

Anticonvulsant Effect of *Persea americana* Mill (Lauraceae) (Avocado) Leaf Aqueous Extract in Mice

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Various morphological parts of *Persea americana* Mill (Lauraceae) (avocado) are widely used in African traditional medicines for the treatment, management and/or control of a variety of human ailments, including childhood convulsions and epilepsy. This study examined the anticonvulsant effect of the plant's leaf aqueous extract (PAE, 50–800 mg/kg i.p.) against pentylenetetrazole (PTZ)-, picrotoxin (PCT)- and bicuculline (BCL)-induced seizures in mice. Phenobarbitone and diazepam were used as reference anticonvulsant drugs for comparison. Like the reference anticonvulsant agents used, *Persea americana* leaf aqueous extract (PAE, 100–800 mg/kg i.p.) significantly ($p < 0.05$ – 0.001) delayed the onset of, and antagonized, pentylenetetrazole (PTZ)-induced seizures. The plant's leaf extract (PAE, 100–800 mg/kg i.p.) also profoundly antagonized picrotoxin (PCT)-induced seizures, but only weakly antagonized bicuculline (BCL)-induced seizures. Although the data obtained in the present study do not provide conclusive evidence, it would appear that 'avocado' leaf aqueous extract (PAE) produces its anticonvulsant effect by enhancing GABAergic neurotransmission and/or action in the brain. The findings of this study indicate that *Persea americana* leaf aqueous extract possesses an anticonvulsant property, and thus lends pharmacological credence to the suggested ethnomedical uses of the plant in the management of childhood convulsions and epilepsy. Copyright © 2006 John Wiley & Sons, Ltd.

Keywords: *Persea americana* leaf; aqueous extract; anticonvulsant activity.

INTRODUCTION

Persea americana Mill (family: Lauraceae), commonly known as: 'avocado', 'avocado pear', 'Mexican avocado' and so on, is a medium-sized, single-stemmed, terrestrial, erect, perennial, deciduous, evergreen tree of 15–20 m in height. Although a native of Central America (Mexico), *Persea americana* is now found in most tropical and subtropical countries of the world. The branches are fissured and grey, but the twigs are green and smooth. The 15–25 cm long and 10–20 cm broad leaves with well-developed petioles, are spirally arranged, often clustered near the branch ends, narrowly to broadly elliptical or obovate, and are usually pointed at the tip (Ross, 1999). The greenish-yellow flowers are borne on branched compact panicles which are shorter than the leaves. The often pear-shaped, one-seeded fruits are variable in size and shape according to the variety, up to 18 cm long and usually shiny and green, or brownish when ripe; with flesh soft, oily, greenish or yellow pulp surrounding one large, loose round seed (Ross, 1999). Avocado is now cultivated commercially as a fruit crop

in many countries of the world. In many parts of Africa, the fruits of avocado are much sought after by human beings and some other animals as a valuable foodstuff. Besides the fixed oil, the fruit pulp contains carbohydrates and more protein than any other fruit, while its contents of vitamins A and B are high (Watt and Breyer-Brandwijk, 1962; Ross, 1999).

In addition to the nutritional values of its fruits, the leaves and other morphological parts of *Persea americana* possess medicinal properties, and are widely used in traditional medicines of many African countries. For example, the fruit pulp of the plant is eaten as an aphrodisiac and as an emmenagogue in South Africa (Watt and Breyer-Brandwijk, 1962); while a hot-water extract of the plant's leaves is taken orally as a diuretic and for hypertension in many West African countries (Ross, 1999). In Nigeria, the leaves of *Persea americana* have been used as an effective antitussive, antidiabetic, antihypertensive; and as analgesic and anti-inflammatory remedies (Adeyemi *et al.*, 2002; Antia *et al.*, 2005; Adeboye *et al.*, 1999; Owolabi *et al.*, 2005). In other parts of the world, various parts of *Persea americana* have been employed for a wide range of human ailments. Products of the plant have been effectively used for the management, control and/or treatment of amenorrhoea, anaemia, insomnia, hyperlipidaemia, hypertension, diabetes mellitus, diarrhoea, dysentery, gastritis, peptic ulcers, bronchitis, cough, hepatitis and so forth (Ross, 1999; Watt and Breyer-Brandwijk, 1962).

