

PHCOG MAG.: Research Article

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

George K. Ainooson, Eric Woode*, David D. Obiri, George A. Koffour

Department of Pharmacology, Faculty of Pharmacy and Pharmaceutical Sciences, College of Health Sciences, Kwame Nkrumah University of Science & Technology, Kumasi, Ghana.

*Corresponding author: Telephone: +233 244 589793

E-mail: ewoode.pharm@knust.edu.gh, ericwoode@yahoo.com

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. laevis* stem bark in formalin-induced pain, a model of neuropathic pain, in rats. Morphine ($1-10 \text{ mg kg}^{-1} \text{ i.p}$) and stem bark extract of *N. laevis* ($10-300 \text{ mg kg}^{-1} \text{ p.o.}$), dose-dependently decreased both phases of the formalin-induced nociceptive behaviour. Nocifensive response for morphine was four fold higher in the first phase ($\text{ED}_{50}; 1.79 \pm 0.63 \text{ mg kg}^{-1}$) compared to the second ($\text{ED}_{50}; 7.59 \pm 2.26$); 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 ($10-100 \text{ mg kg}^{-1}$) was effective only in the second phase ($\text{ED}_{50} 33.24 \pm 5.20$). 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-arthritis (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.

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

Animals

Sprague-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.

ED₅₀ (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:

$$Y = \frac{a + (b - a)}{(1 + 10^{(\text{LogED}_{50} - X)})}$$

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 (ED₅₀s) 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 ED₅₀ determination. *P* < 0.05 was considered statistically significant in all analysis.

RESULTS

Formalin induced the characteristic nociceptive response (*F*_{1,88} = 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 from 10-60 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 (*F*_{4,40} = 14.95, *P* < 0.0001) and second phase (*F*_{4,180} = 23.53, *P* < 0.0001) (Figure 1); being equipotent in both phases (first phase ED₅₀; 28.28 ± 7.02; second phase ED₅₀; 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 (*F*_{3,32} = 14.89, *P* < 0.0001) and second phases (*F*_{3,144} = 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 (ED₅₀; 1.79 ± 0.63 mg kg⁻¹) (Table 1) compared to the second phase (ED₅₀; 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 (*F*_{3,32} = 0.51, *P* = 0.6810) but produced a dose-dependent analgesic effect in the second phase (*F*_{3,144} = 15.60, *P* < 0.0001; ED₅₀ = 33.24 ± 5.20) (Figure 3).

From ED₅₀ 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: ED₅₀ 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)

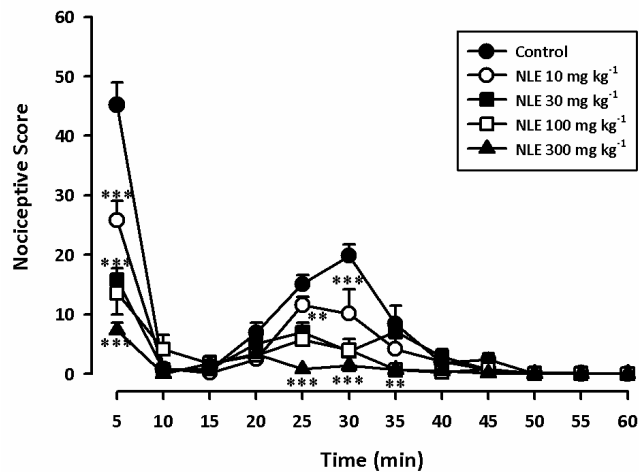


Fig. 1: Effect of NLE (10-300 mg kg⁻¹ p.o.) on the time course of formalin-induced pain in rats. Nociceptive/pain scores are shown in 5min time blocks up to 60 min post formalin injection. Each point represents mean ± S.E.M (n = 5). ****P* < 0.001; ** *P* < 0.01 compared to vehicle-treated group (Two-way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

