Asian Journal of Biochemistry

ISSN 1815-9923

Academic
Journals Inc.

www.academicjournals.com

Asian Journal of Biochemistry 10 (6): 299-305, 2015
ISSN 1815-9923 / DOI: 10.3923/ajb.2015.299.305

Anticonvulsant and Analgesic Properties of Leaf and Root Extracts of *Newbouldia laevis*

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ABSTRACT

Extracts of various parts of *Newbouldia laevis* are used traditionally in Eastern Nigeria in management and treatment of several diseases, including some neurological disorders. This study investigated anticonvulsant and analgesic potentials of leaf and root extracts. The extracts were prepared with deionized water and ethylacetate. Extracts doses of 200, 400, 600 and 800 mg kg^{-1} b.wt. were used for the investigations. Anticonvulsant potential of the extracts against pentylenetetrazole-induced convulsion was tested in albino rats, by measuring time for convulsion onset, duration of convulsion and plasma glucose and Ca^{2+} levels before, during and after convulsion, using diazepam as standard. The hot plate method was used to investigate the analgesic property of the extracts using morphine sulphate as standard. Pretreatment of the animals with different doses of the extracts delayed the onset of convulsion in a dose-dependent manner. Convulsion was not observed in 800 mg kg^{-1} b.wt. of Deionized Water Leaf (DWL) extract and in diazepam pretreated groups. The extracts decreased significantly ($p<0.05$) the severity of convulsion and prolonged the duration of convulsion induced by pentylenetetrazole. The plasma concentration of glucose and Ca^{2+} decreased significantly ($p<0.05$) in the test groups through the course of convulsion, while they did not change significantly ($p>0.05$) in the non-convulsed groups. All the extracts at all doses and 4 mg kg^{-1} of morphine sulphate (an analgesic) showed a significant ($p<0.05$) percentage inhibition against hot plate induced pain. The difference between the analgesic potentials of 800 mg kg^{-1} of DWL extract and 4 mg kg^{-1} morphine sulphate was not significant ($p>0.05$). These findings indicate that the extracts may be effective in management/treatment of convulsion and pains.

Key words: Deionized water, ethylacetate, plasma glucose and Ca^{2+}, pentylenetetrazole, morphine sulphate, *Newbouldia laevis*

INTRODUCTION

Nature has been a source of medicinal agents for thousands of years and an impressive number of modern drugs have been isolated from natural sources. Many of these isolations were based on the uses of the agents in traditional medicine. The plant-based, traditional medicine systems continues to play an essential role in healthcare, with about 80% of the world's inhabitants relying mainly on traditional medicines for their primary health care (Owolabi *et al*., 2007).

The medicinal properties of plants could be based on the antioxidant and antimicrobial antipyretic effects of the phytochemicals in them (Cowan, 1999; Adesokan *et al*., 2008). According to World Health Organization, medicinal plants would be the best source to obtain a variety

of drugs. Therefore, such plants should be investigated to better understand their properties, safety and efficacy (Nascimento *et al*., 2000).

Plants generally produce many secondary metabolites which are biosynthetically derived from primary metabolites and constitute an important source of microbicides, pesticides and many pharmaceutical drugs. From a long period of time medicinal plants or their secondary metabolites have been directly or indirectly playing an important role in the human society to combat diseases (Wink *et al*., 2005).

Secondary metabolites (compounds) have no apparent function in a plant's primary metabolism, but often have an ecological role as pollinator attractants, represent chemical adaptations to environmental stresses or serve as chemical defense against micro-organisms, insects and higher predators and even other plants (allelochemics). Secondary metabolites are frequently accumulated by plants in smaller quantities than the primary metabolites (Karuppusamy, 2009; Sathishkumar and Paulsamy, 2009).

Newbouldia laevis is a medium sized angiosperm in the Bignoniaceae family. It is found tropical Africa and grows to a height of about 10 m with acauliferous habit. It is ever green, though its leaves turn somewhat dark purple during the cold seasons. It is popularly known as the tree of life or fertility tree in Nigeria. Its local Nigerian names include Akoko (Yoruba), Aduruku (Hausa) and Ogirisi (Igbo). The root and leaves are used in the treatment of diseases such as fever, headache, convulsion, epilepsy and manic disorders (Ainooson *et al*., 2009).

