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Wound healing and antiulcer activities of the ethanol extract of *Newbouldia laevis* root bark

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Abstract
The objective of this study was to investigate the wound healing and antiulcer activities of the ethanol extract of *Newbouldia laevis* root bark in rats. Dried root bark of *Newbouldia laevis* was extracted by maceration in ethanol and concentrated in a rotary evaporator. Qualitative phytochemical analysis and lethality study (LD_{50}) were carried out using standard methods. Wound healing effects of an ointment containing 50, 100, and 200 mg/kg of the extract were evaluated in rats using the excision wound model while the antiulcer effects of the ethanol extract at 100 and 200 mg/kg were evaluated in rats using indomethacin induced ulcer model. Results obtained showed that the ethanol extract of *Newbouldia laevis* root bark had significant ($P<0.05$) and dose dependent wound healing and antiulcer effects. Percentage wound healing on day 14 of the study at 50, 100 and 200 mg/kg of the extract were 62, 82 and 95% respectively. Both the wound healing and ulcer protection were comparable with those of standard drugs. Phytochemical analysis showed the presence of alkaloids, glycosides, saponins, tannins, flavonoids, steroids and terpenoids. The LD_{50} value obtained was 1296 mg/kg, indicating the safety of the extract. Results of this study vindicate the folkloric use of the plant in wound healing.

Keywords: *Newbouldia laevis*, Root bark, Healing effect, Antiulcer activity, Ethanol extract

INTRODUCTION

Newbouldia laevis (P. Beauv) stem or boundary tree is locally called 'Aduruku' in Hausa, 'ogirishi' in Igbo and Akoko in Yoruba languages (Hutchinson and Dalziel, 1963). It is a medium sized angiosperm which belongs to the Bignoniaceae family and grows to a height of about 7-8 meters (Usman and Osuji, 2007). It can also be found as a shrub with many stem forming clumps of gnarled branches of about 3 meters in height (Arbonnier, 2004). *Newbouldia laevis* is native to the Guinea savannah and the tropical forests of Africa (Arbonnier, 2004). In Nigeria, extracts of the plant have been found to be effective in the treatment of elephantiasis, dysentery, rheumatic swellings, syphilis, constipation, pile and as vermifuge for round worms (Usman and Osuji, 2007). It has also been found useful for ear ache, sore feet, chest pain, epilepsy and children's convulsion (Akunyili, 2000). The stem bark is chewed and swallowed for stomach pains, diarrhea and tooth ache (Lewis and Manony, 1977). In the present study, the anti ulcer and

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wound healing effects of the ethanol extract of the root bark were investigated.

EXPERIMENTAL

Experimental animals. Adult albino mice (20 – 32g) and rats (160 – 220g) of both sexes obtained from the animal house of the Department of Pharmacology and Toxicology, Madonna University, Elele were used. The animals were housed in standard metallic cages and fed with standard rat chaw and allowed access to drinking water without restriction. All animal experiments were in compliance with the National Institute of Health Guide for care and use of Laboratory Animals.

Chemicals and drugs. All reagents were of analytical grade and were purchased locally from licensed chemical dealers. They included methanol, sodium chloride, cetoseary alcohol, soft paraffin, hard paraffin and wool fat. Neomycin sulphate (Cicatrin powder®) a product of GlaxoSmithKline was purchased locally from a registered pharmacy.

Plant material and extraction. Fresh root bark of *Newbouldia laevis* was harvested at Madonna University, Elele, Rivers state and its identity authenticated by Pharm. Osuala of the Department of Pharmacognosy of the same institution, where a voucher specimen was deposited. The root bark was air dried for seven days and pulverized. 200 g of the pulverized root bark was macerated in 2 litres of ethanol for 48 hours. The extract was filtered using Whatman No. 1 filter paper and concentrated using a rotary evaporator.

Phytochemical Screening. The crude extract was screened for the presence of alkaloids, glycosides, saponins, tannins, flavonoids, and terpenoids, using standard methods (Evans, 2002; Harbone, 1998).

