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EVALUATION OF WOUND HEALING EFFECT OF EXTRACT AND FRACTIONS OF *Newbouldia leavis* ROOT BARK IN 5-FLOUROURACIL IMMUNOCOMPROMISED RATS

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ABSTRACT

Chronic wound is one of the major health challenges affecting a great number of people especially individuals with compromised immune system. There is a need to develop an effective drug that would facilitate rapid wound healing in such individuals.To investigate the wound healing properties of extract and fractions of *Newbouldia leavis* extract in immunocompromsed albino rats.This study assessed the wound healing activity of Chloroform extract, n-hexane (NLHF) and ethylacetate (NLEAF) fractions of *Newbouldia leavis* root bark in 5-fluorouracil-induced immunocompromised rats. 5-flourouracil (30 mg/kg) was used to induce immunosuppression. The extract, fractions and reference drug were topically applied for 15 days. The wound healing activity was studied using the excision wound modelSignificant ($p<0.05$) increases in rate of wound contraction, elevated levels of hydroxyproline, hexosamine and hexuronic acid were observed in treated groups when compared with the untreated groups of immunocompromised rats. The extract (40%) significantly accelerated wound healing with the highest percentage of wound contraction of 98.68 at day 12 when compared with groups treated with silver sulphadiazene (95.48%), 40% NLHF (77.04%), and 40% NLEAF (88.83%). The rats treated with NLEAF exerted greater wound healing activity with significantly ($p<0.05$) increased rate of wound contraction, hydroxyproline, hexosamine and hexuronic acid concentrations compared to the group that

received NLHF.It could be stated that *Newbouldia leavis* extract and fractions significantly promoted wound healing and were able to overcome the wound healing suppressing effect of 5-flourouracil.

Keywords: Wounds, immunocompromised, 5-flourouracil, *Newbouldia leavis*, Silver sulphadiazene

1.0 INTRODUCTION

Wounds have been identified as one of the major health problems, both in terms of morbidity and mortality[1]. The problems associated with wounds are even more pronounced in immunocompromised individuals such as those receiving chemotherapy like 5-flourouracil, cyclophosphamide and other anticancer drugs. This is as a result of the immune suppression effect of chemotherapeutic drugs. A number of chemotherapeutic drugs have been identified to cause immune suppression which manifests in the form of leucopenia, neutropenia, thrombopenia, etc [2-3]. Chemotherapeutic drugs treatment suppresses T-lymphocytes activity leading to a transient reduction in interleukin-12 and interferon (IFN-γ) expression, and this forms the basis of its use of chemotherapy in compromising the immune system of an organism, thereby facilitating immunodeficiency.

The immune system plays a significant role in the natural process of wound healing, and thus immune cells and factors produced by these cells are also involved in the host response to tissue regeneration strategies [4].

Essentially, wound healing has four (4) basic stages which are coagulation and homeostasis, inflammation, proliferation, and wound remodeling with scar tissue formation [5].

Medicinal plants have been identified as the primary healthcare agents over many centuries before the advent of modern medicine. *Newbouldia laevis* is one of such plants with rich medicinal benefits. *Newbouldia laevis*is widely used in African folk medicine for the treatment of malaria and fever, stomach-ache, coughs, sexually transmitted diseases, tooth ache, breast cancer,elephantiasis, dysentery, septic wounds, skin infection, infertility rheumatic swellings and constipation [6-10].

2.0 MATERIALS AND METHODS

2.1 Drugs and Chemicals

All drugs and chemicals used were of analytical grade.

2.2 Collection of Root Bark of *Newbouldia laevis*

Fresh root bark of *Newbouldia laevis* was collected from Ozom Mgbagbu-Owa in Ezeagu Local Government of Enugu State and identified by Mr. Alfred Ozioko of Bioresources Development and

Conservation Programme (BDCP), Nsukka, Enugu State, Nigeria.

2.3 Preparation of Plant Material

The root bark was washed with distilled water, air-dried at room temperature and pulverized into powder for extraction. The powder (500 g) was extracted in 1.5 L of 2:1 chloroform/methanol solution and allowed to stand for 48 hrs at room temperature. The mixture was filtered with Whatman No. 1 filter paper. The two layers formed by the addition of distilled water were separated using separating funnel, and were concentrated using rotary evaporator to get a semi solid extract. The chloroform extract was used for further studies.