Detailed documented information on the applications of extracts of various parts of *Newbouldia laevis* are scarce. Further, like many medicinal plants, many of the uses of the plant by traditional medicine practitioners have not been investigated. Hence, this research investigated anticonvulsant and analgesic properties of leaf and root extracts of *Newbouldia laevis* in albino rats.

MATERIALS AND METHODS

Collection of leaves and roots of *Newbouldia laevis*: Fresh leaves and roots of *Newbouldia laevis* were collected in the month of October, 2014 from Izzi in Abakaliki Local Government of Ebonyi State. The samples were identified by Prof. S.C. Onyekwelu of Department Applied Biology Ebonyi State University, Abakaliki.

Preparation of extracts: The samples were washed with distilled water, air-dried and ground into powder for extractions.

The methods of extraction used by Agbafor (2004) were adopted, utilizing deionized water and ethylacetate as solvents. The extracts were concentrated using rotorevaporator to get gel-like dark brown extracts.

Anticonvulsant property of the extracts: Solutions of extracts and drugs (diazepam and pentylenetetrazole) were prepared with normal saline. A modified method of Amole *et al*. (2009) was used to assess the anticonvulsant property of the extracts.

Experimental animals and handling: Ethical approval for use of animals in research was given by Ebonyi State University Research and Ethics Committee.

Thirty five adult male rats were divided into seven groups (1-7), five in each group. Groups 1, 2, 3 and 4 were given intraperitoneal administration of 200, 400, 600 and 800 mg kg^{-1} b.wt., respectively of aqueous leaf extract. Group 5 was given 10 mg kg^{-1} b.wt. of diazepam

(anticonvulsant drug) intraperitoneally (10). Groups 6 and 7 received normal saline. Groups 1-6 were further administered with 75 mg kg^{-1} b.wt. of pentylenetetrazole (10), the convulsion inducing drug, 30 min after administration of the extract. Group 7, the overall control received normal saline again. All the animals were allowed free accesses to feed and water. The time for the onset of convulsion and duration of convulsion were recorded in convulsing groups. Plasma levels of glucose and calcium ion were measured before extract administration and during and after convulsion. These parameters were also measured in non-convulsing groups at the corresponding times recorded in group 6. The other extracts were used in the same way.

The glucose oxidase method of Trinder (1969) and described by Tietz (2000) was used to measure glucose concentrations, while calcium ion concentration was measured spectrophotometrically by the method of Gindler and King (1972), the methylthymol blue method.

Analgesic studies: The analgesic property of aqueous leaf extract was studied with 30 adult male rats placed in six groups (8-13) of five in each. The test was carried out using a Gallenkamp (England) thermostat hot plate apparatus (cat no: HL-054), maintained at 50°C and the method described by Turner (1965) was employed. Only rats that showed initial nociceptive response within 30 sec were selected for the experiment. The reaction time of the rats to the thermal stimulus was taken to be the interval between the instant the animal reached the hot plate to the time it licked its paw or jump off the hot plate. The test was performed at 30 min before administration and repeated at 30 min post administration.

The final test mean value (Ta) for each treatment group was calculated which represented the after treatment reaction time and was subsequently used to determine the percentage thermal pain stimulus or protection by applying the formula:

$$\text{Protection against thermal stimulus (\%)} = \text{Test mean (Ta)} - \frac{\text{Control mean (Tb)}}{\text{Control means (Tb)}} \times 100$$

The intraperitoneal route was used to administer the extract in a single dose of 200, 400, 600 and 800 mg kg^{-1} b.wt. to groups 8, 9, 10 and 11, respectively. Morphine sulphate (4 mg kg^{-1}, ip) (Aiyelero *et al*., 2009) a reference analgesic was given to group 12 and normal saline to group 13. The other extracts were used the same way.

RESULTS AND DISCUSSION

The results of the anticonvulsant property of the extracts are shown in Table 1-4. Pretreatment of the animals with different doses of the extracts delayed the onset of convulsion

Table 1: Effect of deionized leaf extract on pentylenetetrazole- induced convulsion

