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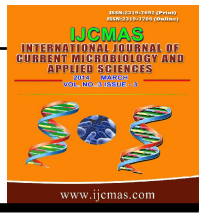

Original Research Article

Anti-inflammatory, phytochemical and acute toxicity study of the flower extract of *Newbouldia laevis*

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Keywords

Newbouldia laevis; anti-nociceptive; anti-inflammatory; intraperitoneal; phyto-chemicals.

A B S T R A C T

Preliminary investigations carried out on the flower and root bark of *Newbouldia laevis* revealed the presence of flavonoids, Tannins, Terpenes, steroids, alkaloids and cardiac glycosides. The ethanol extract of *Newbouldia laevis* flower was investigated for possible anti-nociceptive and anti-inflammatory effect in rats. Acetic acid induced writhing in mice and formalin test in rats were used in the study. The extract caused a significant decrease ($P<0.05$) which was not a dose dependent inhibition on acetic acid induced writhing and the neurogenic pains induced by formalin. The extract at the doses of (25, 50 and 100 mg/kg) showed 59, 71 and 47% inhibitions of the abdominal construction in mice respectively. The highest activity was recorded at lower dose of 50 mg/kg of the acetic acid induced abdominal construction. The intraperitoneal LD_{50} value of the extract was found to be 1264.9 mg/kg body weight in mice. The results from this research corroborated the claim that *Newbouldia laevis* could be used as health remedies for diarrhoea, typhoid fever and abdominal discomforts. *Newbouldia laevis* could be used in making antibiotics but that should be after its toxicological and active ingredient elucidation.

Introduction

The use of plants as medicine is an ancient practice to all societies especially the African society. This practice continues to exist in the developing nations. It is on this basis that researchers keep on working on medicinal plants in order to produce and develop the best medicines for physiological or therapeutic uses (Adesanya et al., 1994). *Newbouldia laevis* is called Aduruku in Hausa, Ogirisi in and Akoko in Yoruba (Ahmadiani et al., 2000). *Newbouldia laevis* is a medium sized angiosperm which belongs to the *Bignoniaceae* family. It grows to a height of about seven to eight up to fifteen meters more, usually a shrub of two to three meters; many streamed forming clumps of gnarled branches (Aladesanmiet al.,1998). *Newbouldia laevis* is native to Africans and grows from Guinea Savannahs to

dense forests, on moist and well-drained soil. It inhabits the secondary forest extending from Senegal to Cameroon, Gabon, Democratic Republic of Congo and Angola (Aladesanmi et al.,1998). In Nigeria, the bark is chewed and swallowed for stomach pains; diarrhoea and tooth ache (Gaertner et al., 1999). 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. It has also been found useful for ear-ache, sore feet, chest pain, epilepsy and children's convulsion (Iwu, 2000). The leaf, stem and fruit have been used for wound dressing and stomach ache (Iwu, 2000). Earlier studies on the leaves and bark of Congolese *Newbouldia laevis* revealed the absence of Flavonoids, Saponnins, Quinononones, Terpenes and steroids (Gafner et al.,1997).

With the increasing population and high industrial activities, the use of *Newbouldia laevis* as a medicine has also increased. As a result, there is need to study the flower root of bark of *Newbouldia laevis*, family of *Bignonniaceae* as a medicinal plant and as a cure for bacterial and fungal infection. The aim of this study was to determine the phytochemicals, acute toxicity and anti-inflammatory properties of the flowers and root bark of *Newbouldia laevis*.

Materials and Methods

Collection and identification of the plant materials

The flower and root bark of the plant were collected from Anyigba village in Kogi State in the month of April, 2013. The plant specimen was identified by Samuel Atumeyi, a lecturer in the Department of Biological Sciences, Kogi State University, Anyigba, Nigeria as *Newbouldia laevis* of the *Bignoniaceae* family.

Experimental animals

Adult wista rats of both sexes weighing between 150-200g and adult Swiss albino mice of both sexes weighing between 55-30g were used for the experiments.

Extraction of the plant materials

The flowers of *Newbouldia laevis* was shade dried and pulverized into powder. About 200g of the powdered form was extracted exhaustively with 95% (v/v) ethanol in water using continuous soxhlet apparatus and the solvent to solid ratio was 15% (w/v). The extract was concentrated under reduced pressure to yield a dark green mass which weighs 30g.

Phytochemical screening

The ethanol flower extract obtained was subjected to preliminary phytochemical screening to identify the chemical constituents. The method of analysis employed were those described by (Trease et al (1989) and Harbone et al (1993).

Acute toxicity study

This was conducted by using the method described by Lorke, 1983. In the initial phase, mice were divided into 3 groups of three and treated with the ethanol flower extract of the plant at doses of 10, 100 and 1000mg extract per kg body weight intraperitoneally (IP injection) and were then observed for 24h for signs of toxicity including death. In the final phase, mice were divided into four groups of mouse each and treated with the ethanol extract at doses of 600, 1000, 1600 and 2900 mg/kg

body weight IP. The median lethal dose (LD50) was calculated from the second phase.

