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Analgesic and Anti-Inflammatory Screening of *Newbouldia laevis* Flower in Rodents

¹H. Usman, ²A.H. Yaro and ³M.M. Garba

¹Department of Chemistry, University of Maiduguri, P.M.B. 1069, Maiduguri, Nigeria

²Department of Pharmacology, Faculty of Medicine, Bayero University, Kano, Nigeria

³Department of Pharmacology and Clinical Pharmacy,
Faculty of Pharmacology Science, ABU Zaria, Nigeria

Abstract: Analgesic and anti-inflammatory effects of the ethanolic flower extract of *Newbouldia laevis* were studied in rodents. Investigations were carried out on acetic acid-induced writhing in mice and carrageenan-induced hind paw oedema in rats. The results showed that the ethanol extract possessed significant ($p < 0.001$) anti-nociceptive activity between 50 and 200 mg kg⁻¹ intraperitoneally (i.p.) in mice and also dose dependent anti-inflammatory activity between 50 and 200 mg kg⁻¹ (i.p.) in rats. These effects were compared favourably with that expressed by ketoprofen (10 mg kg⁻¹ i.p.). From the results obtained, although relatively toxic the extract exhibited highest anti-nociceptive and anti-inflammatory activities at the dosage of 200 mg kg⁻¹ (i.p.). These data corroborate with the traditional use of this plant in the treatment of rheumatic pain and other types of pain reported in traditional medicine.

Key words: Analgesic, anti-inflammatory, bignoniaceae, ketoprofen, *Newbouldia laevis*

INTRODUCTION

The use of plants as medicine is an ancient practice common to all societies especially the African society. These practices continue to exist in the developing nations. It is on this basis that researchers keep on working on medicinal plants in order to produce and/or develop the best medicines for physiological or therapeutic uses (Usman and Osuji, 2007).

Newbouldia laevis (P. beauv) seem or boundary tree called variously as; *Aduruku* in Hausa, *Ogirisi* in Igbo and *Akoko* in Yoruba languages (Hutchinson and Dalziel, 1963) is a medium sized angiosperm of the Bignoniaceae family. *Newbouldia laevis* is native to tropical Africa and grows from Guinea Savannahs to dense forests (Arbonnier, 2004). In Nigeria, the plant has been found to be effective in the treatment of elephantiasis, dysentery, rheumatic swellings, syphilis, constipation, pile and as a vermifuge to round worms (Usman and Osuji, 2007). The root, leaf, stem and fruits have been used variously for febrifuge; wound dressing and stomach ache (Iwu, 2000), including inflamed sores, ulcers and abscesses (Grand, 1989).

Recent phytochemical studies on the root, root bark and stem of this plant revealed the presence of alkaloids, quinoid and phenylpropanoid amongst others (Gafner *et al.*, 1997; Aladesanmi *et al.*, 1998; Germann *et al.*, 2006). There was no extensive report on the presence of compounds from the leaves and flower of this species (Usman and Osuji, 2007; Usman *et al.*, 2007a). Our report on the preliminary phytochemical composition of the flower extract revealed the presence of cardiac and steroidal glycosides, flavonoids; tannins while other phytochemicals such as alkaloids, saponins were not detected (Usman *et al.*, 2007a).

Corresponding Author: H. Usman, Department of Chemistry, University of Maiduguri, P.M.B. 1069, Maiduguri, Nigeria Tel: +234-802-833-2246

From the literature search made so far, there was no report on the present studies on the flower extract of this plant species. Thus, this investigation was conducted in order to ascertain the analgesic and anti-inflammatory efficacy of the ethanolic flower extract of *Newbouldia leavis* using the acetic acid-induced abdominal writhing in mice and carrageenan-induced hind paw oedema in rats.

MATERIALS AND METHODS

Plant Material

The flowers of *Newbouldia laevis* for this study were collected from Kudingi Village-Samaru Zaria, in the month of August 2005. The studies were carried out in May 2007 and the plant specimen was identified by Mr. U.A. Gallah of the Herbarium Unit, Department of Biological Sciences, Ahmadu Bello University, Zaria-Nigeria, where a voucher specimen was deposited.