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Previous studies in Nigeria have shown that leaf extracts of *Persea americana* possess a catalogue of pharmacological activities, including analgesic, anti-inflammatory, antidiabetic and hypoglycaemic, hypotensive and antihypertensive properties (Adeyemi *et al.*, 2002; Antia *et al.*, 2005; Adeboye *et al.*, 1999; Owolabi *et al.*, 2005). The present study was prompted by the claim of some African traditional health practitioners that decoctions, infusions and extracts of *Persea americana* leaves are effective remedies in the management and/or control of childhood convulsions and epilepsy. The core aim of the present study was, therefore, to investigate the anticonvulsant property of *Persea americana* leaf aqueous extract in experimental animal paradigms, with a view to providing a pharmacological justification (or otherwise) for the ethnomedical uses of the plant's leaf in the management, control and/or treatment of childhood convulsions and epilepsy in some rural communities of Africa.

MATERIALS AND METHODS

The experimental protocol used in this study was approved by the Ethics Committee of the University of Durban-Westville, Durban 4000, South Africa and conforms to the *Guide to The Care and Use of Animals in Research and Teaching* (published by the University of Durban-Westville, Durban 4000, South Africa).

Plant material. Fresh leaves of *Persea americana* were collected from a play-ground behind Willowpark Centre along Umbilo Road in Durban, South Africa, between January and June 2002. The leaves were identified by Professor H. Baijnath, the former Chief Taxonomist/Curator of the University of Durban-Westville's Department of Botany, as those of *Persea americana* Mill [family: Lauraceae]. A voucher specimen of the plant has been deposited in the University's Botany Departmental Herbarium.

Preparation of the plant extract. One kilogram of air-dried leaves of *Persea americana* was milled in a Waring commercial blender. The powdered leaf was macerated in distilled water and extracted twice, on each occasion with 2.5 L distilled water at room temperature for 48 h, with occasional shaking. The combined distilled water extracts were concentrated to dryness at 60 ± 1 °C in a rotary evaporator. Freeze-drying and solvent elimination under reduced pressure finally gave 21.50 g (i.e. 2.15% yield) of a light-brown, powdery, *Persea americana* leaf aqueous extract. This crude aqueous extract was used in our study without further purification. Aliquot portions of the leaves' aqueous extract residue were weighed and dissolved in distilled water for use on each day of the experiments.

Animal material. Healthy, male Balb C mice (*Mus domesticus*) weighing 20–25 g were used. The animals were kept and maintained under laboratory conditions of temperature, humidity, and light; and were allowed free access to food (standard pellet diet) and water *ad libitum*. The animals were divided into plant

extract- and reference drug-treated 'test', and distilled water-treated 'control', groups of 10 animals per group. All the animals were fasted for 16 h, but still allowed free access to water, before the commencement of our experiments.

Acute toxicity testing. The median lethal dose (LD_{50}) of *Persea americana* leaf aqueous extract (PAE) was determined in mice by a modified method of Lorke (1983). Mice fasted for 16 h were randomly divided into groups of 10 mice per group. The procedure described in detail earlier by Ojewole (2006) was followed for the determination of the acute toxicity of the plant extract in the mice.

Evaluation of anticonvulsant property. The anticonvulsant testing method of Vellucci and Webster (1984), modified by Amabeoku and Chikuni (1993) and Mahomed and Ojewole (2006), was used to assess the anticonvulsant property of the plant extract (PAE) in mice. Standard convulsant agents, pentylenetetrazole (PTZ, 90 mg/kg i.p.), picrotoxin (PCT, 10 mg/kg i.p.) and bicuculline (BCL, 30 mg/kg i.p.) were used to induce convulsions in the mice. Phenobarbitone (PBT, 20 mg/kg i.p.) and diazepam (DZP, 0.5 mg/kg i.p.) were used as reference anticonvulsant drugs for comparison. Following induction of convulsions in the 'test' mice (with intraperitoneal injections of the convulsant agents), the animals were observed for 30 min for signs of neurological deficits, especially hind-limb tonic seizures or convulsions. Hind-limb tonic extensions of the mice were regarded as manifestations of seizures. The ability of the plant extract (PAE, 100–800 mg/kg i.p.) to prevent the seizures or to delay/prolong the latency or the onset of the hind-limb tonic extensions, was considered as an indication of anticonvulsant activity (Navarro-Ruiz *et al.*, 1995; Amabeoku *et al.*, 1998). Because the plant extract and the reference drugs used in this study were dissolved in distilled water each day at the beginning of our experiments, distilled water (3 mL/kg i.p.)-treated mice were used as 'control' animals.

