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Antinociceptive Effects of *Newbouldia laevis* (P. Beauv.) Stem Bark Extract in a Rat Model

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ABSTRACT
Various parts of *Newbouldia laevis* (fam. Bignoniaceae) are used for pain and several inflammatory conditions in Ghana. This study presents the effect of a hydro-alcoholic extract of *N. leavis* stem bark in formalin-induced pain, a model of neuropathic pain, in rats. Morphine (1-10 mg kg^{-1} *i.p*) and stem bark extract of *N. laevis* (10-300 mg kg^{-1} *p.o.*), dose-dependently decreased both phases of the formalin-induced nociceptive behaviour. Nocifensive response for morphine was four fold higher in the first phase (ED_{50};1.79 ± 0.63 mg kg^{-1}) compared to the second (ED_{50} ;7.59± 2.26); however the response for the extract was similar in both phases (ED_{50}; first phase 28.28±7.02; ED_{50}; second phase 25.07 ± 5.83). Diclofenac (10-100 mg kg^{-1}) was effective only in the second phase (ED_{50} 33.24± 5.20). The potency of the drugs was in the order; morphine > extract > diclofenac for the first phase and morphine > extract ≈ diclofenac for the second phase. The results from this study show that *N. laevis* extract has central and peripheral analgesic properties and thus adds credence to its traditional uses.
KEY WORDS : *Newbouldia laevis,* Anti-nociceptive activity, Anti-inflammatory activity.

INTRODUCTION

Newbouldia laevis (P. Beauv.) Seeman ex Bureau.(Bignoniaceae) is a shrub or small tree growing in regions of wooden savanna and deciduous forest of West Africa. The plant is widely used in African traditional medicine. Commonly called the fence tree or the African border tree, it is locally known in Ghana as *sasanemasa* or *esisimansa* in Akan; *aviati* amongst the Ewes, and *asratso, or hiatso* in Ga (1). The roots and leaves are used in treating elephantiasis and convulsion. The roots and stem are used to treat malaria, while the bark and twigs are used to treat pelvic pain in females. The stem bark is used to treat peptic ulcer, otalgia, skin ulcer, epilepsy, hemorrhoids and constipation. The leaves and stem bark are used for treating cough while the leaves are used to treat orchitis (gonococcal) (1). Olajide *et al*. (2) reported that the methanolic stem bark extracts of *N. laevis* inhibited carrageenan-induced edema in the rat hind paw in a dose-related fashion. The methanolic extract of the stem bark was also found to produce a reduction of yeast-induced pyrexia and significant analgesic activity in acetic acid-induced "writhings" in mice (2). We have earlier presented a preliminary report on the anti-arthritic (3) and antioxidant properties (4) from the hydro-alcoholic stem bark extract. The present study reports of the analgesic effects of the hydro-alcoholic stem bark extract in the formalin test a clinical model for pain.

MATERIALS AND METHODS

Plant material

Bark of *Newbouldia laevis* was collected from the Botanic Gardens, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana.

Preparation of extract

Stem bark of *N. laevis* was air-dried for seven days, powdered and soxhlet-extracted with 70 % v/v of ethanol. The hydro-alcoholic extract was then evaporated to a syrupy mass under reduced pressure, air-dried and kept in a dessicator. This is subsequently referred to as extract or NLE.

Animals

Spraque-Dawley rats (150-200g) were purchased from Noguchi Memorial Institute for Medical Research, University of Ghana, Legon, and maintained in the Animal House of the Department of Pharmacology, Kwame Nkrumah University of Science and Technology (KNUST), Kumasi, Ghana. The animals were housed in groups of 6 in stainless steel cages (34 × 47 × 18 cm) with soft wood shavings as bedding, fed with normal commercial pellet diet (GAFCO, Tema), given water *ad libitum* and maintained under laboratory conditions (temperature 24-28 °C, relative humidity 60-70%, and 12 hour light-dark cycle).

Formalin-induced nociception

The formalin test first described by Dubuisson and Dennis (5) was carried out as described by (6) with a few modifications. Each animal was assigned and acclimatized to test chambers (a perspex chamber 15 × 15 × 15 cm) for thirty minutes before formalin injection (7). The rats were then pre-treated with the test drugs (30 min for *i p*. route and 1 h for oral route) before intraplantar injection of 50 µl of 4 % formalin. The animals were immediately returned individually into the testing chamber. A mirror angled at 45˚ below the floor of the chamber allowed a complete view of the paws. The behavior of the animals over one hour was captured with a digital camera (Sony digital video camera recorder, DCR-DVD 705E) placed directly opposite to the mirror and attached to a computer. Pain response was scored for 60 min, starting immediately after formalin injection.