Extraction Procedure

The plant material was dried under shade for several days and then pulverized into fine powder. About 200 g of the powdered material was extracted exhaustively with 95% (v/v) ethanol in water using continuous soxhlet apparatus. The extract was concentrated under reduced pressure to yield a dark green mass that weighed 38.51 g (19.26% w/w). The crude ethanolic extract was then coded NLF-*Newbouldia leavis* flower extract.

Animals

A total of fifty animals were used for these tests, comprising of twenty five adult male Swiss albino mice that weighed between 18-28 g and also twenty five male Wister rats which weighed between 150-250 g were obtained from the Animal house, Department of Pharmacology and Clinical Pharmacy, ABU Zaria-Nigeria. The animals were kept under well-ventilated conditions with 12 h light/dark cycle (6:00 am-6:00 pm) at room temperature, fed on Standard feeds (Excel feeds Plc. Kaduna, Nigeria) and allowed water *ad libitum*.

Drugs

Ketoprofen (Pfizer, USA), carrageenan, Sigma Chemical (Germany) and acetic acid, BDH reagent (Poole, UK); were obtained from a retail store at Samaru-Zaria, Nigeria. All drugs or reagents were freshly prepared to the desired concentration with distilled water or normal saline just before use.

Acetic Acid-Induced Writhing in Mice

This test was conducted employing the method described by Koster *et al.* (1959), Vongtau *et al.* (2000) and Usman *et al.* (2005). Swiss albino mice were divided into 5 groups of five mice each. Groups 1, 2 and 3 received 50, 100 and 200 mg kg⁻¹ body weight of the extract respectively. The fourth group served as control and was given normal saline equivalent to vehicle given with the extract, while the fifth group was given ketoprofen (10 mg kg⁻¹ body weight). The drug and extract were administered by intraperitoneal (i.p.) route. Thirty minutes later, all the groups were treated with acetic acid (0.06%, 1 mL per 100 g i.p.). Thereafter, mice were placed in an individual cage; the numbers of abdominal constrictions were counted five minutes after acetic acid injection for a period of 10 min. Percentage inhibition of writhing was obtained using the formula:

$$\text{Inhibition (\%)} = \frac{\text{Mean No. of writhing (control)} - \text{Mean No. of writhing (test)}}{\text{Mean No. of writhing (control)}} \times 100$$

(Vongtau *et al.*, 2000; Usman *et al.*, 2005).

Carrageenan-Induced Paw Oedema in Rats

The test was conducted according to the method described by Winter *et al.* (1962). Wister rats were divided into five groups each containing five rats. Groups 1, 2 and 3 received 50, 100 and 200 mg kg⁻¹ body weight of the extract respectively. The fourth group served as control and was given normal saline equivalent to vehicle given with the extract; while the fifth group was given ketoprofen (10 mg kg⁻¹ body weight). The drug and extract were administered by i.p. route. Thirty minutes later, 0.1 mL of freshly prepared carrageenan suspension (1% w/v in 0.9% normal saline) was injected into the sub planter region of the left hind paw of each rat. The paw diameter was measured with the aid of a vernier caliper at 1, 2, 3, 4, 5 h after injection of carrageenan.

Statistical Analysis

All values were expressed as mean±Standard Error of the Mean (SEM). The data of acetic acid and carrageenan tests were analysed statistically by one way analysis of variance (ANOVA) and Student's t-test respectively. The differences between means were considered significant when p<0.05 and p<0.001.

RESULTS

Anti-Nociceptive Study

The extract at doses between 50 and 200 mg kg⁻¹ significantly decreased the number of acetic acid-induced writhes as shown in Table 1. The highest percentage inhibition of 83.74% was observed at extract dose of 200 mg kg⁻¹ while that of 50 mg kg⁻¹ showed 63.41%, indicating a dose-dependent activity pattern; the extract at the dose of 200 mg kg⁻¹ had similar activity profile to that of ketoprofen 10 mg kg⁻¹ exhibiting same percentage inhibition. This result showed that the extract (200 mg kg⁻¹) had exhibited a similar protection to that of the standard drug. All the values were significant (p<0.001) compared to the negative control (normal saline group).