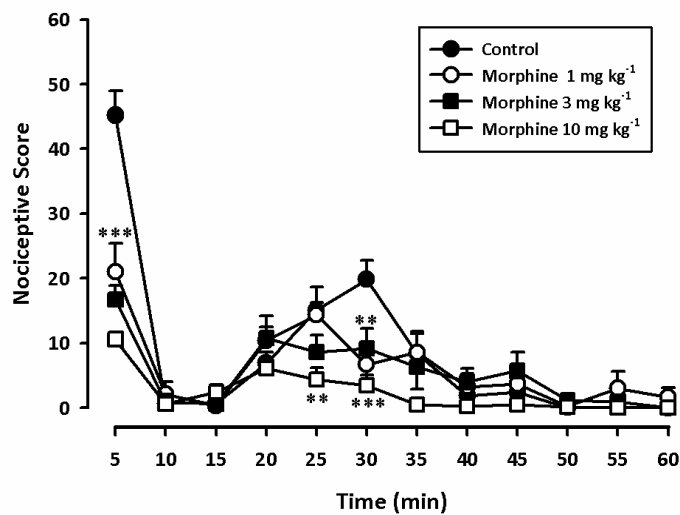


Fig. 2: Effect of morphine (1-10 mg kg⁻¹ i.p.) on the time course of formalin-induced pain in rats. Nociceptive/pain scores are shown in 5min time blocks up to 60 min post formalin injection. Each point represents mean ± S.E.M (n = 5). ****P* < 0.001; ** *P* < 0.01 compared to vehicle-treated group (Two-way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

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

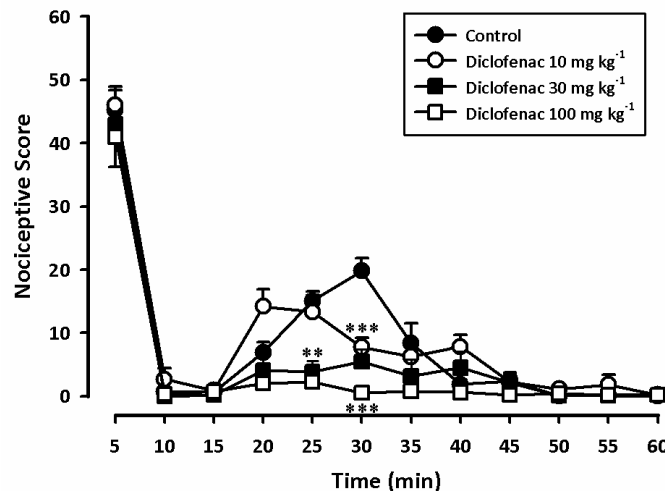


Fig. 2: Effect of diclofenac (10-100 mg kg⁻¹ i.p.) on the time course of formalin-induced pain in rats. Nociceptive/pain scores are shown in 5min time blocks up to 60 min post formalin injection. Each point represents mean $\pm$ S.E.M (n = 5). ***P < 0.001; ** P < 0.01 compared to vehicle-treated group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

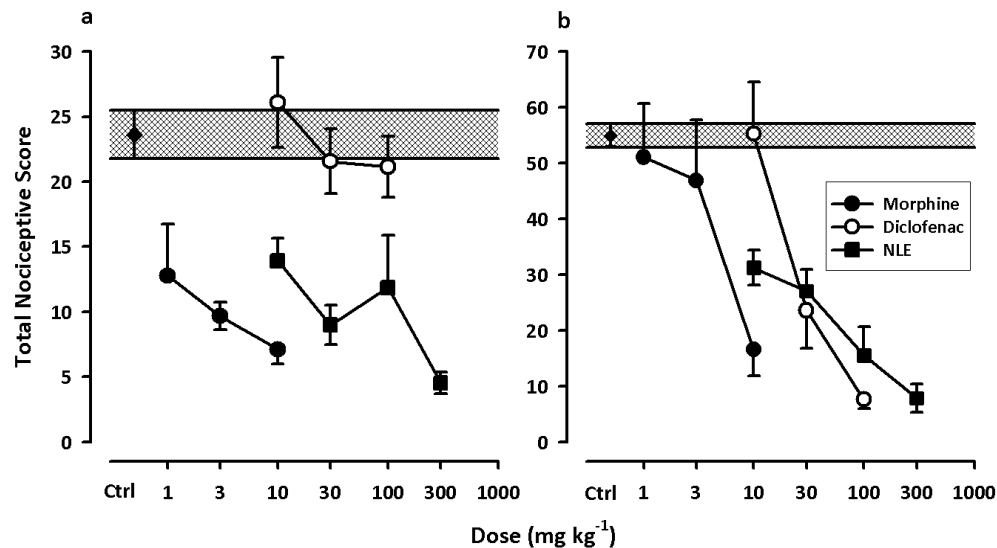


Fig. 4 Dose response curves for the effect of morphine (1-10 mg kg⁻¹ i.p.), diclofenac (10-100 mg kg⁻¹ i.p.) and NLE (10-300 mg kg⁻¹ p.o.) on the total nociceptive score for the first phase (a) and the second phase (b) of the rat formalin test. Each point represents mean $\pm$ S.E.M (n = 5).

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 (amitriptyline) 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 bark 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.

ACKNOWLEDGEMENTS

The authors wish to express their gratitude to Thomas Ansah and George Ofei of the Department of Pharmacology, KNUST, Kumasi for their technical assistance.