Analysis of Data.

The first phase of the formalin test was defined conservatively as 0-10 minutes and the second phase 10-60 minutes post formalin injection (8). A nociceptive score was determined for each 5-minute time block in each phase by measuring the time spent biting/licking the injected paw (9). Tracking of the behavior was done using Behavior Tracker Version 1.5. Average nociceptive score for each 5-minute time block was calculated by multiplying the frequency and time spent in biting/licking. The time-course curves were subjected to two-way (*treatment* × *time*) repeated measures analysis of variance with Bonferroni's *post hoc* test.

ED_{50} (dose responsible for 50% of the maximal effect) for each drug was determined by using an iterative computer least squares method, with the following nonlinear regression (three-parameter logistic) equation:

$$Y = \frac{a + (b - a)}{\left(1 + 10^{(LogED_{50} - X)}\right)}$$

Where, *X* is the logarithm of dose and *Y* is the response. *Y* starts at *a* (the bottom) and goes to *b* (the top) with a sigmoid shape.

The fitted midpoints (ED_{50}S) of the curves were compared statistically using *F* test (10, 11). GraphPad Prism for Windows version 4.03 (GraphPad Software, San Diego, CA, USA) was used for all statistical analyses and ED_{50} determination. $P < 0.05$ was considered statistically significant in all analysis.

RESULTS

Formalin induced the characteristic nociceptive response ($F1,88 = 469$, $P<0.0001$) exhibited as biting or licking of the injected paw. The response to pain was biphasic as previously reported (5, 12), consisting of an initial intense response to pain beginning immediately after formalin injection and rapidly decaying within 10 min after formalin injection (first phase). This was then followed by a slowly rising but longer-lasting response from10-60 min after formalin injection with maximum effect at approximately 20-30 min after formalin injection (second phase) (9, 13).

The extract, NLE (10-300 mg kg^{-1}) significantly and dose-dependently decreased formalin-induced nociceptive behavior in both the first ($F4,40 = 14.95$, $P < 0.0001$) and second phase ($F4,180 = 23.53$, $P < 0.0001$) (Figure 1); being equipotent in both phases (first phase ED_{50}; 28.28±7.02; second phase ED_{50}; 25.07 ± 5.83) (Table 1). Similarly morphine (1-10 mg kg^{-1}), an opioid agonist significantly attenuated the formalin-induced nociceptive behavior in both the first ($F3,32= 14.89$, $P < 0.0001$) and second phases ($F3,144=4.65$, $P < 0.0161$) in a dose-dependent fashion (Figure 2). The nocifensive response for morphine was approximately four fold lower in the first phase (ED_{50};1.79±0.63 mg kg^{-1}) (Table 1) compared to the second phase (ED_{50};7.59±2.26) (Table 1). In contrast to morphine and NLE, diclofenac (10-100 mg kg-1) was not effective in the first phase ($F3,32= 0.51$, $P = 0.6810$) but produced a dose-dependent analgesic effect in the second phase ($F3,144 = 15.60$, $P < 0.0001$; $ED_{50} = 33.24±5.20$) (Figure 3).

From ED_{50} values (Table 1) obtained from dose response curves (Figure 4) the rank order of potency was; morphine > extract > diclofenac for the first phase and morphine > extract ≈ diclofenac for the second phase. NLE was found to be approximately fifteen fold less potent than morphine in the first phase. In the second phase, NLE was three fold less

Table 1: ED_{50} values for N. laevis extract, diclofenac and morphine in the formalin test

Drug	ED_{50} (mg kg^{-1})	
	Phase 1	*Phase 2*
NLE	33.15±14.27	28.41±6.20.
Morphine	1.90±0.63*†††	7.63±4.67‡
Diclofenac	-	34.87±10.20

*P < 0.05 compared to morphine phase 2; †††P<0.001 compared NLE phase 1; ‡P<0.05 compared to *NLE* phase 2 (One-way ANOVA followed by Neuman-Keul's *post hoc* test)

