Vol. 2 no. 2, pp. 93-99 (September 2005)
http://www.ajol.info/journals/jpb

Journal of
PHARMACY AND
BIORESOURCES

Some *in vivo and in vitro* studies of the aqueous leaf extract of *Phyllanthus muellerianus* (Euphorbiaceae) in laboratory animals

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Received 22nd December 2004; Revised 10th May 2005; Accepted 2nd August 2005

Abstract

The aqueous leaf extract of *Phyllanthus muellerianus* (5–30 mg/kg, ip) was found to induce behavioural sedation/sleep in young chicks (2-day old) resulting in decreased locomotor activity as well as pecking behaviour. The extract (0.16-1.4 mg/ml) relaxed the rabbit and rat ilea with the rabbit ileum being more responsive. The effect of the extract on rat ileum was antagonized mainly by propranolol and to a lesser extent by phenoxybenzamine (0.16 μg/ml). At doses ranging from 5–30 mg/kg, the extract produced a rise in cat blood pressure (BP) which increased with increase in dose. The rise in BP was also blocked by (2 μg/kg) phenoxybenzamine and propranolol. The extract (5-30 mg/kg) produced a significant ($P<0.002$) analgesia compared with the control and was found to possess anti-inflammatory properties which were dose dependent. The results of these studies showed that the aqueous extract of *Phyllanthus muellerianus* possesses pharmacological properties that bear relevance to its therapeutic claims by local users.

Keywords: *Phyllanthus muellerianus*; Analgesia; Blood pressure; Inflammation; Sedation

Introduction

Many important drugs used in medicine today were directly or indirectly derived from plants. It is estimated that about 40% of all drugs in developing countries are derived from plants and also 65% (majority) of the people living in developing countries still depend on traditional medicine as compared to orthodox medicine. *Phyllanthus muellerianus* is a glabrous shrub or woody climber found growing in deciduous and secondary forests. It is sometimes arborescent and armed with recurved stipular thorns. The leaves are ovate or ovate- elliptic, mucronate and nearly 3 x 1.5 inches. The base of the leaf is rounded and glabrous. The flowers are in copious inflorescences, minute, greenish-white, in slender axillary racemes or fascicles having five stamens. The fruits are

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ISSN 0189-8442

numerous and about 0.125 inches in diameter (Irvine 1961).

Preliminary investigation of *Phyllanthus muellerianus* by Paris (1970) showed that the bark contains tannins, no alkaloids and is not poisonous to animals (Oliver, 1960). Adesida *et al.*, (1972) showed that the bark contains 22β-hydroxyfriedel – 1 – ene and 1 β -22 – β-hydroxyfriedelin. The leaves have been used for the treatment of toothache (Kerharo and Bouquet, 1961) fever and dysentery (Kersting 1961), ophthalmia and conjunctivitis (Dalziel 1955, gonorrhoea (Culwick, 1961). *Phyllanthus muellerianus* is commonly used in local medicine alone or in combination with other herbs in various parts of Africa, but little or nothing has been reported on its pharmacological profile. This is the motivation for the present study and the present work was designed to investigate some of its pharmacological profiles and to authenticate some of its therapeutic values and claims by local users.

Experimental

Plant collection and identification. The leaves of *Phyllanthus muellerianus* were collected from Area C of Ahmadu Bello University, Zaria, Nigeria. The plant was identified and authenticated by a staff of the Herbarium unit of the Department of Biological Sciences, Faculty of Sciences, Ahmadu Bello University, Zaria. A voucher specimen was deposited for future reference at the Herbarium.

Extraction. The fresh leaves of *Phyllanthus muellerianus* were dried in open air under a shade for a period of about four weeks prior to extraction. The water extract of the leaves was obtained by decoction in accordance with the general process described in USPXII. The dried leaves were coarsely powdered and 10g of the powdered leaves were soaked in 100ml of cold distilled water for 24 hours, boiled for 15 minutes and then allowed to cool at 40 °C. This was centrifuged and the clear supernatant decanted to yield an extract of 4.0% w/v, which was used for the studies.

Drugs. Propranolol and phenoxybenzamine were obtained from Sigma Chemical Company, USA. Drugs were freshly prepared to the desired concentration with distilled water just before use. The extract was also freshly prepared using distilled water.

In vivo studies

(i) Behavioural studies. Two-day old chicks (white ranger cockerel obtained locally from Arewa Agricultural Enterprises, Zaria, Nigeria) weighing 30-51 g were used for this study. The chicks were placed in separate transparent perspex cages and allowed to acclimatize to laboratory conditions for 1 hour prior to administration of the extract. The experiments were performed in a quiet room free from external disturbances with ambient temperature of 27°C±3 °C. Different concentrations of the extract (10–30 mg/ml) were administered intraperitoneally (i.p.) as solutions in distilled water while the controls were injected only with distilled water. The chicks were scored for behavioural variables such as, locomotion, pecking, head/neck jerk/ depression and sleep. The behavioural variables were authenticated by EEG and EMG recordings.