2.4 Phytochemical Screening

The screening for some chemical constituents of the plant stem bark chloroform extract was carried out as described by Harbone [11] and Trease and Evans [12]. Quantitative analysis was carried out as described by Harbone [11] and Soni and Sosa [13].m

2.5 Fractionation using Column Chromatography

The chloroform extract was dissolved in HPLC grade n hexane and ethylacetate respectively, and the solutions were applied to a column chromatographic column (30 mm diameter × 450 mm height) filled with silica gel (60-120 Mesh). The dissolved extracts were then evaluated with n hexane and ethylacetate respectively. The flow rate was 1 ml/min. The elutes were then concentrated using rotary evaporator.

2.6 Treatment Protocols

Thirty two (32) adult male albino rats, weighing 120 to 200 g were obtained from the animal house of the Faculty of Veterinary Medicine, University of Nigeria, Nsukka. Animals were kept in clean aluminum cages and placed in a well-ventilated house under standard laboratory condition. All the rats were allowed free access to rat pellet and water before and throughout the experiment. The rats were handled according to the guidelines of the National Institute of Health on the case and use of laboratory animals (NIH, 1985).

Summary of groups and treatment:Group A: Untreated; Group B: Standard drug (silver sulfadiazene); Group C: 20% NLE; Group D: 40% NLE; Group E: 20% NLHF; Group F: 40% NLHF; Group G: 20% NLEAF; Group F: 40% NLEAF

KEY:

NLE: *Newbouldia laevis* extract; NLHF: *Newbouldia laevis* n-hexane fraction; NLEAF: *New bouldia laevis* ethylacetate fraction

2.7 INDUCTION OF IMMUNOSUPPRESSION

30 mg/kg b.w of 5-flourouracil was prepared and injected intraperitoneally for five (5) days prior to excision. After excision, 5 mg/kg b.w was administered

every two days throughout the experimental period.

2.8 EXCISION WOUND MODEL

The excision was carried out using a modified method described by Suguna et al. [14]. A wound was created on the dorsal interscapular region of each animal by excising a predetermined circular area of 10×10 mm skin under ether anesthesia. Wounds were left open and the different treatments were applied topically twice a day (once in the morning and night respectively) [15].

2.9 MEASUREMENT OF WOUND AREA

Wound closure was calculated as a percentage of the reduction in wound area [1].

$$\text{Percentage of wound closure} = \frac{\text{Initial wound area} - \text{specific day wound area}}{\text{Initial wound area}} \times 100$$

2.10 Epithelialization Period

The epithelialization time was measured from the initial day to the day when the scab fell off from the wound surface exclusive of leaving a raw wound behind as described by Rashed et al. [16]

2.11 Estimation of Connective Tissue Parameters

The method described by Murthy et al., [1]was used to prepare the connective tissues for evaluation. A quantity (250 mg) of wet tissue was dried at 50°C for 24 h. It was weighed and kept in glass stoppered test tubes. To each tube containing 40 mg of the dried granulation tissue, 1 mL of 6 N HCl was added. The tubes were then kept on boiling water bath for 24 h (12 h each day for two days) for hydrolysis. The hydrolysate was then cooled and excess of acid was neutralized using 10 N NaOH using phenolphthalein as indicator. The volume of neutral hydrolysate was diluted to a concentration of 20 mg/ml with distilled water. The final hydrolysate was used for the estimation of hydroxyproline, hexosamine, and hexuronic acid. Hydroxyproline, hexosamine and hexuronic acid were determined using the methods described by Newman and Logan[17], Dische and Borenfreund[18] and Bitter and Muir [19].

2.10 Data Analysis

Values were expressed as Mean ± standard deviation. Statistical differences were evaluated using a one way analysis of variance (ANOVA), followed by Duncan's Multiple Range Test. Results were statistically significant at $P < 0.05$.

3.0 RESULTS

3.1 Qualitative Phytochemical Evaluation of *Newbouldia laevis* Root Bark Extract

The phytochemical evaluation of the extract showed that the phytoconstituents occurred in the following order: terpenoids> saponins> alkaloids > flavonoids > cardiac glycosides > tannins.