Animal group	Time for onset of convulsion (min)	Duration of convulsion (min)	Change in plasma glucose (mg dL^{-1})	Change in plasma Ca^{2+} (mg dL^{-1})
1 DWL (200 mg kg^{-1})	9.15±0.7^{a}	9.10±0.6^{a}	-42.45±2.09^{a}	-6.06±0.51^{a}
2 DWL (400 mg kg^{-1})	15.30±1.2^{b}	12.55±0.9^{a}	-36.22±3.12^{a}	-4.84±0.63^{a}
3 DWL (600 mg kg^{-1})	26.25±2.2^{c}	18.78±1.5^{b}	-23.55±2.64^{b}	-2.28±0.70^{b}
4 DWL (800 mg kg^{-1})	Nil	Nil	-1.86±0.33^{c}	-0.85±0.06^{c}
5 Daz (10 mg kg^{-1})	Nil	Nil	-1.47±0.61^{c}	-0.69±0.03^{d}
6 Pent (75 mg kg^{-1})	4.20±0.5^{d}	8.33±1.5^{a}	-79.74±3.40^{d}	-13.45±1.20^{e}
7 (normal saline)	NA	NA	+3.16±0.59^{e}	+0.72±0.05^{f}

Values are Mean±SD, N = 5. Values in the same column bearing different superscripts differ significantly ($p<0.05$), DWL: Deionized water leaf, Daz: Diazepam, Pent: Pentylenetetrazole, NA: Not applicable

Table 2: Effect of deionized root extract on pentylenetetrazole-induced convulsion

Animal group	Time for onset of convulsion (min)	Duration of convulsion (min)	Change in plasma glucose (mg dL^{-1})	Change in plasma Ca^{2+} (mg dL^{-1})
1 DWR (200 mg kg^{-1})	7.35±0.7[a]	12.10±0.6[a]	-49.67±1.75[a]	-7.84±0.48[a]
2 DWR (400 mg kg^{-1})	11.30±1.2[b]	13.55±0.9[a]	-40.60±3.35[a]	-5.09±0.52[a]
3 DWR (600 mg kg^{-1})	20.25±2.2[c]	17.78±1.5[b]	-25.70±2.33[b]	-2.97±0.51[b]
4 DWR (800 mg kg^{-1})	27.25±2.2[c]	20.78±1.5[b]	-11.55±2.02[c]	-1. 88±0.19[b]
5 Daz (10 mg kg^{-1})	Nil	Nil	-1.47±0.61[d]	-0.69±0.03[c]
6 Pent (75 mg kg^{-1})	4.20±0.5[d]	8.33±1.5[c]	-79.74±3.40[e]	-13.45±1.20[d]
7 (normal saline)	NA	NA	+3.16±0.59[f]	+0.72±0.05[e]

Values are Mean±SD, N = 5. Values in the same column bearing different superscripts differ significantly ($p<0.05$), DWR: Deionized water root, Daz: Diazepam, Pent: Pentylenetetrazole, NA: Not applicable

Table 3: Effect of Ethylacetate Leaf extract on pentylenetetrazole- induced convulsion

Animal group	Time for onset of convulsion (min)	Duration of convulsion (min)	Change in plasma glucose (mg dL^{-1})	Change in plasma Ca^{2+} (mg dL^{-1})
1 EAL (200 mg kg^{-1})	5.22±1.3[a]	10.05±0.6[a]	-56.71±2.60[a]	-9.79±1.03[a]
2 EAL (400 mg kg^{-1})	12.58±0.8[b]	14.12±1.3[b]	-51.45±1.85[a]	-7.70±1.62[a]
3 EAL (600 mg kg^{-1})	14.77±2.4[b]	15.35±0.8[b]	-32.07±2.50[b]	-3.90±0.60[b]
4 EAL (800 mg kg^{-1})	30.81±1.7[c]	16.44±1.6[b]	-17.74±2.42[c]	-2.69±0.21[b]
5 Daz (10 mg kg^{-1})	Nil	Nil	-1.47±0.61[d]	-0.69±0.03[c]
6 Pent (75 mg kg^{-1})	4.20±0.5[a]	8.33±1.5[a]	-79.74±3.40[e]	-13.45±1.20[a]
7 (normal saline)	NA	NA	+3.16±0.59[f]	+0.72±0.05[d]

Values are Mean±SD, N = 5. Values in the same column bearing different superscripts differ significantly ($p<0.05$), EAL: Ethylacetate leaf, Daz: Diazepam, Pent: Pentylenetetrazole, NA: Not applicable

Table 4: Effect of ethylacetate root extract on pentylenetetrazole-induced convulsion