Acute toxicity test. Lorke's (1983) method was used to determine the acute toxicity of the ethanol extract of *Newbouldia laevis* root bark. In the first stage, fifteen mice of both sexes were randomly divided into three groups of five mice each and administered orally 10, 100 and 1000 mg/kg aqueous extract. The mice were observed for 24 hours for effects of toxicity and mortality. When no death occurred, a further three groups of five mice each were administered 1200, 1400 and 1800 mg/kg of the extract orally. The animals were observed for 48 hours for effects of toxicity and the number of deaths was recorded. The LD_{50} was calculated as the geometric mean of the highest dose that the animals survived and the lowest dose that killed the animals.

Indomethacin induced ulcers. Sixteen adult albino rats were randomly divided into four groups of four animals each. They were fasted for 18 hours but allowed free access to water prior to the commencement of the experiment. Group A (control group) received 0.5 ml normal saline. Groups B and C received 100 mg/kg and 200 mg/kg of the extract respectively. Group D (reference group) received 100 mg/kg cimetidine. The route of administration for all the agents was oral.

Thirty minutes after the administration of extract, ulcer was induced by oral administration of indomethacin (30 mg/kg) to all the groups of animals. After 8 hours the animals were sacrificed and their abdomen was cut open along the greater curvature and rinsed under a stream of water. The lesions on the gastric mucosa were observed with a hand lens (x 10) (Aguwa and Mittal, 1981), and scored 0-4 using an arbitrary scale where 0 = no lesions; 0.5 = hyperemia; 1 = one or two lesions; 2 = severe lesions; 3 = very severe lesions and 4 = mucosa full of lesions (Cashin *et al.*, 1979). The number of ulcer spots in the glandular portion of the stomach was counted in both control and drug treated animals and the ulcer index calculated.

Wound induction. Twenty albino rats of both sexes were randomly divided into five groups

(A-E) of 4 animals each. The animals were anaesthesized with chloroform and excision wounds of 1.5 cm in diameter created on the depilated thoracic region (Morton and Malone, 1972). The animals were treated as follows:

Group A received the ointment base only
Groups B, C and D received ointment containing 50, 100 and 200 mg of the extract respectively.
Group E (reference) received cicatrin ointment (neomycin sulfate).

The route of administration for all the animals was topical and the frequency was once daily, starting from day 1 after wound induction and continued until the 6^{th} day post wounding. The wound diameter was measured daily and the epithelization period recorded at the end of the study. The percentage wound contraction was calculated using the following formula:

% wound contraction = (Healed area/Total wound area) x 100

Healed area = Original wound area - Present wound area

Statistical analysis. The results were presented as mean ± SEM and differences between means were analysed using one way analysis of variance (ANOVA). The level of significance was at $p < 0.05$.

RESULTS

Phytochemical screening. Phytochemical screening of the ethanol extract of *Newbouldia laevis* root bark revealed the presence of alkaloids, glycosides, saponins, flavonoids, steroids and terpenoids (Table 1).

Acute toxicity tests. One death each was recorded at the dose of 1400 and 1800 mg/kg of the extract. Before dying the animals exhibited general body weakness. No death was recorded at 10, 100 and 1000 mg/kg. The LD_{50} was therefore 1296 mg/kg.

Effect of extract on excision wound. Table 2 summarizes the effect of the ethanol extract of *Newbouldia laevis* root bark on excision wound model in rats. As time progressed, there was a dose dependent reduction in wound sizes of the treatment groups (Groups B, C and D) compared to the control group (Group A). Group B (50 mg extract) and Group C (100 mg extract) recorded significant ($P < 0.05$) reduction in wound sizes compared to group A animals treated with the ointment base only. The reduction in wound sizes in group D treated with ointment containing 200 mg extract was observed as from day 2 and continued on till day 6 . Group C animals recorded significant ($P < 0.05$) reduction in wound sizes as from day 4 until day 6. Animals in group B which received 50 mg of the extract recorded significant ($P < 0.05$) reduction in wound sizes only on day 12 with a mean wound size of 0.57 ± 0.03 cm.

Effect of extract on indomethacin induced ulcers in rats. The ethanol extract of *Newbouldia laevis* root bark significantly ($P < 0.05$) protected the rats from indomethacin induced ulcers at all dose levels (Table 3), with the highest protection (62.5%) recorded at 200 mg/kg as against 50% recorded for the 100 mg/kg dose level.