3.2 Effects of Crude Extract, Standard drug (silver sulphadiazene), n Hexane and Ethylacetate Fractions of *Newbouldia laevis* Root Bark Extract on Wound Area and Percentage Contraction

Table 2 shows the effect of crude extract, standard drug (silver sulphadiazene), n-hexane and ethylacetate fractions of *Newbouldia laevis* root bark extract on the wound area, percentage wound contraction and epithelization period. Topical treatment of immunocompromised rats with *Newbouldia laevis* extract and the different fractions accelerated wound healing as shown in Table 2.

3.3 Effects of Crude Extract, Standard drug (silver sulphadiazene), n Hexane and Ethylacetate Fractions of *Newbouldia laevis* Root Bark Extract on Wound Healing Parameters

Table 3 shows the results of effects of crude extract, standard drug (silver sulphadiazene), n hexane and ethylacetate fractions of *Newbouldia laevis* root bark extract on connective tissue parameters (hydroxyproline, hexuronic acid and hexosamine). Hydroxyproline, hexosamine and hexuronic acid contents of dry granulous tissues were significantly ($p<0.05$) increased in NLE, NLHF and NLAEF treated wound when compared with the control group.

Table 1: Phytochemical Constituents of *Newbouldia laevis* Root Bark Extract

Phytochemical Constituents	Bark Extracts (mg/g)	
Terpenoids	+++	6.06±0.37
Saponins	+++	5.59±0.49
Alkaloids	++	4.50±0.26
Flavonoids	++	4.17±0.32
Glycosides	+	3.07±0.40
Tannins	+	2.71±0.28

n = Mean±SD of triplicate determination
+++ = Highly present
++ = Moderately present
+ = Slightly present

Table 2: Effects Different Treatments on Wound area and Percentage Contraction

Treatment	Wound Area in mm^2/ rat (% Contraction)						Epithelization Period (Days)
	DAY 1	DAY 3	DAY 6	DAY 9	DAY 12	DAY 15	
Untreated	314.29±0.00^{a} (0.00)	254.17±0.02^{h} (19.13)	208.88±0.93^{g} (33.54)	183.38±0.06^{g} (41.65)	143.21±0.02^{h} (54.43)	90.19±1.90^{g} (71.30)	19.75±0.96^{e}
Silver sulphadiazene	314.29±0.00^{a} (0.00)	202.14±0.03^{b} (35.68)	171.49±0.95^{b} (45.44)	86.35±0.09^{b} (72.53)	14.20±0.15^{b} (95.48)	0.00^{a} (100)	11.50±0.56^{b}
20% NLE	315.08±1.58^{a} (0.00)	228.22±0.08^{d} (27.57)	178.42±0.05^{c} (43.37)	89.34±0.13^{c} (71.65)	46.23±0.04^{d} (85.33)	23.18±1.96^{c} (92.64)	13.75±0.10^{c}
40 % NLE	314.29±0.00^{a} (0.00)	189.21±0.08^{a} (39.80)	160.52±0.38^{a} (48.93)	60.41±0.12^{a} (80.78)	4.15±0.04^{a} (98.68)	0.00^{a} (100)	9.25±0.50^{a}
20% NLHF	315.88±1.83^{a} (0.00)	248.12±0.03^{g} (21.45)	189.69±0.89^{f} (39.95)	142.85±2.25^{f} (54.78)	84.27±0.08^{g} (73.32)	52.38±2.07^{f} (83.42)	15.75±0.50^{d}
40% n NLHF	314.29±0.00^{a} (0.00)	234.12±0.05^{e} (25.51)	180.73±0.92^{d} (42.50)	120.16±0.08^{d} (61.77)	72.16±1.74^{e} (77.04)	30.91±1.55^{d} (90.17)	14.50±0.58^{c}
20% NLEF	314.29±0.00^{a} (0.00)	236.14±0.03^{f} (24.87)	183.42±0.03^{e} (41.64)	125.60±1.12^{e} (60.04)	80.21±0.16^{f} (74.48)	43.63±0.99^{e} (86.12)	14.75±0.50^{c}
40% NLEF	314.29±0.11^{a} (0.00)	208.18±0.11^{c} (33.76)	178.76±0.89^{c} (43.12)	88.93±0.82^{c} (71.70)	35.12±0.03^{c} (88.83)	6.12±0.07^{b} (98.05)	12.25±0.50^{b}