REFERENCES

1. N.R. Mshana, K. Abbiw, I. Addae-Mensah, E. Adjanohoun, M.R.A. Ahyi, J.A. Ekpere, E.G. Enow-Orock, Z.O. Gbile, G.K. Noamesi, M.A. Odei, H. Odunlami, A.A. Oteng-Yeboah, K. Sarpong, A. Soforowa and A.N. Tackie *Traditional Medicine and Pharmacopoeia. Contribution to the Revision of Ethnobotanical and Floristic Studies in Ghana*. Organization of African Unity/Scientific, Technical & Research Commission: Accra, 2000.
2. O.A. Olajide, S.O. Awe and J.M. Makinde. Pharmacological studies on *Newbouldia laevis* stem bark. *Fitoterapia* **68**(5): 439-443 (1997).
3. E. Woode, M. Duwiewua, C. Ansah, G. A. Koffuor, D. D. Obiri, F. A. Amissah, and G. K. Ainooson. Anti-inflammatory and analgesic effects of *Newbouldia laevis* stem bark extract. *Proceedings of the 2nd Scientific Meeting Western Africa Network of Natural Products Research Scientists* (Wannpres), Elmina, Ghana, 1st-4th August, 2006.
4. E. Woode, M. Duwiewua, C. Ansah, G. A. Koffuor, D. D. Obiri, A. Y. Mensah, and G. K. Ainooson. Antioxidant properties of *Newbouldia laevis* stem bark extract. *Proceedings of the 2nd Scientific Meeting Western Africa Network of Natural Products Research Scientists* (Wannpres), Elmina, Ghana, 1st-4th August, 2006.
5. D. Dubuisson and S.G. Dennis. The formalin test: a quantitative study of the analgesic effects of morphine, meperidine, and brain stem stimulation in rats and cats. *Pain* **4**: 161-174 (1977).
6. A.B. Malmberg and T.L. Yaksh. Antinociceptive Actions of Spinal Nonsteroidal Antiinflammatory Agents on the Formalin Test in the Rat. *JPET* **263**(1): 136-146 (1992).
7. L. Bardin and F.C. Colpaert. Role of spinal 5-HT_{1A} receptors in morphine analgesia and tolerance in rats. *Eur J Pain* **8**: 253-261 (2004).
8. E.J. Chesler, J. Ritchie, A. Kokayeff, W.R. Lariviere, S.G. Wilson and J.S. Mogil. Genotype-dependence of gabapentin and pregabalin sensitivity: the pharmacogenetic mediation of analgesia is specific to the type of pain being inhibited. *Pain* **106**: 325-335 (2003).
9. K. Hayashida, T. Takeuchi, H. Shimizu, K. Ando and E. Harada. Lactoferrin enhances opioid-mediated analgesia via nitric oxide in the rat spinal cord. *Am J Physiol - Regul Integr and Comp Physiol* **285**: R306-R312 (2003).
10. J.R. Miller. GraphPad Version 4.0. Step-by-Step Examples. GraphPad Software Inc., San Diego, CA (2003).
11. H.J. Motulsky and A. Christopoulos. *Fitting model to biological data using linear and nonlinear regression. A practical guide to curve fitting*. GraphPad Software Inc.: San Diego, CA, (2003).
12. H. Wheeler-Aceto, F. Porreca and A. Cowan. The rat formalin test: comparison of noxious agents. *Pain* **40**: 229-238 (1990).
13. Y.X. Wang, S.S. Bowersox, M. Pettus and D. Gao. Antinociceptive Properties of Fenfluramine, a Serotonin Reuptake Inhibitor, in a Rat Model of Neuropathy. *JPET* **291**(3): 1008-1016 (1999).
14. T.T. Chau. Analgesic Testing In Animal Models. In: *Pharmacological Methods in the Control of*