Table 1. Phytochemicals present in the ethanol extract of *Newbouldia laevis* root bark.

Phytochemical	Inference
Alkaloids	+
Glycosides	+
Saponins	+
Tannins	+
Flavonoids	+
Terpenoids	+

+ Present - Absent

Table 2: Effects of ethanol extract of *Newbouldia laevis* root bark on excision wounds in rats

Treatment	Wound Sizes (cm) ± SEM						
	Day 0	Day 2	Day 4	Day 6	Day 8	Day 10	Day 12
Group A (Ointment base)	1.50 ±0.00	1.50±0.00	1.33± 0.03	1.17± 0.03	1.00± 0.06	0.95± 0.05	0.87±0.03 (42)
Group B (50 mg Extract)	1.50 ±0.00	1.50± 0.00	1.27± 0.03	1.00± 0.06	0.8 ± 0.09	0.70± 0.06	0.57±0.03* (62)
Group C (100 mg Extract)	1.50 ±0.00	1.47 ±0.05	0.97±0.09*	0.87±0.03*	0.6 ± 0.33*	0.43±0.03*	0.27±0.03* (82)
Group D (200 mg Extract)	1.50 ±0.00	1.37±0.03*	0.97±0.09*	0.56±0.03*	0.2 ± 0.03*	0.13±0.03*	0.07±0.03* (95)
Group E (100 mg Cicatrin ointment)	1.5 ± 0.00	1.40 ±0.06	1.17± 0.15	0.93± 0.15	0.6 ± 0.14	0.50 ±0.12	0.30±0.12 (80)

Each value is mean ± SEM of 4 rats. Figure in parenthesis represents % wound healing at the end of the study.

Table 3: Effects of ethanol extract of *Newbouldia laevis* root bark on indomethacin induced ulcers in rats

Treatment	Dose (mg/kg)	Ulcer index	% ulcer protection
Control (Normal saline)	-	4.0 ± 0.00	0
Extract	100	2.0 ± 0.00*	50
Extract	200	1.5 ± 0.00*	62.5
Cimetidine (Reference standard)	100	1.0 ± 0.00	75

DISCUSSION

The results of the phytochemical tests are in agreement with Usman and Osuji (2007); and Oliver-Bever (1986). Results of the acute toxicity test showed that with a very high LD_{50} of 1296 mg/kg, the extract is very safe for use in traditional medicine.

Experimental assessment of the wound healing and antiulcer activities of the ethanol extract of the root bark of *Newbouldia laevis* showed increased rate of wound healing and protection of gastric mucosa from ulcerogenic effects of indomethacin in treated animals. While wounds occur under acute conditions and are healed through a natural process that may be prolonged without treatment, peptic ulcers are amplified and sustained by their pathophysiological mechanisms (Okoli *et al*., 2009). Whatever is the basic amplification mechanism of the disease process, treatment with wound healing or antiulcer agents respectively stimulates or accelerates healing (Okoli *et al*., 2009). Results of this study have established the protective effects of ethanol extract of the root bark of *Newbouldia laevis* against indomethacin induced gastric ulceration in rats which is caused by the inhibition of the synthesis of endogenous cytoprotective prostaglandins (Lanza, 1998). The protective effect of the extract against indomethacin induced gastric ulceration in rats was significant and dose dependent, with the percentage protection of the 200 mg/kg group higher (62.5 %) than the 100 mg/kg group which gave a protection of 50 %. Cytoprotection by drugs has been considered to be due to the generation of prostaglandins by antiulcer drugs when used in their non-antisecretory doses (Robert *et al*., 1979). Experimental assessment of the wound healing activity of the ethanol extract of the root bark of *Newbouldia laevis* showed increased rate of wound healing. Topical application of the extract at 100 and 200 mg/kg significantly ($P < 0.05$) accelerated the

process of wound healing, compared to the control group which received only the ointment base. Wound healing involves regeneration of specialized cells by proliferation of surviving cells and connective tissue response characterized by the formation of granulation tissue (Whaley and Burt, 1996). The wound healing and ulcerogenic protective effects of the extract can be attributed to its phytoconstituents. Tannins by virtue of their antioxidant, anti-inflammatory, astringent and antimicrobial properties could be responsible for the wound healing and ulcerogenic protective activities of the extract (Manjunath *et al*., 2006). The antioxidant property of flavonoids (Manjunath *et al*., 2006) could also be responsible for the wound healing and antiulcer activities of the extract. In conclusion, the ethanol extract of the root bark of *Newbouldia laevis* has both wound healing and ulcer healing effects against experimentally induced wound and ulcer in rats.