Anti-Inflammatory Activity

The anti-inflammatory activity of flower extract of *Newbouldia laevis* against acute paw oedema (carrageenan-induced) is presented in Table 2 and 3 and the results showed that NLFE caused a slight inhibition of carrageenan-induced oedema over a period of 5 h. This effect appeared to be

Table 1: Effects of ethanolic extracts of *Newbouldia laevis* flower on acetic acid-induced writhing in mice

Treatments (i.p.)	Dose (mg kg ⁻¹)	No. of abdominal constrictions (mean±SEM)	Inhibition (%)
Normal saline	-	24.6±2.36	-
Extract	50	9.0±1.22*	63.41
Extract	100	8.2±0.86*	66.67
Extract	200	4.0±0.71*	83.74
Ketoprofen	10	4.0±0.70*	83.74

One way ANOVA; df = 4, 24; F = 42.558; n = 5; *: p<0.001

Table 2: Effect of ethanolic extracts of *Newbouldia laevis* flower on carrageenan-induced oedema in rats

Treatments (i.p.)	Dose (mg kg ⁻¹)	Mean paw diameter (cm) at various time (h)				
		1	2	3	4	5
Normal saline	-	0.188±0.010	0.254±0.030	0.230±0.020	0.198±0.020	0.200±0.020
Extract	50	0.134±0.010*	0.158±0.010*	0.164±0.010**	0.156±0.010**	0.126±0.010*
Extract	100	0.128±0.010*	0.168±0.010*	0.164±0.010**	0.166±0.010**	0.090±0.010*
Extract	200	0.132±0.010*	0.164±0.010*	0.156±0.010**	0.116±0.010*	0.090±0.010*
Ketoprofen	10	0.080±0.020*	0.050±0.010*	0.070±0.010*	0.104±0.030*	0.112±0.030*

*: p<0.05; **: p>0.05 along each column

Table 3: Percentage inhibition expressed by ethanolic extract of *Neboulidia laevis* flower and ketoprofen on carrageenan-induced oedema in rats

Treatments (i.p.)	Dose (mg kg ⁻¹)	Percentage inhibition at various time (h)					Mean inhibition (%)
		1	2	3	4	5	
Extract	50	28.7	37.8	28.7	21.2	37.0	30.68±6.86
Extract	100	31.9	33.9	28.7	16.2	55.0	33.14±14.02
Extract	200	29.8	35.4	32.2	41.1	55.0	38.70±10.05
Ketoprofen	10	57.4	80.3	69.6	47.3	44.0	59.72±15.23

dose-dependent. Maximum inhibitory effects of 55.0% was observed 5 h post treatment with the extract at a dose of 100 and 200 mg kg⁻¹, the drug (standard ketoprofen) indicated 80.3% as the maximum inhibitory effect 2 h post treatment. However, the mean percentage inhibition of these effects was found to be 33.14, 38.70 and 59.72 for 100, 200 and 10 mg kg⁻¹ extract and ketoprofen respectively. All the results were significant (p<0.001) compared to normal saline group (control).

DISCUSSION

Earlier reports on the phytochemical constituents of this extract (Usman *et al.*, 2007a) revealed the presence of cardiac and steroidal glycosides, flavonoids, tannins among others while phytochemical constituents such as alkaloids, saponins was not detected. The earlier report on the acute toxicity studies by Usman *et al.* (2007a), revealed that the extract had an intraperitoneal LD₅₀ of 1264.9 mg kg⁻¹ in mice and thus, found the toxicity index of the extract in mice to be relatively moderately.

The method employed in this study also called abdominal constriction response, is very sensitive and able to detect anti-nociceptive effects of compound(s) at dose level that may be inactive in other methods like tail-flick test (Collier *et al.*, 1968; Bentley *et al.*, 1981; Usman *et al.*, 2005, 2007b). The abdominal constrictions response is postulated to partly involve local peritoneal receptors (Bentley *et al.*, 1983; Usman *et al.*, 2007b). The extract NLFE significantly (p<0.001) reduced the number of abdominal constriction induced by acetic acid in mice. The effects observed were in a dose-dependent manner between 50-200 mg kg⁻¹; the higher dosage (200 mg kg⁻¹) exhibited similar activity as presented by ketoprofen 10 mg kg⁻¹. The activity was in line with the findings of Usman *et al.* (2007b) that as the concentration of these plant bioactive components increases, so also protection against abdominal writhes induced by acetic acid. It is therefore, pertinent to say that the extract contains active analgesic compound(s) when isolated in its pure form.