Values are mean±SD. Values in the same column having different superscripts differ significantly (P < 0.05) NLE = *Newbouldia laevis* extract; NLHF= *Newbouldia laevis* hexane fraction; NLEF= *Newbouldia laevis* ethylacetate fraction

Table 3: Effects of different doses of the extract, n Hexane and Ethylacetate fractions on connective tissue parameters

Groups	Connective Tissue Parameters		
	Hydroxyproline (µg/mg protein)	Hexosamine (µg/mg protein)	Hexuronic acid (µg/mg protein)
Untreated	27.33±10.94^{a}	18.48±0.25^{a}	31.19±4.84^{a}
Standard drug	95.22±4.55^{f}	40.81±2.63^{f}	79.14±4.98^{d}
20% NLE	72.77±1.59^{d}	27.96±0.61^{e}	60.28±4.89^{c}
40% NLE	104.64±6.96^{g}	41.20±1.24^{f}	85.65±4.75^{e}
20% NLHF	38.49±5.17^{b}	15.92±0.90^{b}	34.22±3.51^{a}
40% NLHF	51.60±3.04^{c}	25.38±1.63^{d}	43.52±2.56^{b}
20% NLEF	48.71±2.01^{c}	21.96±0.25^{c}	47.43±1.37^{b}
40% NLEF	86.22±1.03^{e}	43.17±1.14^{g}	83.65±2.74^{e}

Values are mean±SD, n=4.Values in the same column having different superscripts differ significantly (P < 0.05) NLE = *Newbouldia laevis* extract; NLHF= *Newbouldia laevis* hexane fraction; NLEF= *Newbouldia laevis* ethylacetate fraction

4.0 DISCUSSION

The phytochemical contents of the chloroform extract of *Newbouldia laevis* root bark as shown in Table 1 indicated the presence of alkaloids, sterols, tannins, terpenoid and flavonoids. Flavonoids and terpenoids have been identified to possess antioxidant effect [20] and this could be beneficial to wound healing by scavenging excessive reactive oxygen species that might be present in the wound area. Triterpenoids, flavonoids and tannins promoting the wound healing by multiple mechanisms such as wound contraction, increased rate of epithelialization, and prevention of secondary bacterial infection

[21] Etsuo et al. [22] reported that reactive oxygen species induced cell death can result from oxidative processes such as membrane lipid peroxidation, enzyme inhibition, protein oxidation and DNA and RNA damage and thus undesirable for wound healing. Thus, the presence of flavonoids and other antioxidants play an important role in facilitating wound healing possibly by creating a homeostatic condition and also regulating the activities of free radicals.

Wound healing is a biological process which results in the restoration of tissue integrity [23]. Wound healing involves complex activities of blood cells, tissues, soluble mediators, cytokines and several growth factors [24]. However, wound healing can be impeded by several clinical factors including hypoxia, infection, diabetes mellitus, certain medication etc, and any interruption in the process will lead to the formation of non-healing chronic wounds [25, 24]. Though immune system provides a conclusive environment to regeneration and repair for wounded tissue, immunosuppression leads to delayed wound healing. However, an impairment of inflammation response (one of the components of immune system and essential part of wound healing which occurs shortly after injury) by any drug can interrupt the healing cascade [23].

In this study, the decrease in percentage wound contraction observed in the untreated immunocompromised rats indicated a delay in wound healing which could be attributed to impairment of appropriate inflammatory response by 5-flourouracil induced immunosuppression, consequently resulting in slow healing process as shown by decrease in percentage contraction of untreated group.

Healing process depends to a large extent on the regulated biosynthesis and deposition of new collagens and their subsequent maturation [26]. Collagen is the predominant extracellular protein in the granulation tissue of a healing wound and it liberates free hydroxyproline and its peptides when it is cleaved. Hydroxyproline content has been used as an indicator of collagen turn-over [1]. Significant elevated content of hydroxyproline observed in NLE, NLHF and NLEAF treated rats as shown in table 3 suggests faster collagen turn over resulting in speedy healing with simultaneous rise in the wound breaking strength of the treated rats.