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

- Inflammation* J.Y. Chang, A.J. Lewis (eds), pp 195-212. New York: Alan R. Liss, Inc., 1989.
15. S.H. Ferreira, B.B. Lorrenzetti and F.M.A. Correa. Central and peripheral antialgesic action of aspirin like drugs. *Eur J Pharmacol* **53**: 39-48 (1978).
16. D. Le Bars, G. Manuela and S.W. Cadden. Animal Models of Nociception. *Pharmacol Rev* **53**: 597-652 (2001).
17. L.V. Costa-Lotufo, D.F. De Lucena, M. Andrade-Neto, J.N.S. Bezerra, L.K.A.M. Leal, F.C.F. De Sousa and G.S. De Barros Viana. Analgesic, Antiinflammatory and Central Depressor Effects of the Hydroalcoholic Extract and Fractions from *Aeolanthus suaveolens*. *Biol. Pharm. Bull* **27**(6): 821-824 (2004).
18. S.M.M. Vasconcelos, G.R. Oliveira, M.M. De Carvalho, A.C.P. Rodrigues, E.R. Silveira, M.M.F. Fonteles, F.C.F. Sousa and G.S.B. Viana. Antinociceptive Activities of the Hydroalcoholic Extracts from *Erythrina velutina* and *Erythrina mulungu* in Mice. *Biol. Pharm. Bull* **26**(7): 946-949 (2003).
19. K. Vissers, V. Hoffmann, F. Geenen, R. Biermans and T. Meert. Is the Second Phase of the Formalin Test Useful to Predict Activity in Chronic Constriction Injury Models ? A Pharmacological Comparison in Different Species. *Pain Practice* **3**(4): 298-309 (2003).
20. F.V. Abbott. Peripheral and central antinociceptive actions of ethylketocyclazocine in the formalin test. *Eur J Pharmacol* **152**: 93-100 (1988).
21. C. Courteix, P. Bourget, F. Caussade, M. Bardin, F. Coudore, J. Fialip and A. Eschaliere. Is the Reduced Efficacy of Morphine in Diabetic Rats Caused by Alterations of Opiate Receptors or of Morphine Pharmacokinetics. *JPET* **285**(1): 63-70 (1998).
22. J. Szolcsanyi, K. Bolcskei, A. Szabo, E. Pinter, G. Petho, K. Elekes, R. Borzsei, R. Almasi, T. Szuts, G. Keri and Z. Helyes. Analgesic effect of TT-232, a heptapeptide somatostatin analogue, in acute pain models of the rat and the mouse and in streptozotocin-induced diabetic mechanical allodynia. *Eur J Pharmacol* **498**: 103-109 (2004).
23. K. Yashpal and T.J. Coderre. Influence of formalin concentration on the antinociceptive effects of anti-inflammatory drugs in the formalin test in rats: separate mechanisms underlying the nociceptive effects of low and high-concentration formalin. *Eur J Pain* **2**: 63-68 (1998).
24. I.A. Bukhari, R.A. Khan, A.H. Gilani, A.J. Shah, J. Hussain, and V.U. Ahmad. The Analgesic, Anti-inflammatory and Calcium Antagonist Potential of *Tenacetum artemisioides*. *Arch Pharm Res* **30**(3): 303-308 (2007).
25. S. Yano, Y. Suzuki, M. Yuzurihara, Y. Kase, S. Takeda, S. Wantanabe, M. Aburada, and K. Miyamoto. Antinociceptive effect of methyleugenol on formalin-induced hyperalgesia in mice. *Eur J Pharmacol* **553**: 99-103 (2006).
26. M.I. Ortiz, J.E. Torres-Lopez, G. Castaneda-Hernandez, R. Rosas, G.C. Vidal-Cantu, V. Granados-Soto. Pharmacological evidence for the activation of K⁺ channels by diclofenac. *Eur J Pharmacol* **438**: 85-89 (2002).
27. J. Mino, V. Moscatelli, O. Hnatyszyn, S. Gorzalczy, C. Acevedo and G. Ferraro. Antinociceptive and antiinflammatory activities of *Artemisia copa* extracts. *Pharmacol Res* **50**: 59-63 (2004).
28. J.H. Rosland, A. Tjolsen, B. Maehle and D.K. Hole. The formalin test in mice. Effect of formalin concentration. *Pain* **42**: 235-242 (1990).
29. S. Amos, L. Binda, H. Vongtau, B. Chindo, J. Abbah, N. Sambo, E.M. Odin, S.K. Okwute, P. Akah, C. Wambebe and K. Gamaniel. Sedative effects of the methanolic leaf extract of *Newbouldia laevis* in mice and rats. *Boll Chim Farm* **141**(6): 471-475 (2002).
30. J. Sawynok and A. Reid. Antinociception by tricyclic antidepressants in the rat formalin test: differential effects on different behaviours following systemic and spinal administration. *Pain* **93**: 51-59 (2001).
31. R.D. Macpherson. Pharmacological basis of contemporary pain management. *Pharmacol Ther* **88**: 163-185 (2000).
32. J.D. Schim, and P. Stang. Overview of Pain Management. *Pain Practice* **4**(1S): S4-S18 (2004).
33. T.S. Sindrup Jensen. Efficacy of pharmacological treatments of neuropathic pain: an update and effect related to mechanism of drug action. *Pain* **83**: 389-400 (1999).
34. F., R. Irvine, *Woody plants of Ghana*. Oxford University Press: London, 1961.
35. B. Oliver-Bever. *Medicinal plants in tropical West Africa*. Cambridge University Press: Cambridge, 1986.