Data analysis. Data are presented as means (\pm SEM). Data from distilled water-treated 'control' mice were used as baseline values. The differences between the data obtained with the plant extract- and reference anticonvulsant drug-treated 'test' mice, and the data obtained with distilled water-treated 'control' animals, were subjected to one-way analysis of variance (ANOVA; 95% confidence interval), followed by Scheffe's multiple range comparison test. The proportion of mice convulsing was analysed by Chi-square test (Bienvenu *et al.*, 2002). In all cases, values of $p \leq 0.05$ were taken to imply statistical significance.

RESULTS

Intraperitoneal administrations of graded doses of *Persea americana* leaf aqueous extract in mice gave an LD_{50} value of 1336 ± 103 mg/kg. This finding probably suggests that the plant aqueous extract is relatively safe in, or non-toxic to, mice.

Table 1. Effects of *Persea americana* leaf aqueous extract (PAE), phenobarbitone (PBT) and diazepam (DZP) on pentylenetetrazole (PTZ)-induced seizures in mice

PTZ	Treatment dose (mg/kg i.p.)			No. convulsed/ No. used	% Animals not convulsed (i.e. % animals protected)	Latency of tonic convulsion (min) (mean \pm SEM)
	PAE	Phenobarbitone	Diazepam			
90	–	–	–	10/10	0	8.01 \pm 0.35
90	100	–	–	7/10	30	11.30 \pm 1.44 ^a
90	200	–	–	6/10	40	14.52 \pm 2.23 ^a
90	400	–	–	5/10	50	17.48 \pm 2.36 ^b
90	800	–	–	3/10	70	20.39 \pm 2.41 ^c
90	–	20	–	0/10 ^d	100	0.00 ^c
90	–	–	0.5	0/10 ^d	100	0.00 ^c

^a $p < 0.05$; ^b $p < 0.01$; ^c $p < 0.001$ vs pentylenetetrazole control (PTZ, 90 mg/kg i.p.).

^d $p < 0.001$ vs pentylenetetrazole control (PTZ, 90 mg/kg i.p.); Chi-square test.

Table 2. Effects of *Persea americana* leaf aqueous extract (PAE), phenobarbitone (PBT) and diazepam (DZP) on picrotoxin (PCT)-induced seizures in mice

PCT	Treatment dose (mg/kg i.p.)			No. convulsed/ No. used	% Animals not convulsed (i.e. % animals protected)	Latency of tonic convulsion (min) (mean \pm SEM)
	PAE	Phenobarbitone	Diazepam			
10	–	–	–	10/10	0	10.30 \pm 0.46
10	100	–	–	7/10	30	12.15 \pm 1.28 ^a
10	200	–	–	6/10	40	14.56 \pm 2.33 ^a
10	400	–	–	5/10	50	16.35 \pm 2.52 ^b
10	800	–	–	3/10	70	18.44 \pm 2.73 ^b
10	–	20	–	1/10 ^d	90	24.41 ^c
10	–	–	0.5	1/10 ^d	90	27.20 ^c

^a $p < 0.05$; ^b $p < 0.01$; ^c $p < 0.001$ vs picrotoxin control (PCT, 10 mg/kg i.p.).

^d $p < 0.001$ vs picrotoxin control (PTZ, 10 mg/kg i.p.); Chi-square test.