Hexosamine and hexuronic acid which are components of glycosaminoglycans are matrix molecules which act as ground substratum for the synthesis of new extracellular matrix [1]. Glycosaminoglycans are known to stabilize the collagen fibres, and their ability to bind

and alter protein-protein interaction has been identified as important determinants of cellular responsiveness in development, homeostatsis and disease [27].

The result of connective tissue parameters such as hexosamine, hexuronic acids and hydroxyproline as shown in Table 3 indicated significant ($p<0.05$) increases in concentration of these substances (hexosamine, hexuronic acid and hydroxypoline) in the treatment groups when compared with the untreated group. The group that received 40% extract showed the highest concentration of hydroxypoline and hexuronic acid respectively when compared with the groups that received 40% ethylacetate and n hexane fraction respectively. Similarly, there was a dose dependent significant ($p<0.05$) increase in wound healing of the group that received the standard drug when compared to the untreated group. Effective wound healing is normally characterized by an increase in hydroxyproline, hexuronic acid and hexosamine in the wound site.

Collagen deposition normally increases during wound healing and hence determines in part the extent of wound healing. Wound healing depends on the regulated biosynthesis, deposition of new collagens and their subsequent maturation [26]. The glycosaminoglycans such as hexuronic acid and hexosamine are known as major components of the extracellular matrix of skin. They are also known to demonstrate hygroscopic and viscoelastic properties which play important roles for dermal tissue function [28]. Collagen molecules are synthesized, laid down at the wound site and become cross-linked to form fibres [1]. In addition, glycosaminoglycan plays important roles in collagen fibre stabilization by enhancing electrostatic and ionic interactions in the collagen fibres thereby facilitating the alignment and characteristic sizes of the collagen fibres [1].

The wound healing ability of the different treatments could also be due to their ability to stimulate interleukin-8, an inflammatory α-chemokine which has been identified to influence the function and recruitment of various inflammatory cells, fibroblasts and keratinocytes. Also, interleukin-8 possibly increases the gap junctional intracellular communication in cultured fibroblasts, and induces a more rapid maturation of granulation tissue [29]. In addition, inflammatory cells facilitate the migration and proliferation of endothelial cells resulting to neovascularization of connective tissue cells which synthesize extracellular matrices including collagen, and of keratinocytes which induce reepithelization of the wounded tissue [30].

CONCLUSION

The results of the study indicated that extract and the different fractions of

Newbouldia laevis root bark expressed a significant wound healing properties as shown by the significant decreases in wound area and increases in percentage wound contraction. The wound healing activities of the various treatment were also reflected by the increase in the concentrations of the various extracellular matrix parameters (hydroxyproline, hexosamine andhexuronic acid) monitored.

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REFERENCES

[1] Murthy S, Gautam MK., GoelS., Purohit V, Sharma H, Goel RK, Evaluation of in vivo wound healing activity of *Bacopa moniera* on different wound models in rats. Biomed Res Int., 2013, 2013, 1-9.

[2] Mintzer DM, Billet SN, Chmielewski L. Drug-Induced Hematologic Syndromes. Adv Hematol, (2009). Article ID 495863, 11 pages.

[3] Bhatt S, Sollom A. Drug-induced neutropenia-pathophysiology, clinical features and management. Ann Clin Lab Sci, 34, 2004, 131-137.

[4] Atala A, Irvine DJ, Moses M, Shaunak S. Wound healing versus regeneration: Role of the tissue environment in regenerative medicine. MRS Bull. 35, 2010, 10.

[5] Velner, T, Bailey, Smorkolj V. The wound healing process. An overview of the cellular and molecular mechanisms, J Int Med Res, 37(5), 2009, 1528-1542.

[6] Iwu MM."Traditional Igbo medicine." Institute of African Studies, University of Nigeria, Nsukka, 1983, 122-144.

[7] Akerele JO, Ayinde BA, Ngiagah J. Phytochemical and antibacterial evaluations of the stem bark of *Newbouldia leavis* against isolates from infected wounds and eyes. Trop J Pharm Res. 10, 2011, 211-218.

[8] Okeke AO. Three minute herbal treatment to reduce dental caries with a *Newbouldia leavis* based extract. Am. J. Undergrad Res, 2, 2003, 1-4.