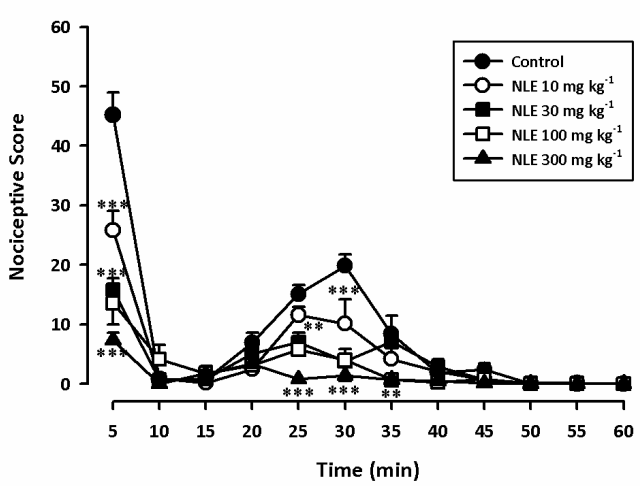

Fig. 1: Effect of NLE (10-300 mg kg^{-1} p.o.) on the time course of formalin-induced pain in rats. Nociceptive/pain scores are shown in 5min time blocks up to 60 min post formalin injection. Each point represents mean ± S.E.M (n = 5). ***P < 0.001; ** P < 0.01 compared to vehicle-treated group (Two-way ANOVA followed by Bonferroni's post hoc test)

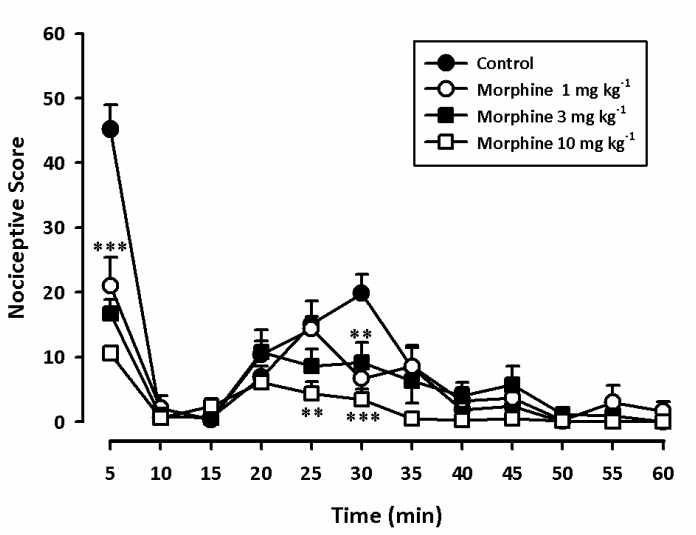

Fig. 2: Effect of morphine (1-10 mg kg^{-1} i.p.) on the time course of formalin-induced pain in rats. Nociceptive/pain scores are shown in 5min time blocks up to 60 min post formalin injection. Each point represents mean ± S.E.M (n = 5). ***P < 0.001; ** P < 0.01 compared to vehicle-treated group (Two-way ANOVA followed by Bonferroni's post hoc test)

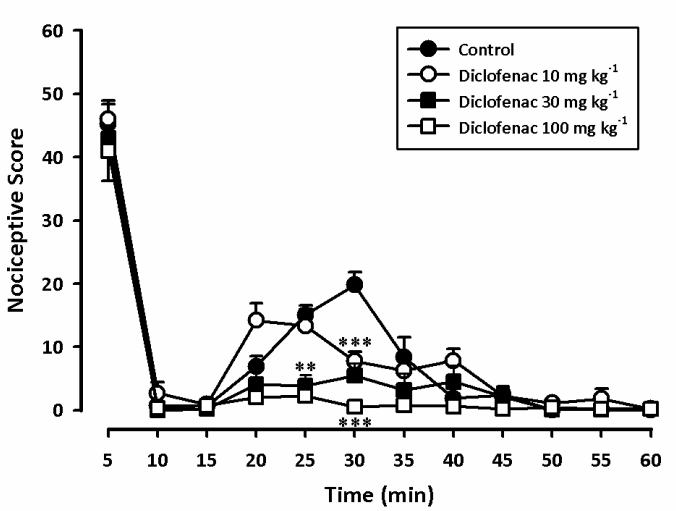

*Fig. 2: Effect of diclofenac (10-100 mg kg^{-1} i.p.) on the time course of formalin-induced pain in rats. Nociceptive/pain scores are shown in 5min time blocks up to 60 min post formalin injection. Each point represents mean ± S.E.M (n = 5). ***P < 0.001; ** P < 0.01 compared to vehicle-treated group (Two-way ANOVA followed by Bonferroni's post hoc test).*