Effect of *P. americana* leaf aqueous extract (PAE) on pentylenetetrazole (PTZ)-induced seizures

Pentylenetetrazole (PTZ, 90 mg/kg i.p.) produced hind-limb tonic seizures in all the 10 mice used. *Persea americana* leaf aqueous extract (PAE, 100–800 mg/kg i.p.) produced a dose-related, significant ($p < 0.05$ – 0.001) protection of the mice against PTZ-induced seizures (Table 1). The plant extract (PAE, 100–800 mg/kg i.p.) significantly delayed ($p < 0.05$ – 0.001) the onset of, and antagonized, PTZ-induced seizures. The reference anticonvulsant drugs used, phenobarbitone (PBT, 20 mg/kg i.p.) and diazepam (DZP, 0.5 mg/kg i.p.), also profoundly delayed the onset of, and significantly antagonized ($p < 0.001$), PTZ-induced seizures (Table 1).

Effect of *P. americana* leaf aqueous extract (PAE) on picrotoxin (PCT)-induced seizures

Picrotoxin (PCT, 10 mg/kg i.p.) produced hind-limb tonic seizures in all the 10 mice used. *Persea americana* leaf aqueous extract (PAE, 100–800 mg/kg i.p.) produced a dose-related, significant ($p < 0.05$ – 0.001) protection of the mice against PCT-induced seizures (as in the PTZ-induced tonic seizures) (Table 2). Furthermore, the plant extract (PAE, 100–800 mg/kg i.p.) significantly delayed ($p < 0.05$ – 0.001) the onset of PCT-induced seizures (as in the PTZ-induced tonic seizures). The reference anticonvulsant drugs used, phenobarbitone

(PBT, 20 mg/kg i.p.) and diazepam (DZP, 0.5 mg/kg i.p.), profoundly delayed the onset of, and significantly antagonized ($p < 0.001$), PCT-induced seizures (Table 2).

Effect of *P. americana* leaf aqueous extract (PAE) on bicuculline (BCL)-induced seizures

Bicuculline (BCL, 30 mg/kg i.p.) produced hind-limb seizures in all the 10 mice used. *Persea americana* leaf aqueous extract (PAE, 100–800 mg/kg i.p.) produced dose-related protection of the mice against BCL-induced seizures (Table 3). However, relatively low doses of the plant extract (PAE, ≤ 100 mg/kg i.p.) did not significantly alter ($p < 0.05$) the onset of BCL-induced seizures, whereas, relatively high doses of the plant extract (PAE, ≥ 200 – 800 mg/kg i.p.) significantly delayed ($p < 0.05$ – 0.01) the onset of BCL-induced seizures. The two reference anticonvulsant drugs used, phenobarbitone (PBT, 20 mg/kg i.p.) and diazepam (DZP, 0.5 mg/kg i.p.), profoundly antagonized and significantly delayed ($p < 0.001$) the onset of BCL-induced seizures (Table 3).

DISCUSSION

The results of the present laboratory animal study indicate that aqueous leaf extract of *Persea americana*

Table 3. Effects of *Persea americana* leaf aqueous extract (PAE), phenobarbitone (PBT) and diazepam (DZP) on bicuculline (BCL)-induced seizures in mice

BCL	Treatment dose (mg/kg i.p.)			No. convulsed/ No. used	% Animals not convulsed (i.e. % animals protected)	Latency of tonic convulsion (min) (mean \pm SEM)
	PAE	Phenobarbitone	Diazepam			
30	–	–	–	10/10	0	7.41 \pm 0.36
30	100	–	–	8/10	20	10.08 \pm 1.25
30	200	–	–	7/10	30	12.50 \pm 2.04 ^a
30	400	–	–	6/10	40	14.78 \pm 2.43 ^b
30	800	–	–	5/10	50	16.64 \pm 2.70 ^b
30	–	20	–	0/10 ^c	100	0.00 ^c
30	–	–	0.5	0/10 ^c	100	0.00 ^c

^a $p < 0.05$; ^b $p < 0.01$; ^c $p < 0.001$ vs bicuculline control (BCL, 30 mg/kg i.p.).

^d $p < 0.001$ vs bicuculline control (BCL, 30 mg/kg i.p.); Chi-square test.