Test for anti-nociceptive study

The Acetic acid induced writhing test in mice as describe by Koster *et al*., (1959) was employed. Twenty five (25) Swiss albino mice were divided into 5 groups of 5 mice each. The first group was given 10 ml/kg of Normal saline IP and served as control; groups 2 received 25, 50 and 100 mg of extract per kg body weight IP respectively. Thirty minutes later, mice in all the groups were treated with Acetic acid (0.6% v/v, 1ml per 100g body weight IP). Five minutes after Acetic acid injection mice were placed in individual cage and number of abdominal contractions was counted for each mouse for a period of 10 minutes. Percentage inhibition of writhing was calculated using the formula.

Inhibition (%) = Mean No. of writhing (control) – Mean No. of writhing (test) / Mean number of writhing (control) X100

Anti-inflammatory study

Increase in the rat hind paw edema induced by sub-planter injection of aphlogistic agent was used as the measure of acute inflammation (Winter *et al*., 1963). The phlogistic agent employed in this study was formalin (50µl of 2.5% v/v). The rats were randomly divided into five groups (n=5). Thirty minutes before the injection of formalin, the groups were treated with the single extract intraperitoneally. The groups are as follows: group 1: normal saline (10ml/kg as a negative control), group 2: 20mg/kg of Diclofenac 3, 4 and 5 received ethanol flower extract of *Newbouldia laevis* at the doses of 25, 50 and 100mg/kg respectively. Inflammation of the hind paw was induced by single injection of formalin into the sub planter surface of the left hind. Paw diameter (cm) was measured at 1, 2, 3, 4 and 5h after formalin injection. Paw diameter after administration of the phlogistic agent was measured using digital venier caliper.

Results and Discussion

Phytochemical analysis

The freshly prepared extracts revealed the presence of Alkaloids, Tannins, Saponins, flavonoids and steroids.

Acute toxicity study (LD_{50})

Signs of toxicity were first noticed after 4-6 h of extract administration. There was decreased sensitivity to touch and jerking. Also there was decreased feed intake, tachypnea and prostration after 10 h of extract administration. The median lethal dose (LD_{50}) in mice was calculated to be 1264.9 mg/kg body weight.

Anti-nociceptive study in mice

The extract demonstrated a significant ($P < 0.05$) anti-nociceptive activity at all the doses (25, 50 and 100 mg/kg body weight I.P) tested compared to control normal saline. The activity resides more at the lowest dose 50 mg/kg body weight I.P that was found to have the highest percentage (71%) inhibition of the abdominal constriction induced by acetic acid in mice as shown Table.4 .

Anti-inflamatory study

The extract was found to have a significant ($P< 0.05$) inhibitory effect on the formalin

– induced edema in rats at all the doses (25, 50 and 100 mg/kg body weight I.P) tested in rats when compared to the normal saline control, as represented in Table 5. The activity resides more at the lowest dose of 25 mg/kg with 42.5% inhibition after 5 h of extract administration. Also in regard to the other doses 50 and 100 mg/kg), there was also significant decreased to 40.3% and 36.8% respectively after 5 h of extract administration as shown in Table 5. The phytochemical screening of ethanol flower of *Newbouldia laevis* as represented in table 1 showed the presence of tannin, saponin, flavonoid, alkaloid, steroid and terpenoid. Acute toxicity studies are carried out to evaluate the safety of a drug or chemical substances. The figure serves as a guide in selection of doses for pharmacological studies. The ethanol flower extract of *Newbouldia laevis* was found to have shown a significant ($P<0.05$) anti – nociceptive effect at all the doses tested. Although, the inhibitory effect on the acetic-induced writhing in mice was not dose- dependent, the percentage inhibition at a dose of 50 mg/kg body weight of extract was found to be highest with 71% inhibition while that of the Diclofenac is 74%. The abdominal constriction method is a very sensitive one and can detect anti-nociceptive effect of substances at a dose that can be detected by other methods, such as the tail-flick test (Osagie and Eka, 1998). Abdominal constriction responses were found to be partly involved with local peritoneal receptors (Bentley *et al*., 1981). The results of the acetic acid induced writhing strongly suggest that the mechanism of action of this extract may be linked partly to lipoxygenases and cyclo-oxygenases. The activity demonstrated by the extract might be due to the presence of flavonoids and tannins that were present in the extract. This was supported by other workers who found that flavonoids and tannins were found to have anti-nociceptive and anti-inflammatory activities (Ahmadiani *et al*., 2000). The significant ($P<0.05$) anti –inflammatory activity exhibited by the extract at all the doses used (25, 50, and 100 mg/kg body weight extract I.P) against edema induced by formalin in rats compared to the control group was an indication that, the plant might serve as a useful source of anti-inflammatory agent. The highest activity resides at the dose of 50 mg/kg with the mean percentage inhibition of 42.5 after 5 h of extract administration. This anti-inflammatory effect of the extract observed might be due to the presence of flavonoids in the plant.