Animal group	Time for onset of convulsion (min)	Duration of convulsion (min)	Change in plasma glucose (mg dL^{-1})	Change in plasma Ca^{2+} (mg dL^{-1})
1 EAR (200 mg kg^{-1})	5.14±1.3[a]	10.05±0.6[a]	-68.88±2.57[a]	-12.04±1.55[a]
2 EAR (400 mg kg^{-1})	9.58±0.8[b]	14.12±1.3[b]	-59.70±2.40[a]	-9.87±1.44[a]
3 EAR (600 mg kg^{-1})	12.77±2.4[b]	15.35±0.8[b]	-42.85±3.62[b]	-4.77±0.65[b]
4 EAR (800 mg kg^{-1})	18.81±1.7[c]	16.44±1.6[b]	-25.88±1.76[c]	-3.79±0.72[b]
5 Daz (10 mg kg^{-1})	Nil	Nil	-1.47±0.61[d]	-0.69±0.03[c]
6 Pent (75 mg kg^{-1})	4.20±0.5[a]	8.33±1.5[a]	-79.74±3.40[a]	-13.45±1.20[a]
7 (normal saline)	NA	NA	+3.16±0.59[e]	+0.72±0.05[d]

Values are Mean±SD, N = 5. Values in the same column bearing different superscripts differ significantly ($p<0.05$), EAR: Ethylacetate root, Daz: Diazepam, Pent: Pentylenetetrazole, NA: Not applicable

(rapid and repeated turning followed by falling, stiffening and jerking of limbs). Convulsion was not observed after 40 min of treatment with pentylenetetrazole in 800 mg kg^{-1} b.wt. of DWL extract and in diazepam pretreated groups (groups 4 DWL and 5, respectively). Comparing the pretreated and untreated groups, this effect on time of onset of convulsion was significant ($p<0.05$) at all doses of DWL extract and 400-800 mg kg^{-1} b.wt. of other extracts. The effect was linearly dose-dependent.

The extracts decreased the severity of convulsion and prolonged significantly ($p<0.05$) the duration of convulsion induced by pentylenetetrazole. From the tables, the plasma concentration of glucose and Ca^{2+} decreased significantly ($p<0.05$) in the test groups, through the course of convulsion. The effect was also linearly dependent on the dose of extracts. There was no significant change ($p>0.05$) in the values of these parameters in the groups that did not show any sign of convulsion. Comparing 800 mg kg^{-1} of the extract with diazepam (classical anticonvulsant drug), these effects were not statistically significant ($p>0.05$).

The ability of the extracts to delay or prevent pentylenetetrazole-induced convulsion in the animals indicates that they possess anticonvulsant property. Pentylenetetrazole may elicit convulsion by inhibiting gabaergic mechanisms (De Sarro *et al*., 1999). The inhibition of GABA neurotransmitter and the enhancement of the action of glutamic acid have been shown to be the

underlying factors in convulsion (Rang *et al*., 2005). Standard anticonvulsant drugs, such as diazepam are believed to produce their effects by enhancing GABA-mediated inhibition in the brain (Rang *et al*., 2005). It is therefore suggested that the anticonvulsant effect shown by the extracts against pentylenetetrazole induced convulsion might be due to the activation of GABA neurotransmission by the chemical constituents of the extracts. The most effective dose of the extracts was 800 mg kg^{-1} of DWL extract. This dose prevented pentylenetetrazole-induced convulsion.

The decrease in plasma glucose and Ca^{2+} during and after convulsion recorded in this research has been reported by Hegde *et al*. (2009) in mice treated with root extract of *Carissa carandas* Linn. The circulatory level of glucose decreases during convulsion in order to meet the energy demand of the process (Rogawski and Loscher, 2004). During the rapid and repeated contraction of muscles that accompany convulsion, blood level of Ca^{2+} decreases because Ca^{2+} are withdrawn from circulation into cells to aid the contractile process (Robert and Darly, 2000).

These parameters did not change significantly in the non-convulsing groups, suggesting that there were no "Latent" convulsion in these groups. The actual chemical constituent (s) responsible for this anticonvulsant property is not known at this point. However, some alkaloids, monoterpenes and flavonoids also have protective effects against chemically induced convulsions (Librowski *et al*., 2000; Johnston and Beart, 2004). The phytochemicals, alkaloids, tannins, saponins, flavonoids, anthraquinones, terpenoids and cardiac glycosides have been found in extracts of the plant (Oloyede, 2004; Agbafor *et al*., 2015).

