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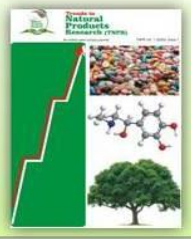

Antinociceptive Activity of Methanol Leaf Extract of *Newbouldia laevis* in Wistar Rats

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Abstract: Side effects attributable to currently available pain killers necessitated the search for novel analgesics with higher safety index. In Nigerian traditional medicine, *Newbouldia laevis* is reputed for alleviating pain. The present study investigated the methanol extract of *N. laevis* for antinociceptive potential in Wistar rats. Dried and pulverized leaves (550 g) of *N. laevis* were extracted with 80 % methanol (1.65 L) by cold maceration. Acute toxicity and preliminary phytochemical analysis of the extract were done following standard methods. Analgesic models adopted for the study included: acetic acid-induced writhing reflex, hot plate test, tail immersion and formalin tests. In each of the models thirty adult Wistar rats were assigned to five groups (n = 6). Group 1 (control) received distilled water (5 ml/kg) while group 2 rats were dosed with 20 mg/kg tramadol (reference drug). Groups 3-5 received 200, 300 and 400 mg/kg of the extract, respectively. All treatments were administered orally. The acute toxicity test produced no death even at highest dose of 5000 mg/kg. The preliminary phytochemical analysis revealed the presence of alkaloids, flavonoids, glycosides, polyuronoids and tannins. The extract significantly reduced abdominal constrictions in the treated rats, with 800 mg/kg evoking 44 % antinociceptive activity against 89 % anticociception achieved by tramadol. In the hot plate and tail immersion tests, the extract caused no significant difference in the rats' response to noxious stimuli. However, during the second phase of the formalin test, the extract significantly reduced the number of flinches in the treated rats when compared with the control. The results of this study showed that *N. laevis* methanol leaf extract possesses significant peripheral

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antinociceptive property. This validates its use in ethno medicine to minimize or abolish pain.

Introduction

Virtually all animal and human diseases are associated with pain, which may be mild, moderate or severe. Pain is usually a warning sign or early signal of an underlying disease (Ezeja *et al*., 2011). It is naturally protective, but comes with varying degrees of discomfort and may result in loss of function of the affected parts of the body (Aiello and Mays, 1998). Conventional drugs used to minimize or abolish pain are categorized into narcotic or central acting analgesics and the non-steroidal anti-inflammatory drugs (NSAIDs) which act peripherally (Rang *et al*., 2007).Although these agents are potent pain killers, their analgesic actions are linked with numerous undesirable effects, which include: gastrointestinal irritation, ulceration and bleeding, nephrotoxicity, respiratory depression, decrease in body temperature, tolerance and physical dependence (Harvey and Champe, 2009). These adverse effects, coupled with high cost of the synthetic/orthodox analgesics necessitate the search for novel pain killers with higher safety index. *Newbouldia laevis* is commonly called 'boundary tree' and belongs to the family *Bignoniaceae*. It is a small tree and attains a height of 15 m. The plant is native to sub-Saharan Africa (Boakye-Gyasi *et al*., 2013). Among the major tribes in Nigeria, *N. laevis*is is called 'ogirisi' (Igbo), 'aduruku' (Hausa) and 'akoko' (Yoruba) (Usman *et al*., 2008). The root and stem bark of *N. laevis* are used to treat malaria, elephantiasis, pelvic pain in women, constipation, epilepsy and haemorrhoids in folklore medicine (Ainooson *et al*., 2009). Decoction of the leaf and root of the plant also serve as remedy for rheumatism (Boakye-Gyasi *et al*., 2013). Some researchers have reported numerous pharmacological activities of *N. laevis*, which include: anti-arthritic and antioxidant activities of the ethanol stem bark extract (Woode *et al*., 2008), antinociceptive effect of the stem bark (Ainooson *et al*., 2009), antidiabetic effect of the ethanol leaf extract (Owolabi *et al*., 2011), antibacterial activity of the leaf extract (Okeke, 2003), anti-inflammatory and analgesic activities of the stem bark extract (Olajide *et al*., 1997). The present work investigated the methanol leaf extract of *N. laevis* for antinociceptive potential, using albino rat model.

Methodology

Plant collection and extraction

Leaves of *N. laevis* were harvested from its habitat in Ubakala Abia State, Nigeria. Identification of the plant was done in the Department of Botany, Michael Okpara University of Agriculture, Umudike. A representative specimen with identification number: MOUAU/VPP/2018/113 was deposited in the Institution's herbarium. The leaves were air-dried and pulverized using an electric blender. Five hundred and fifty grams of the coarse powder was extracted with 1.65 L of 80 % methanol, by cold maceration method. The extract was oven-dried (40 °C) after passing through a rotary evaporator, and stored as methanol extract of *Newbouldia laevis* (MENL) at 4 °C until time of use (Madubuike and Asuzu, 2015).