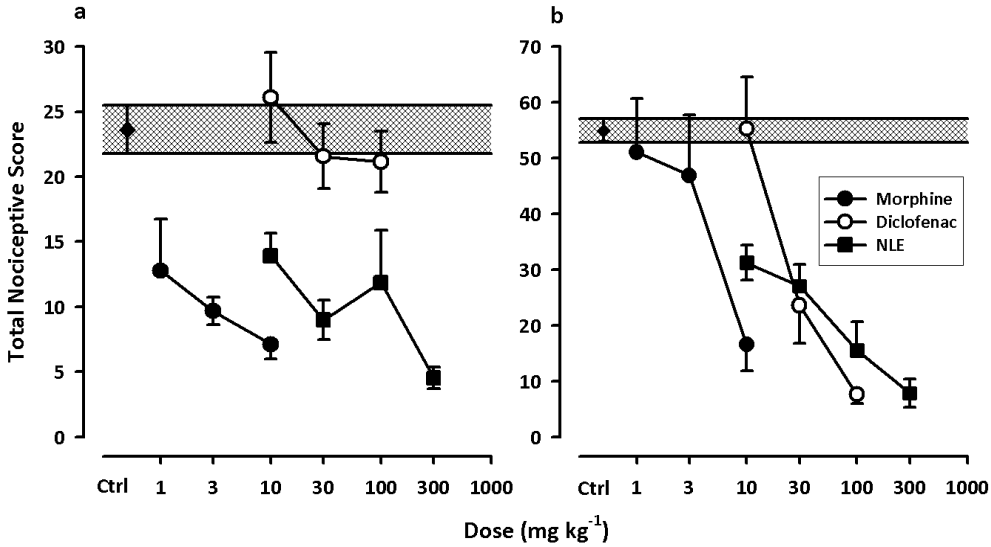

Fig. 4 Dose response curves for the effect of morphine (1-10 mg kg^{-1} i.p.), diclofenac (10-100 mg kg^{-1} i.p.) and NLE (10-300 mg kg^{-1} p.o.) on the total nociceptive score for the first phase (a) and the second phase (b) of the rat formalin test. Each point represents mean ± S.E.M (n = 5).

potent than morphine but equipotent compared to diclofenac in attenuating formalin-induced nociception.

DISCUSSION

Several models are used to evaluate the analgesic effect of drugs. The stimulus may be thermal (tail flick, tail immersion, and hot plate tests), mechanical (tail or paw pressure tests), electrical (stimulation of paw, tail or dental pulp) or chemical ('writhing'and, formalin tests) (14-16). However, the formalin test first described by Dubuisson and Dennis (5) has been shown as the most predictive of acute pain (16) and a valid model of clinical pain (17-19). The formalin test, is a well characterized and accepted method in pre-clinical screening of analgesics (19-20). Intradermal injections of formalin into the rat paw resulted in a biphasic nociceptive response evidenced by flinching, licking or biting of the injected paw as reported earlier (5, 12). An analgesic drug would tend to decrease the incidence of flinching, licking or biting of the injected paw (21). It is suggested that the first phase of the formalin response is due to the direct stimulation of nociceptors by formalin, sensitive to central analgesics (14, 16, 22) whereas the second

phase involves inflammatory components with release of different pain mediating substances that possibly activate small afferents (6, 16, 23), hence sensitive to centrally acting analgesics, NSAIDs (6, 16, 23), and corticosteroids (18). The NSAID, diclofenac was effective in only the second phase of the formalin test as previously described in various experiments (24, 25, 26). This is characteristic of cyclo-oxygenase inhibitors and therefore consistent with NSAIDs (6, 24, 27, 28). The analgesic properties exhibited by the extract and morphine in both the first and second phases is characteristic of analgesics with central effects (16, 27). Extracts of *N. laevis* stem bark are reported to have anticonvulsant (2) and sedative (29) activities. The central analgesic properties of *N. laevis* in the formalin test is supported by the revelation that some tricyclic antidepressants (amitryptiptyline) and anticonvulsants (gabapentin and lamotrigine) have exhibited analgesic activity in the formalin test (8, 19, 30). It is not uncommon these days for clinicians to treat pain with analgesics and co-analgesics such as antidepressants, antiepileptic/anticonvulsants, local anesthetics and antiarrhythmics (19, 31, 32, 33,). These findings lend credibility to the traditional use of stem back extract of *N. laevis* in convulsion, epilepsy, inflammation and pain (1, 34, 35). It is worth mentioning that the analgesic effect of *N. laevis* observed in the second phase may be a combination of its central as well as antiinflammatory effects. This assertion is supported by the fact that the extract had exhibited potent antiarthritic (3) and antioxidant (4) effects in earlier studies conducted in our lab.

CONCLUSION

All together, the observations of this study have shown that NLE has both central and peripheral analgesic properties.

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