The results of the analgesic studies are presented in Table 5. All the extracts at all doses and 4 mg kg^{-1} of morphine sulphate (an analgesic) showed significant ($p<0.05$) percentage inhibition against hot plate induced pain. The effect was linearly dose-dependent. The difference between the analgesic potentials of 800 mg kg^{-1} of DWL extract and 4 mg kg^{-1} morphine sulphate was not significant ($p>0.05$). The hot plate method is one of the most common tests of nociception that is based on a phasic stimulus of high intensity (Mandegary *et al*., 2004). Pain induced by thermal stimulus of the hot plate is specific for centrally mediated nociception (Parkhouse and Pleuvry, 1979). The ability of the extracts to prolong the reaction latency to pain thermally-induced in the rats by the hot plate further suggests central analgesic activity. The extracts, at the doses tested, were shown to possess anti-nociceptive activity. The difference between the analgesic potential of

Table 5: Effect of the extracts on reaction time and percentage inhibition of hot plate induced pain

Animal group	RTBA (s)	RTAA (s)	Inhibition (%)
14 DWL (200 mg kg^{-1})	1.73±0.52^{a}	3.15±0.32^{a}	75.29^{a}
15 DWL (400 mg kg^{-1})	1.47±0.13^{a}	4.22±0.24^{a}	125.67^{b}
16 DWL (600 mg kg^{-1})	1.80±0.43^{a}	6.70±0.60^{b}	258.29^{c}
17 DWL (800 mg kg^{-1})	1.60±0.50^{a}	9.85±0.18^{c}	426.74^{d}
14 DWR (200 mg kg^{-1})	1.98±0.61^{a}	3.03?±0.52^{a}	62.03^{a}
15 DWR (400 mg kg^{-1})	1.82±0.56^{a}	3.94±0.40^{a}	110.70^{b}
16 DWR (600 mg kg^{-1})	1.76±0.24^{a}	5.18±0.84^{b}	186.10^{c}
17 DWR (800 mg kg^{-1})	1.98±0.33^{a}	6.80±1.02^{b}	263.64^{e}
14 EAL (200 mg kg^{-1})	1.68±0.70^{a}	2.94±0.61^{a}	57.22^{a}
15 EAL (400 mg kg^{-1})	1.80±0.49^{a}	4.04±0.55^{a}	116.04^{b}
16 EAL (600 mg kg^{-1})	1.65±0.56^{a}	5.67±1.01^{b}	203.21^{c}
17 EAL (800 mg kg^{-1})	1.70±0.50^{a}	5.95±0.45^{b}	218.18^{c}
14 EAR (200 mg kg^{-1})	1.84±0.60^{a}	3.02±0.70^{a}	61.50^{a}
15 EAR (400 mg kg^{-1})	1.78±0.44^{a}	4.11±0.75^{a}	119.79^{b}
16 EAR (600 mg kg^{-1})	1.68±0.45^{a}	4.89±0.15^{a}	161.50^{c}
17 EAR (800 mg kg^{-1})	1.84±0.62^{a}	6.44±0.63^{b}	244.39^{e}
18 MS (4 mg kg^{-1})	1.76±0.27^{a}	10.68±1.74^{c}	471.12^{d}
19 (normal saline)	1.97±0.21^{a}	1.87±0.32^{d}	NA

Values are Mean±SD, N = 5. Values in the same column bearing different superscripts differ significantly ($p<0.05$), DWL: Deionized water leaf, DWR: Deionized water root, EAL: Ethylacetate leaf, EAR: Ethylacetate root, MS: Morphine sulphate, NA: Not applicable

morphine sulphate (4 mg kg^{-1}, ip), a reference analgesic and that of 800 mg kg^{-1} DWL extract, the most effective dose was not significant ($p>0.05$). The analgesic effect of the extracts was also observed to be linearly dose-dependent. The exact chemical constituent (s) of the extracts responsible for this analgesic property is not known at this point. However, flavonoids, saponins and tannins have been shown to exert analgesic effect on acetic acid induced writhing test (Calixto *et al*., 2000; Shin *et al*., 1997).

CONCLUSION

The leaves and roots of *Newbouldia laevis* contain pharmacologically active compounds which are responsible for their medicinal applications. The extracts may be useful in management and treatment of nervous system related disorders such as convulsion, anxiety, pains, etc. Efforts to purify the extracts and identify the exact chemical compounds responsible for these pharmacological activities are currently in progress in our laboratory.

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