Preliminary phytochemical analysis of MENL

A qualitative phytochemical analysis of the extract was done by following the procedures outlined by Harbourne (1991) and Trease and Evans (1996).

Animals

Eight-weeks old albino rats bred in the Laboratory Animal Unit of the College of Veterinary Medicine, Michael Okpara University of Agriculture, Umudike, were procured for the study. The rats weighed 95.6 ± 3.12 g. Stainless steel rat cages were used to house them, while pelleted feed (Vital feed®, Nigeria) and clean drinking water were served *ad libitum* to the rats. The experimental procedures were approved by the institution's Research Ethics Committee (Approval No. MOUAU/CVM/REC/201804), and the rats were managed according to National Institute of Health (NIH) Guidelines for Care and Use of Laboratory Animals (NIH, 2011).

Acute toxicity test

The up-and-down-procedure was employed for the determination of oral acute toxicity of MENL. Three (3) albino rats were dosed orally with 5000 mg/kg of MENL. The rats had free access to feed and drinking water for 14 days during which they were monitored for evidence of toxicity and death (OECD, 2008).

Acetic acid-induced writhing reflex

Thirty rats were randomly assigned to 5 groups (n = 6) and treated as follows: group 1 (negative control) were given distilled water (5 ml/kg) orally. Group 2 received 20 mg/kg tramadol (reference drug) orally, whereas groups 3-5 were treated with 200, 400 and 800 mg/kg MENL, also following the oral route. One hour after extract/drug administration, all the rats were injected intraperitoneal with 10 ml/kg of 0.6 % acetic acid. The number of writhes (characteristic abdominal constrictions) exhibited by each rat for 20 minutes was recorded (Farouk *et al*., 2008). The percentage antinociceptive activity was calculated as:

$$\frac{number\ of\ writhes\ in\ control - number\ of\ writhes\ in\ treated}{number\ of\ writhes\ in\ control} \times 100$$

Hot plate test

Thirty rats were divided into 5 groups of 6 rats per group. Group 1 rats served as control and were given 5 ml/kg of distilled water orally. Rats in group 2 were given 20 mg/kg tramadol, while groups 3-5 received 200, 400 and 800 mg/kg of MENL, respectively, following the oral route. One-hour post-drug/extract administration, the rats were individually placed on a hot plate maintained at 55 °C and the latency period or pain reaction time (PRT) was recorded with the help of a stop watch. The animals' reaction to noxious stimulus considered were: jumping, licking and withdrawal of the hind feet. The cut off time for latency of response was taken as 20 seconds to prevent damage of the paws (Shalheen *et al*., 2000).

Tail immersion test

This was conducted following procedures outlined by Pandurangan *et al*., (2013). Thirty rats were assigned to 5 groups of 6 rats each. Rats in group 1 served as the negative control and were given 5 ml/kg of distilled water orally. Rats in group 2 were given 20 mg/kg tramadol, while groups 3-5 received 200, 400 and 800 mg/kg of MENL, respectively, following the oral route. After 1 h of administering the drugs and extract about 2.5 cm of the tail of each rat was immersed in water bath maintained at 50 °C. The PRT which is the period taken for the rat to flick its tail was recorded.

Formalin test

A modified method of Hunskaar and Hole (1987) was adopted for the formalin test. Thirty rats were randomly allocated into 5 treatment groups (1-5) of 6 rats per group. Group 1 (negative control) received 5 ml of distilled water while group 2 got 20 mg/kg tramadol. Groups 3-5 were dosed with 200, 400 and 800 mg/kg of MENL, respectively. All the treatments were administered orally. One hour later, pain was induced in all the rats by subcutaneous injection of 50 µl of 5% formalin solution into the plantar surface of the left hind paw. Nociceptive response was quantified at 5 min intervals (post-formalin injection) for 40 min, by counting the number of flinches of the injected paw (Han *et al.*, 2012).

Statistical analysis

The One Way Analysis of Variance (ANOVA) was used to analyse the data obtained from the study. Separation of the variant means was done by the Least Significance Difference of the various groups. Values of $p < 0.05$ were accepted as significant.

Results

Extraction

The extract (MENL) weighed 26.4 g, representing 4.8 % w/w of the dried, pulverized plant material. The extract was semi-solid, greenish-brown and had a pleasant smell.

Preliminary phytochemical analysis

The result shows that MENL contains the following constituents: alkaloids, glycosides, flavonoids, tannins and polyuronoids (Table 1)
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Table 1: Phytochemical screening of MENL

Phytoconstituents	Inference
Alkaloids	+
Flavonoids	+
Glycosides	+
Polyuronoids	+
Resins	-
Saponins	-
Tannins	+

+ present; - absent

Acute toxicity test

The extract, at the maximum dose of 5000 mg/kg caused neither death nor signs of acute toxicity in the rats throughout the fourteen days' observation period.

