to deformities and contractures (Subrahmanyam, 1996).

Unfortunately, the management of burns still remains a matter of debate and an ideal dressing for wounds has not been discovered.

Considering therefore, the growing interest in herbal medicine for its economic values, ethnomedicine has become an increasing part of academic research in Nigeria and elsewhere. In this regard, literatures and researchers have documented the application of plant products in medicine and for general physiological purposes (Usman and Osuyi, 2007).

Of interest however, is *Newbouldia laevis* - one of the plants with magical effects, which, according to Idu et al. (2009), is commonly, called 'Tree of Life'. *Newbouldia laevis* is a medium sized angiosperm which belongs to the Bignoniaceae family and grows to a height of about 7-8 (up to 15) meters (Usman and Osuyi, 2007). The plant is often grown as an ornamental, has shiny dark green leaves, bears large showy terminal purple flowers and easily propagated by cutting (Burkill, 1985). It is more or less a sacred or symbolic tree, well planted as a fence and often permitted to grow into a stockade.

Scientifically, *Newbouldia laevis* has been reported to have medicinal value ranging from anti inflammatory, antioxidant, anti-microbial, anti-fungi, analgestic and wound healing properties (Aladesanmi et al., 1998; Stefan et al., 1998; Chukwujeku et al., 2005; Kuete et al., 2007; Usman and Osuyi, 2007; Akerele et al., 2011). Specifically, the stem bark mixed with clay and red pepper has been reported to be effective against pneumonia, fever, cold, cough and for treating different illness like bone lesions (Idu et al, 2009).

Therefore, based on the comparable properties of honey and *Newbouldia Laevis*, this study was designed to investigate the influence of methanolic extract of *Newbouldia laevis* stem bark on ulcerated soft tissue injuries of Wistar rat in comparism with the wound healing potentials of honey.

#### MATERIALS AND METHODS

**Experimental animal:** A total of sixteen Wistar rats of both sexes (weighing ranging from 225g-300g) were used for this study. The animals were kept in cages in the animal house of the Department of Anatomy, University of Benin, Benin City, Nigeria. The animals were categorized into four experimental groups (A-D) of four rats each. They were allowed to acclimatize for three weeks, during which they were fed with live stock growers mash and water.

**Plant collection and identification:** *Newbouldia laevis* stem bark was obtained from Mr. Igbinosun's compound at 19, Oba Ewuare's street, off Federal Government Girls College Road, Ugbowo, Benin City, Edo State, Nigeria. It was taken to the Department of Botany, University of Benin, Benin City for identification and authentication by Prof. M. Idu.

**Extract preparation:** The collected *Newbouldia laevis* stem bark was air dried at atmospheric temperature for 14 days and grinded with a grinding machine to obtain the powdered form. Five kilogram (5kg) was weighed into 250ml of methanol and subjected to maceration for 48 hours. The macerated extract was decanted to separate it from the residue. Four kilogram (4kg) of concentrated extract was yielded.

Study Duration: The duration of the experiment was two weeks (14 days).

**Wound creation.** Before the wound creation was performed, the animals were anesthetized with chloroform via inhalation. It was then positioned in sternal recumbency and the hair on the back of the rats shaved. The skin was sterilized with methylated spirit and allowed to dry. The skin and the underlying cutaneous trunci muscle were excised with a dissecting scalpel and scissors to create a 1.0 cm x 1.0 cm full-thickness wounds. Hemorrhage was controlled by sterile surgical sponge compresses and they were then allowed to recover from the anesthetic.

**Experimental design:** Group A constituted the control while in group B to D animals wound were created. For two weeks, wound from the animals in group B serving as the untreated control while those for group C and D were assigned to receive 20% methanolic extract of *Newbouldia laevis* stem bark and 20% honey respectively.

After application of the topical treatments, the wound areas were then bandaged with sterile non adherent pads and porous adhesive tape. The wounds were cleansed with sterile saline solution and the topical applications were done every other day until the completion of the study duration.

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**Data / sample collection and analysis:** Weight was measured in each group after acclimatization and at the end of the study for comparism. At the end of the study, biopsy was taken from the wound of animals in each group and assessed histologically for features of wound healing.

The weight data were analysis using SPSS (version 10) and the initial and final weight compared using the student t test at a confidential interval of 95% and P valve of 0.05 considered statistically significant. Tissue micrographs from animals in each group were also presented and compared.

### RESULTS

Table 1 shows the mean weight before the experiment compared with that after the experiment. Although animals in group A and C presented mean body weight gain while those of group B and D presented mean body weight lost, the difference before and after experiment was not statistically different in all the groups. Specifically, group A and C have percentage body weight gain of +8.89g and + 2.28g at the end of the experiment while group B and D have percentage weight loss of -1.39g and -11.59g.

Figure 1 micrograph is a representation showing the normal histological features of the skin. Figure 2 is a micrograph presenting skin features in 14 days an untreated wound in rats. The tissue micrograph of the wound on the rat treatment with methanolic extract of *Newbouldia laevis* stem bark in group C shows detailed wound healing with granulation tissue in the dermis (A) and complete epithlialization of the epidermis (B) (Figure 3). The micrograph of the wound on the rat treated with honey in (group D) shows tissue granulation in the dermis (A) and an incomplete epithelialization of the epidermis (B) (Figure 4).