The carrageenan-induced oedema is commonly used as an experimental animal model for acute inflammation that has been believed to be biphasic, the first of which is mediated by the release of histamine and 5-HT and the release of kinin and prostaglandins in the later phase (Castro *et al.*, 1968; Mazumder *et al.*, 2003). In this study, the mean paw diameters of the rats were measured to determine the extent of protection by the tests samples. The results from this study indicated that the mean anti-inflammatory activity was found to be dose-dependent; significant differences (p<0.05, p<0.01 and p<0.001) between normal saline and the extract treated groups were observed at the early hours of treatment (1-2 h) and later at fifth hour post treatment; but no significant differences was noticed at the third and fourth hour (except 200 mg kg⁻¹ which was significantly different at p<0.05). There was significant differences observed throughout the tests between normal saline and the ketoprofen treated groups. The trend of the activities of the extract and that of ketoprofen was found to be similar at the earlier stages of the study; since the onset of protection were both observed at the second hours post treatment, while the anti-inflammatory activity of the extract behaved sinusoidally and reached peak at the fifth hour; that of the drug (ketoprofen) declined in a normal distribution pattern in later hours after treatment. Thus, the Mechanism of Actions (MOA) of the analgesic and anti-inflammatory effects expressed by the extract studied could be said to have similar properties and MAO with some groups of analgesic/anti-inflammatory agents (NSAIDS) including ketoprofen.

The co-existence of both anti-nociceptive and anti-inflammatory effects seen with this extract is well defined for various Non-Steroidal Anti-Inflammatory Drugs (NSAIDS) particularly Salicylates and their derivatives (Musa *et al.*, 2007). It is therefore, interesting to note that the extract behaves like NSAIDS, since it was able to potently exhibit anti-inflammatory effects at the later phase just as does prostaglandins. It is hence, possible that both the analgesic and anti-inflammatory effects observed with this extract may be attributed to its flavonoids and/or tannins as reported earlier by Ahmadiani *et al.* (1998), Ahmadiani *et al.* (2000), Viana *et al.* (2003) and Usman *et al.* (2005), including some other phytochemicals. Flavonoids have also been reported to potently inhibit prostaglandins, a group of powerful pro-inflammatory signalling molecules (Manthey, 2000). Although, the exact chemical constituents responsible for these effects exhibited by this extract still remain speculative; the activities could be relevant to those earlier reported by similar secondary metabolites. Meanwhile, as far as we know, this is the first report on the analgesic and anti-inflammatory effects of extract from the part of this plant.

CONCLUSIONS

From the results obtained, it can be concluded that though relatively toxic, the plant extract possesses both anti-nociceptive and anti-inflammatory activity dose-dependently. These effects could therefore, be a point for the use of the plant traditionally as a remedy for rheumatic and other pain types. The authors wish to suggest that, further work be carried out on this plant extract with the view of isolating and characterizing the possible phytochemical agent(s) responsible for such activities.

REFERENCES

- Ahmadiani, A., M. Fereidoni, S. Semnani, M. Kamalinejad and S. Saremi, 1998. Anti-nociceptive and anti-inflammatory effects of *Sambucus ebulus* rhizome extract in rats. *J. Ethnopharmacol.*, 61: 229-235.
- Ahmadiani, A., J. Hosseiny, S. Semnani, M. Javan, F. Saeedi, M. Kamalinejad and S. Saremi, 2000. Anti-nociceptive and anti-inflammatory effects of *Eleagnus angustifolia* fruit extract. *J. Ethnopharmacol.*, 72: 287-292.
- Aladesanmi, A.J., R. Nia and A. Nahrstedt, 1998. New pyrazole alkaloids from the root bark of *Newbouldia leavis*. *Planta Med.*, 64: 90-91.
- Arbonnier, M., 2004. *Trees, Shrubs and Lianas of West African Dry Zones*. CIRAD, Margraf Publishers GMBH MNHN, Cote D'Ivoire, pp: 194.
- Bentley, G.A., S.H. Newton and J. Starr, 1981. Evidence for an action of morphine and the enkephallins on sensory nerve endings in the mouse peritoneum. *Br. J. Pharmacol.*, 32: 295-310.
- Bentley, G.A., S.H. Newton and J. Starr, 1983. Studies on the anti-nociceptive action of α -agonist drugs and their interaction with opiod mechanisms. *Br. J. Pharmacol.*, 79: 125-134.
- Castro, J.H., H. Sasame, H. Sussman and P. Buttette, 1968. Diverse effects of SKF-52 and antioxidant on CCl_4 -induced changes in liver microbial cyt. P-450 content and ethyl-morphine metabolism. *Life Sci.*, 7: 129-136.
- Collier, H.O.J., L.G. Dinneen, C.A. Johnson and C. Schneider, 1968. The Abdominal constriction response and its suppression by analgesic drugs in the mouse. *Br. J. Pharmacol.*, 32: 295-310.
- Gafner, S., J.L. Wolfender, M. Nianga and K. Hostettmann, 1997. Phenylpropanoid glycosides from *Newbouldia laevis* roots. *Phytochemistry*, 44: 687-690.
- Germann, K., M. Kaloga, D. Ferreira, J.P. Marais and H. Kolodziej, 2006. Newbouldioside A-C phenylethanoid glycosides from the stem bark of *Newbouldia leavis*. *Phytochemistry*, 67: 805-811.