(ii) Analgesic studies Mice weighing between 30–37 g were challenged with pain sensation, 45 minutes after administration of the extract/drug by delivering an applied force from analgesiometer (Ugo Basile Biological Research apparatus, VIA A. Campiglio, 9-20133 Millan, Italy, Cat No.720) to the animal's paw. The tendency of the animal to withdraw its paw from the instrument was an index of pain sensation. The threshold of pain sensation was recorded from the instrument. The controls were injected with distilled water only. Graded doses (10-30 mg/kg) of paracetamol were also used.

(iii) Anti-inflammatory studies. Egg albumin has been shown to induce inflammation when injected into living tissues (Winter *et al.*,

1962). Two doses (i.p.) of the aqueous extract (15 & 30 mg/kg) were tested for their anti-inflammatory effect in mice treated with egg albumin and the readings recorded using Plethysmometer at 10 min. interval for 2 hours. The readings were compared with those of standard drug, acetyl salicylic acid (15 and 30 mg/kg). The controls were injected with the egg albumin only. A pre-treatment time of 30 min. was allowed after drug treatment before readings were recorded. The test was performed using fresh egg albumin induced hind paw oedema as a model for acute inflammation (Winter *et al.*, 1963). The paw circumference was used to estimate the degree of inflammation and percentage inhibition (Akah and Nwambie, 1994).

(iv) Cat blood pressure. Adult cats (2.0-2.76 kg) were anaesthetized with pentobarbitone (40 mg/kg i.p.) and the femoral vein and carotid artery cannulated. Arterial blood pressure was monitored continuously from carotid artery via a (Narco-Biosystem) pressure transducer (type P-1A). Drugs were injected through the femoral vein and the effects recorded on a 4-Channel® physiograph curtilinear paper at a speed of 0.025 cm/sec.

In vitro studies

2-3 cm strips of rabbit and rat ilea were suspended in an organ bath under a tension of 0.5 g containing Tyrode solution aerated with pure oxygen and maintained at 37 °C. Muscle contractions were measured using a microdynamometer and its isotonic transducer (Ugo Basile). The preparations were allowed to equilibrate for 30 minutes before dosing with the extract or drugs.

Results and Discussion

The aqueous extract (5–30 mg/kg) (i.p.) was found to induce behavioural changes in 2-day chicks. It produced sedation at lower doses (5-10 mg/kg) and behavioural sleep at higher doses (15-30 mg/kg) lasting for about 50 minutes with a dose of 30 mg/kg. This consequently resulted in a decrease in both gross locomotor activity as well as pecking behaviour. (Fig.1). The onset of action decreased with increase in dose at 9 min, for 5 mg/kg, 2 min. for 15 mg/kg and 1 min. for 30 mg/kg. These observations suggest that the aqueous extract of *Phyllanthus muellerianus* exerts a dose dependent depressant effect on the central nervous systems (CNS) of young chicks.

The depressant effect of the extract on the CNS of chicks was validated by Electroencephalography (EEG) and Electromyography (EMG) recordings (Results in the press 2005). The extract 15 mg/kg depressed both the EEG and EMG of 2-day old chicks. The EEG of the hyperstriatum (HS), optic tectum (OT), pontine recticular formation (RF) and muscle activity (EMG) conformed to that of behavioural sleep. The behavioural effect of *Phyllanthus muellerianus* may be central in origin as both the EEG and EMG were depressed. The short onset of action especially at a higher dose of 30 mg/kg confirms a direct depressant effect on different brain regions of the young chicks.

The extract produced analgesic effect, which was less in potency than the standard paracetamol preparation at equal doses (Fig.2). The analgesic effect of the extract was similar to that produced by paracetamol since both drugs were subjected to the same treatment and gave similar responses. However the analgesic effect of paracetamol at 10, 20 and 30 mg/kg compared with the extract was insignificant. Like all non-narcotic analgesics, the extract may be acting peripherally with some central effects to produce this effect. The analgesic effect of the extract justifies its use in toothache and fever (Kerharo and Bouquet, 1961), Kersting (1961) by local users.

The extract (15 and 30 mg/kg) showed anti-inflammatory properties, which were dose dependent. It significantly ($P<0.002$) reduced the effect of egg albumin on the rat paw

oedema compared with the control. On dose-dose bases, the standard preparation of acetyl salicylic acid (Aspirin) was superior to the extract in arresting inflammations induced by albumin (Fig.3).

The prostaglandins (PGs) have been implicated in all inflammatory responses in virtually all tissues of the body. The anti-inflammatory action of salicylate-like drugs are attributable to their ability to suppress PGs formation by inhibiting cyclo-oxygenase and thus prevent the synthesis of PGs. Hence the inhibition of cyclo-oxygenase may well explain the analgesic and anti-inflammatory activities of this extract. The anti-inflammatory effect of this extract strongly justifies its therapeutic use by local healers in the treatment of conjunctivitis and ophthalmia (Dalziel, 1955).