[9] Akunyili DN. Anticonvulsant activity of the ethanol extract of *Newbouldia leavis*. 2nd NAAP Scientific Conference, 2000, 155-158.

[10] Owolabi OJ, Amaechina FC, Okoro M. Effect of ethanol leaf extract of *Newbouldia laevis* on blood glucose levels of diabetic rats. Trop J. Pharma Res 10, 2011, 249-254.

[11] Harborne JB. 'Phytochemical methods: A guide to modern technique of plant analysis. London: Champman and Hall, 1998.

[12] Trease GE, Evans WC. Pharmacognosy. 15th Ed. Saunder Publishers, London, 2002, 42 - 44, 221 -229, 246 – 249, 404 -306, 331-332, 391-393.

[13] Soni A, Sosa S. Phytochemical analysis and free radical scavenging potential of herbal and medicinal plant extracts. J. Pharmacogn. Phytochem. 2, 2013, 22-24.

[14] Suguna L, Sivakumar P, Chandrakasan G. Effect of *Centella asiatica* extract on dermal wound healing in rats. Indian J Exp Biol. 34, 1996, 1208-1211.

[15] Tramontina VA, Machado MA, Nogueira Filho Gda R, Kim SH, Vizzioli MR, Toledo S. Effect of bismuth subgallate (local hemostatic agent) on wound healing in rats. Histological and histometric findings. Braz. Dent. J. 13, 2002, 11-16.

[16] Rashed AN, Afifi FU, Disi AM. Simple evaluation of the wound healing activity of a crude extract of *Portulaca oleracea* L. (growing in Jordan) in Musmusculus JVI-1.J. Ethnopharmacol, 88, 2003, 131–136.

[17] Newman RE, Logan MA. "The determination of hydroxyproline," J. Biol. Chem. 184, 1950, 299–306.

[18] Dische Z, Borenfreund E. A spectrophotometric method for the micro determination of hexoamines. J. Biol. Chem. 184, 1950, 517-522.

[19] Bitter T, Muir HM. "A modified uronic acid carbazole reaction," Anal Biochem. 4, 1962, 330–334.

[20] Schroeter H, Boyd C, Spencer JPE, et al. MAPK signaling in neurodegeneration: Influences of flavonoids and of nitric acid. Neurobiol Aging, 23, 2002, 861–880.

[21] Lodhi S, Singhai AK. Wound healing effect of flavonoid rich fraction and luteolin isolated from *Martyniaannua* Linn. On streptozotocin induced diabetic rats. *Asian Pac J Trop Med.*6, 2013, 253–259.

[22] Etsuo N, Yorihiro Y, Erika K, Keizo S. Membrane damage due to lipid oxidation. Am. J. Clin. Nutr. 53, 1991, 2015-2055.

[23] Alistair Y, McNaught C. The physiology of wound healing. Surg, 29, 2011, 475-479.

[24] Saini S, Dhiman A, Nanda S. Traditional Indian medicinal plants with potential wound healing activity: A review. Int J PharmaSci Res 7, 2016, 1809-1819.

[25] Mackay D, Miller AL. Nutritional support for wound healing. Altern Med Rev. 8, 2003, 359-377.

[26] Agarwal PK, Singh A, Gaurav K, Goel S, Khanna HO, Goel RK. Evaluation of wound healing activity of extracts of plantain bann. (*Musa sapientum* var paradisiacal) in rats. Indian J Exp Biol. 4, 2009, 32-40.

[27] Trowbridge JM, Gallo RL. Dermatansulphate: New function from an old glycosaminoglycan. Glycobiology, 12, 2002, 117R-125R.

[28] Shetty S, Udupa S, Udupa L. Evaluation of antioxidant and wound healing effects of alcoholic and aqueous extracts of *Ocimum sanctum* linn in rats. Evid Based Complement Altern med 5, 2007, 95-101.

[29] Moyer KE, Saggers GC, Ehrlich HP. Effects of interleukins on granulation tissue maturation. J Cell Physiol. 193, 2002, 173-179.

[30] Clark RAF. Cutaneous wound repair. In: L.A. Goldsmith (Ed), Physiology, Biochemistry and Molecular Biology of the skin Oxford, UK, 1991, pp. 576-601.