Formalin test is a well-established valid model for the study of central sensitization events at the spinal level after peripherial inflammatory state (Hill, 1997; Liu, 2004;Okwu, 2001 and Silver et al., 1998). The two distinct phases in formalin test are due to direct effect of formalin on nocieception and due to inflammation with the release of serotonin, histamine, prostaglandins and least to some degree, the sensitization of central nociceptive neurons (Sofowora, 1992 and Sofowora, 1993). Stimulation of upload receptors has also been suggested as a possible mechanism of action against neurogenic pain (Gaertner *et al*., 1999). The ability of the extract to inhibit the neurogenic phase suggests that it possess central analgesic activity. Analgesic and anti-inflmmatory effects have been observed in flavonoids as well as tannins (Ahmadiani *et al*., 2000). Certain flavonoids possess potent inhibitory activity against a wide array of

Table.1 Phytochemical screening of the ethanol flower extract of *Newbouldia laevis*

Constituents	Remarks
Tannins	+
Saponins	+
Flavonoids	+
Alkaloids	+
Steroids	+
Terpenoids	+

Key: + (present) and -(Absent)

Table.2 The LD_{50} of the different doses of extract of *Newbouldia laevis* flower extract administered intraperitoneally to Mice during the first phase of acute toxicity study.

Group	Dose (mg/kg)	Deaths
1	10	0/3
2	100	0/3
3	1000	1/3

Table.3 The LD_{50} of the different doses of extract of *Newbouldia laevis* flower extract administered intraperitoneally to Mice during the second phase of acute toxicity study.

Group	Dose (mg/kg)	Deaths
1	600	0/1
2	1000	0/1
3	1600	0/1
4	2900	0/1

Table.4 Effect of ethanol flower extract of *Newbouldia laevis* given intraperitoneally on acetic acid–induced abdominal writhing contraction in mice

Treated (I.P)	Dose (mg/kg)	No. of abdominal Contractions	% Inhibition
Normal saline	10	22.8 ± 6.87	-
Piroxicam	20	6.0 ± 1.73[a]	73
Newbouldia laevis	25	9.42 ± 2.19[a]	59
Newbouldia laevis	50	6.60± 2.42[a]	71
Newbouldia laevis	100	12.0± 3.42[a]	47

Values of abdominal contraction are mean ± SEM. N=5

Values are statistically significant compared to control at a= P< 0.05

Table.5 Paw oedema diameter (cm) exhibited by ethanol flower extract of *Newbouldia laevis*

Treatment/groups	Dose (Mg/kg)	Oedema diameter (cm)	Oedema diameter (cm)	Oedema diameter (cm)	Oedema diameter (cm)	Oedema diameter (cm)
N=5		1h	2h	3h	4h	5h
Normal saline	10ml/kg	0706±0.662	0.810±0.062	0.822±0.574	0.836±0.475	0.882±0.185
Diclofenac	20	0.602±0.201^{ns}	0.610±0.0190^{a}	0.610±0.00837^{a}	0.630±0.0263^{a}	0.640±0.0243^{a}
Newbouldia laevis	25	0.510±0.0170^{ns}	0.500±0.0707^{a}	0.516±0.172^{a}	0.410±0.0243^{a}	0.366±0.0291^{a}
Newbouldia laevis	50	0.612±0.404^{ns}	0.586±0.0478^{a}	0.508±0.0206^{a}	0.328±0.883^{a}	0.314±0.0370^{a}
Newbouldia laevis	100	0.604±0.0343^{ns}	0.564±0.0448^{a}	0.492±0.0491^{a}	0.424±0.0225^{a}	0.438±0.0317^{a}

Each value is mean ± SEM of 5 rats

^{a}P<0.05; statistically significant when compared to control.

^{ns}:Statistically not significant when compared to control.

Table.6 Percentage inhibition of paw oedema exhibited by ethanol flower extract of *Newbouldia laevis.*

Treatment	Percentage inhibition at 1h	Percentage inhibition at 2h	Percentage inhibition at 3h	Percentage inhibition at 4h	Percentage inhibition at 5h	Mean percentage Inhibition
Extract 25mg/kg	27.7	38.2	38.2	50.9	58.5	42.5
Extract 50mg/kg	13.3	27.6	38.2	60.7	61.8	40.3
Extract 100mg/kg	14.4	30.3	40.1	49.2	50.3	36.8

enzymes such as protein kinase C, protein tyrosine kinases, phospholipase, phosphodiesterases and others (Silver et al., 1998). Other flavonoids potently inhibit prostaglandins, a group of powerful pro-inflammatory signaling molecules. Inhibition of these key enzymes provides the mechanism by which flavonoids inhibit inflammatory processes (Liu, 2004). Deleterious effects of excessive release of nitric oxide (NO) have been implicated in tissue damage and inflammation. Tannic acid and polyphenols have been shown to be potent inhibitors of NO synthetase activity and NO production, independent of their antioxidant activity (Osagie and Eka, 1998). The analgesic and anti-inflammatory properties observed might thus be related in part to the flavonoids content of this plant.

The results from this research show that *Newbouldia laevis* flower extract could be a potential anti-inflammatory drug.

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