(PAE) possesses anticonvulsant activity in mice. To the best of our knowledge, this is the first report on the anticonvulsant activity of *Persea americana* in biomedical literature, although other investigators have reported analgesic and antiinflammatory (Adeyemi *et al.*, 2002), hypoglycaemic (Antia *et al.*, 2005), vasorelaxant (Owolabi *et al.*, 2005) and hypotensive (Adeboye *et al.*, 1999) effects of the plant's leaf extracts in various experimental animal models.

In Africa, febrile convulsion among infants and young children is a common phenomenon, especially in the rural communities. Some of the infantile and/or childhood convulsions often result in death. Although there are a number of synthetic anticonvulsant drugs currently available for use in the management, control and/or treatment of individuals with epilepsy, most of these synthetic drugs are not only inaccessible and unaffordable, but they also possess many toxic adverse effects. There is, therefore, a dire need for the development of cheap, effective and safe anticonvulsant agents from plants and other natural sources.

The results of the present study provide evidence in favour of the anticonvulsant activity of *Persea americana*, and show that aqueous leaf extract of the plant possesses anticonvulsant activity in the experimental animal model used. The effectiveness of the plant extract in the experimental convulsion paradigm used probably suggests that the plant could be used in both *petit* and *grand mal* types of epilepsy. The plant's leaf extract was relatively more effective in PTZ- and PCT-induced convulsions than in BCL-induced seizures. In general, the average onset and duration of convulsion was markedly delayed and reduced, respectively. These findings tend to suggest that *Persea americana* leaf aqueous extract might have inhibited and/or attenuated PTZ-, PCT- and BCL-induced seizures of the mice used by enhancing, or in some ways interfering with, GABAergic action and/or neurotransmission.

Persea americana has been reported to contain many bioactive chemical compounds, including: polyphenolics, tannins, coumarins, flavonoids, triterpenoids, phytosterols (especially, β -sitosterol), biotin, α -tocopherol, carotene, ascorbic acid, scopoletin, quercetin, oils, organic acids and inorganic substances such as calcium, magnesium, zinc, phosphorus, and so forth (Watt and

Breyer-Brandwijk, 1962; Ross, 1999). However, our present state of knowledge of the chemical constituents of the extract is limited. It is, therefore, impossible for us at this stage, to identify with certainty, the anticonvulsant constituent/s of PAE. Although we speculate that one or more of the major chemical constituents of the plant [namely: polyphenols, tannins, coumarins (especially scopoletin and other coumarins), flavonoids, triterpenoids and phytosterols (Watt and Breyer-Brandwijk, 1962; Ross, 1999)], may possibly account for the observed anticonvulsant activity of the plant extract, there are no sufficient scientific data or evidence at present, to justify this speculation. However, experimental evidence obtained in the present laboratory animal study show that the leaf aqueous extract of *Persea americana* significantly delayed the onset of seizures induced by pentylenetetrazole (PTZ), and also significantly antagonized picrotoxin (PCT)-induced seizures. Since PTZ- and PCT-induced seizures have been shown to be due to inhibition and/or attenuation of GABAergic neurotransmission (Leonard, 2000; Rang *et al.*, 2003; Katzung, 2004), it is not unreasonable to speculate that *Persea americana* leaf aqueous extract probably produces its anticonvulsant activity by enhancing GABAergic neurotransmission and/or action. The observed anticonvulsant activity of the plant extract may also be due, at least in part, to its ability to depress the central nervous system (CNS) by one or more of the known mechanisms of anticonvulsant action (MacDonald and Kelly, 1994), which may include altered Na^+ - K^+ ATPase expression (Kang *et al.*, 2004), pyridoxamine-5'-phosphate (PMP) metabolism (An *et al.*, 2004), and inhibition of expression of inducible nitric oxide (Wellard and Morgan, 2004). In conclusion, the findings of the present laboratory animal study lend pharmacological support to the suggested anecdotal, ethnomedical uses of *Persea americana* leaf in the management, control and/or treatment of epilepsy and childhood convulsions in some rural communities of Africa.

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