REFERENCES

Aguwa, C.N. and Mittal G.C. (1981). A study of antiulcer activity of aqueous extract of leaves of Pyrenacantha staudtii (Family Icacinaceae) using various models of experimental gastric ulcer in rats. *Eur. J. Pharmacol*., 74: 215 – 219.

Akunyili, D.N. (2000). Anticonvulsant activity of the ethanolic extract of Newbouldia laevis. 2nd NAAP Scientific Conference, 155 – 8.

Arbonnier, M. (2004) Trees, Shrubs and Lianas of West Africa dry zones (1st edition). CIRAD, Margraf publishers GMBH MNHN, USA, pp. 1 – 574.

Cashin, C. H., Dawson, W. and Kitchen, E.A. (1979). The Pharmacology of Benoxaprofen (2,4 – chlorophenyl-methyl-5-benzoxazole acetic acid) LRC L3694, a new compound with anti-inflammatory activity apparently unrelated to inhibition of prostaglandin synthetase. *J. Pharm. Pharmacol*., 29: 330 – 336.

Evans, W.C. (2002). Trease and Evans' Pharmacognosy, 13th edition. Balliere Tindall, London. Pp. 30 – 48.

Harbone, J.B.C. (1998). Phytochemical methods: A guide to modern technique of plant analysis (2nd edition), Chapman and Hall, London, p. 120.

Hutchinson, J. and Dalziel, J.M. (1963). Flora of West Tropical Africa, Vol II. Crown agents for Oversea Governments and Administration 4, Millbank, London, S.W. 1. Pp. 435 – 436.

Lanza, F.L. (1998). A guideline for the treatment and prevention of NSAID – induced ulcers. *Amer. J. Gastroenterol.* 90: 2037 – 45.

Lewis, W.H. and Manony, P.F.E (1977). Medical Botany; Plants affecting man's health. John Wiley and Sons, New York, U.S.A., p. 240.

Lorke, D. (1983). A new approach to practical acute toxicity testing. Arch. Toxicol., 54: 251 – 287.

Manjunath, B.K., Vidya, M., Krishna, V. and Mankani, K.L. (2006). Wound healing activity of Leucas hirta. *Indian J. Pharma. Sci.* 5:380-384.

Morton, J.J.P. and Malone, M.H. (1972). Evaluation of Vulnerary activity by an open procedure in rats. *Arch Int. Pharmacodyn*. pp. 117 – 126.

Okoli, C.O., Ezike, A.C., Akah, P.A., Udegbunam, S.O., Okoye, T.C., Mbonu, T.P. and Ugwu, E. (2009). Studies on wound healing and antiulcer activities of extract of aerial parts of Phyllanthus niruri L. (Euphorbiaceae). *Amer. J. Pharmacol. Toxicol*., 4(4): 118 – 126.

Oliver-Bever, B. (1986). Medicinal plants in Tropical West Africa. Cambridge University Press, London, pp. 117 -8, 168.

Robert, A., Nezmin, J.E., Lancaster, C. and Hancher, A. J. (1979). Cytoprotection by prostaglandins in rats: Prevention of gastric necrosis produced by HCl, NaOH, hypertonic NaCl and thermal injury. *Gastroenterology*, 76:439-43.

Usman, H. and Osuji, J.C. (2007). Phytochemical and in vitro antimicrobial assay of the leaf extract of Newbouldia laevis. *Afri. J. Trad. Compl. Altern. Med.*, Vol. 4 (4): 476 – 480.

Whaley, K. and Burt, A.D. (1996). Inflammation, healing and repair. In: Muir's textbook of pathology, MacSween, R.M.N. and Whaley, K. (Eds), 13th edition. Arnold, London, pp. 112 – 165.