Acetic acid-induced writhing reflex

The result showed that MENL at the doses tested significantly ($p < 0.05$) inhibited acetic acid-induced abdominal constrictions in the rats in a dose-related manner. The highest dose of MENL (800 mg/kg) achieved 44 % antinociceptive effect against 89 % caused by the reference drug (Table 2).

Table 2: Effect of MENL on acetic acid-induced writhing reflex in rats

Groups	Treatment	Number of abdominal constrictions	Percentage inhibition
1	Distilled water (10 ml/kg)	41.6± 3.12	0
2	Tramadol (20 mg/kg)	04.5± 0.13*	89
3	MENL (200 mg/kg)	32.0 ± 1.24*	24
4	MENL (400 mg/kg)	27.0 ± 2.31*	35
5	MENL (800 mg/kg)	23.2 ± 1.52*	44

*$p < 0.05$ when compared with the control

Hot plate test

The effect of the varying doses of MENL on time latency in the hot plate test did not differ significantly ($p < 0.05$) in the treated rats when compared with the negative control group (Table 3).

Table 3: Effect of MENL on time latency in hot plate test in Wistar rats

Group	Treatment	Time latency (s), mean ± S. E. M.		
		Pre-treatment	Post-treatment	
		0 min	30 min	60 min
1	Distilled water (10 ml/kg)	4.35 ± 0.63	4.47 ± 0.62	4.45 ± 0.32
2	Tramadol (20 mg/kg)	3.98 ± 0.34	15.25 ± 1.53*	13.43 ± 0.95*
3	MENL (200 mg/kg)	4.36 ± 0.47	4.98 ± 0.55	4.74 ± 0.56
4	MENL (600 mg/kg)	4.30 ± 0.58	5.01 ± 0.35	4.92 ± 0.41
5	MENL (800 mg/kg)	4.42 ± 0.81	5.66 ± 0.64	5.31 ± 0.83

*$p < 0.05$ when compared with the control

Tail immersion test

The result showed that there was no significant difference between the time latency in the MENL-treated rats when compared with the negative control (Table 4).

Table 4: Effect of MENL on Tail immersion test

Group	Treatment	Time latency (s), mean S. E. M.
1	Distilled water (10 ml/kg)	3.62 ±1.77
2	Tramadol (20 mg/kg)	7.79 ± 1.08*
3	MENL (200 mg/kg)	3.65 ± 1.53
4	MENL (600 mg/kg)	4.01 ± 1.34
5	MENL (800 mg/kg)	4.26 ± 1.01

*$p < 0.05$ when compared with the control

Formalin test

The effect of MENL on formalin test is presented in figure 1. The result showed that MENL, at the doses tested did not cause significant ($p < 0.05$) antinociceptive activity in the early (acute) phase, when compared with the control. However, in the late (tonic/facilitated) phase the number of flinches was significantly reduced in the MENL-treated groups and in the group treated with tramadol, when compared with the control. The antinociceptive activity of MENL was most pronounced 35 min post-formalin induction of pain, with 800 mg/kg MENL evoking 16.35 ± 1.53 flinches/min by the treated rats, against 28.40 ± 3.20 flinches/min exhibited by the negative control rats. This represents 42 % pain inhibition by 800 mg/kg MENL, though compares less favourably with the reference drug (20 mg/kg tramadol) which evoked a maximum 91 % pain inhibition in the late phase (Figure 1)