 Table 1: Mean weight of untreated rats and rats treated with methanolic extract of Newbouldia laevis and honey before and after the experiment.

Group	Weight (grams) After acclimatization	Weight (grams) After experiment	Weight changes	% weight change
А	270±15.94	294±6.25	24	8.89
В	287±16.4	283±5,95	- 4	- 1.39
С 🔨 🕤	263±12.5	269±23.1	6	2.28
D	276±10.28	244±25.77	- 32	- 11.59

Values are mean ± standard deviation. % weight gain = weight gain/weight after acclimatization x 100.

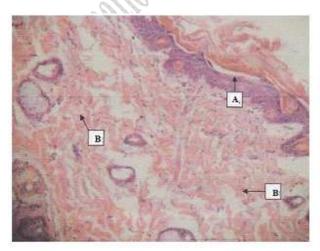


Figure 1: Normal Skin (H&E x100) showing epidermis (A) and dermis with appendages (B)

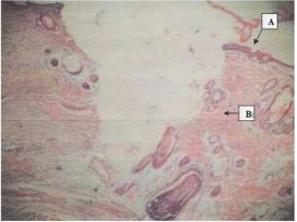


Figure 2: Skin (H&E x40) showing a V-shaped wound involving the epidermis (A)

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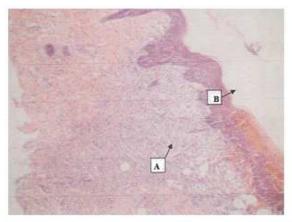


Figure 3: Skin section (H&E x100) treated with Newbouldia laevis stem bark showing wound healing with granulation tissue in the dermis (A) and complete epithelization of the epidermis (B).

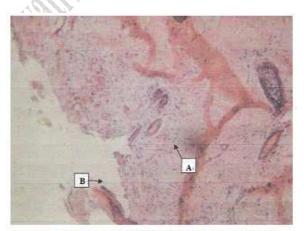


Figure 4: Skin section (H&E x100) treated with honey showing granulation tissue in the dermis (A) and in complete epithelization of the epidermis

## DISCUSSION

This study shows that there was no significant difference (P>0.05) in the weights of the experiment animals compared with control implying that the administered regimen was compatible with the feeding pattern of the animals as reported by Ronald et al., (2006). Statistically, the results show that wound has a weight reducing influence that was more evident amongst the honey treated group that in the *Newbouldia laevis* treated group. This finding is supported by the report of Demling and DeSanti (1999), that endocrine response to trauma results in a net catabolic state and an exaggerated hepatic gluconeogenesis, with losses in lean body mass (weight) similar to the observation made in this study.

Also, the healing potentials of honey observed in this study is in line with that reported by Olaitan et al. (2007) who showed that honey has wound healing properties including hygroscopicity, hyperosmolarity, acidity, peroxide content, and antibiotic activities.

Comparatively however, the result of this study reveals that the wound healing potentials of honey is not as efficacious as *Newbouldia laevis*. Though honey-treated lesions exhibited signs of granulation in the dermis, it was only partial and re-epithelization was incomplete within the experimental period. Nevertheless, the wound healing potentials of honey has earlier been highlighted by Vardi et al. (1998).

On the other hand, treatment with *Newbouldia laevis* showed complete re-epithelization and full granulation of dermis. This attribute appears consistent with that reported by Omokhelin et al., (2001) that herbal plants containing alkaloids, carbohydrates, tannin, flavonoids, saponin, and glycosides, are potential remedies for pain, ulcer, arthritis, rheumatism and other inflammatory condition.

Most importantly, the findings of this study reveal that surface injuries without treatment are associated with delayed healing, possibly due to infection, as observed in the non-treated group unlike those of the honey and newbouldia laevis extract treated groups. The antimicrobial potentials of honey and possibly that *Newbouldia laevis* may account for this observation. Furthermore, the assertion by Demling and Desanti, (1999) that one of the outcomes of trauma following an endocrine response is weight loss implies also, that wound healing can as well be compromised by weight loss. This is in line with the report by Thomas (1997), Williams and Baibbl (2003), Hurd (2004) and Langemo et al., (2006), that there is a relationship between weight and wound healing emphasizing that weight loss is discouraged during wound healing.

Our findings suggest therefore that, *newbouldia laevis* extract can be recommended for the treatment of soft tissue injuries. However, further work needs to be done to ascertain the active components of *newbouldia laevis* and the mechanism of action as regards this reported property.

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## AUTHOR(S) CONTRIBUTION

Omokpo K.I., performed this study with assistance from Oriaghan E.A., Esekie O.T. and Isalar E.L. The materials search, statistical analysis and interpretation and the first draft of this manuscript were prepared by Omokpo K.I. and Oriaghan E.A. However, all authors were involved in the final presentation of this manuscript.