- Grand, A., 1989. Anti-infectious phytotherapy of the tree-savannah, senegal (West Africa) III: A review of the phytochemical substance and antimicrobial activity of 43 species. *J. Ethnopharmacol.*, 25: 315-338.
- Hutchinson, J. and J.M. Dalziel, 1963. *Flora of West Tropical Africa Vol. 2. Crown agents for oversea government and administration.* 4, Millbank, London, S.W., 1: 435-436.
- Iwu, M.M., 2000. *Handbook of African Medicinal Plants.* CRC Press, Inc., London, pp: 19.
- Koster, R., M. Anderson and E.J. De-Beer, 1959. Acetic acid used for analgesic screening. *Fed. Proc.*, 18: 412.
- Manthey, J.A., 2000. Biological properties of flavonoids pertaining to inflammation. *Microcircul.* 7: S 29-34.
- Mazumder, U.K., M. Gupta, L. Manikandan, S. Bhattacharya, P.K. Halder and S. Roy, 2003. Evaluation of anti-inflammatory activity of *Vernonia cinerea* Less. extract in mice. *Phytomedicine*, 10: 185-188.
- Musa, Y.M., A.K. Haruna, M. Ilyas, A.H. Yaro, A.A. Ahmadu and H. Usman, 2007. Analgesic and anti-inflammatory effects of the leaf extracts of *Pseudocedrella kotschyii* Harms (Meliaceae). *J. Pharmacol. Toxicol.*, 2: 542-550.
- Usman, H., A.K. Haruna, M. Ilyas, A.H. Yaro, A.A. Ahmadu and Y.M. Musa, 2005. Anti-nociceptive and anti-inflammatory effects of the leaves extracts of *Celtis integrifolia* Lam. (Ulmaceae). *Res. J. Sci.*, 11: 101-112.
- Usman, H. and J.C. Osuji, 2007. Phytochemical and *in vitro* antibacterial assay of the leaf extract of *Newbouldia leavis*. *Afr. J. Trad. CAM.*, 4: 476-480.
- Usman, H., A.H. Yaro and M.M. Garba, 2007a. Phytochemical and anticonvulsant screening of the ethanolic flower extracts of *Newbouldia laevis* (Bignoniaceae) in mice. *J. Pharmacol. Toxicol.* (In Press).
- Usman, H., Y.M. Musa, Y.A. Geidam and D.M. Abore, 2007b. Phytochemical and anti-nociceptive studies of the root bark extracts of *Maytenus senegalensis* (Lam.) in mice. *J. Life Environ. Sci.*, 9: (In Press).
- Viana, G.S.B., M.A.M. Bandeira and F.J.A. Matos, 2003. Analgesic and anti-inflammatory effects of chalcones isolated from *Myracrodruon urundeuva* Allemão. *Phytomedicine*, 10: 189-195.
- Vongtau, H.O., S. Amos, L. Binda, S.D. Kapu, K.S. Gamaniel, O.F. Kunle and C. Wambebe, 2000. Pharmacological effects of the aqueous extract of *Neorautanenia mitis* in rodents. *J. Ethnopharmacol.*, 72: 207-214.
- Winter, C.A., E.A. Risley and G.W. Nuss, 1962. Carrageenan-induced oedema in hind paw of the rats as assay for anti-inflammatory drugs. *Exp. Biol. Med.*, 111: 544-547.