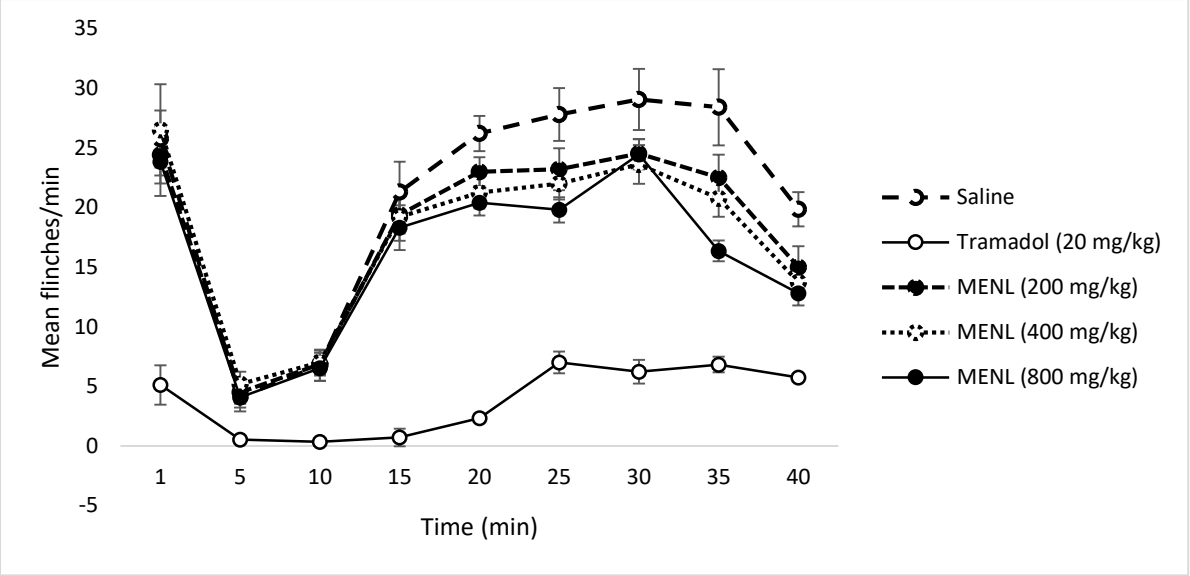

Figure 1. Time course of MENL on flinching response of Wistar rats to formalin-induced nociception.

Discussion

Animal models used in screening drugs for analgesic activity usually involves assessing the animals' response to noxious stimuli (Rang *et al.,* 2007). The stimulus may be chemical, mechanical, thermal etc. (Ainooson *et al*., 2009). In this study, acetic acid and formalin (chemical) as well as hot plate and water bath (thermal) were used to induce pain in the rats.

The acetic acid-induced writhing reflex model is commonly employed for testing the antinociceptive potential of substances (Bentley *et al*., 1983). Nociception in this model is caused by triggering localized inflammatory response resulting in the synthesis of prostaglandin via the cyclooxygenase pathway of arachidonic acid metabolism (Ahmed *et al*., 2006, Madubuike and Asuzu 2015). Pain is thus generated by prostaglandin and other endogenous mediators (Onansanwo and Elegbe 2006). In this study, MENL significantly reduced the number of abdominal constrictions (writhes) in the test rats. This is an indication of the analgesic or antinociceptive activity of *N. laevis* (Machioro *et al*., 2005). In addition, suppression of writhing response induced by acetic acid suggests that the antinociceptive action of MENL is mediated through inhibition of prostaglandin biosynthesis (Ferdous *et al*., 2008).

In the hot plate and tail immersion tests, MENL did not produce any significant antinociceptive effect. This suggests that the extract lacks central analgesic activity, since both models are only sensitive to centrally-acting analgesics (Wolfe and MacDonald, 1994).

The formalin-induced nociception consists of two different nociceptive states and separate mechanisms underlie the two phases of behavioural response. The early phase lasts for about 5 min and seems to result from the immediate and intensive

increase in activity of the primary afferent fibers induced by formalin, reflecting an acute pain (Han *et al*., 2012). On the other hand, the late phase (15-30 min post formalin injection) appears to be the outcome of the activation of wide dynamic range neurons in the dorsal horn, in addition to a continuous low level flow in the primary afferent fibers (Pug and Sorkin, 1996). Hence, the late phase represents a facilitated state of prominent and intensified noxious state despite a decreased level of afferent input (Han *et al*., 2012). This pain model is a valuable tool for assessing the effects of several analgesic agents on these two types of pain. It is widely reported that central analgesics ameliorate pain in both phases while peripherally-acting antinociceptive agents are effective only at the late phase (Shibata *et al*., 1989; Shoaib *et al*., 2016). In this study, the different doses of MENL significantly reduced flinching response by the rats, post formalin injection only in the late phase of the experiment, also suggesting that the antinociceptive activity of MENL is mediated peripherally.

Alkaloids and flavonoids are secondary metabolites of several plants that have been reported to possess strong analgesic activity (Raquibul *et al*., 2010; Vaishali *et al*., 2012; Shoaib *et al*., 2016). Their presence in MENL could be responsible for antinociceptive effect of the extract observed in the present study. The acute toxicity testing of MENL was aimed at determining its LD_{50} and to subsequently serve as a guide for selecting appropriate doses for the study. However, the absence of mortality and any other sign of acute toxicity implies that the oral LD_{50} of MENL in rats is greater than 5000 mg/kg (which is the maximum dose of MENL administered to the rats). It also implies that the extract, at the doses administered (200, 400 and 800 mg/kg) was well tolerated by the rats.

Conclusion

The results of this study show that the methanol extract of *Newbouldia laevis* possess peripheral antinociceptive activity. This finding supports the traditional use of leaves of *N. laevis* for pain relief.

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