The extract produced a rise in cat blood pressure (B.P), which increased with increase in dose. (Fig.4). 2 μg/kg phenoxybenzamine and propranolol blocked this increase. This may suggest some central β-adrenergic property of the extract in raising the B.P. It is also possible that the extract may have peripheral α-adrenoceptor effect to cause vasoconstriction leading to increased peripheral resistance and subsequently the B.P.

The extract produced dose-dependent relaxation of rabbit and rat ilea with the rabbit ileum being more responsive. This effect on rat ileum was antagonized mainly by propranolol and phenoxybenzamine to a lesser extent (Fig.5a and b). This further supports the earlier suggestion that the aqueous extract of *Phyllanthus muellerianus* possesses pharmacological properties that bear relevance to its therapeutic claims by local users.

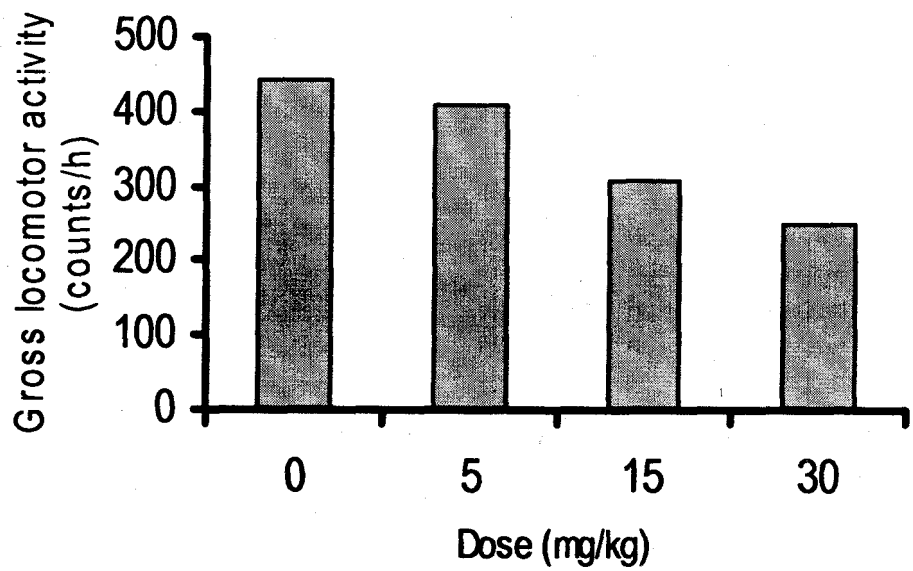


Fig. 1: Effect of *Phyllanthus muellerianus* aqueous extract on gross locomotor activity in two-day old chicks

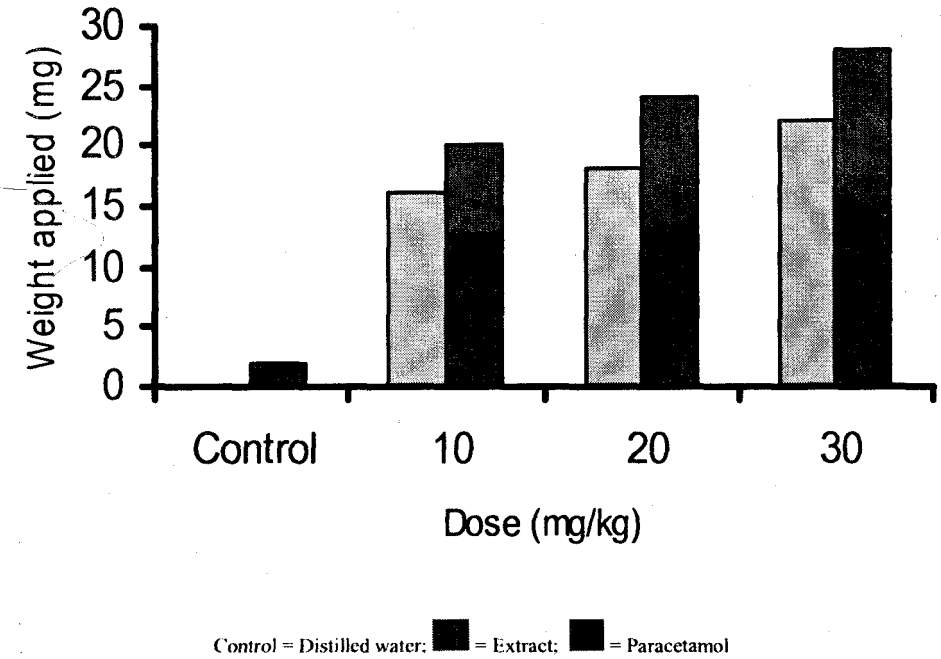


Fig. 2: Analgesic effect of the aqueous extract of *Phyllanthus muellerianus* on rats

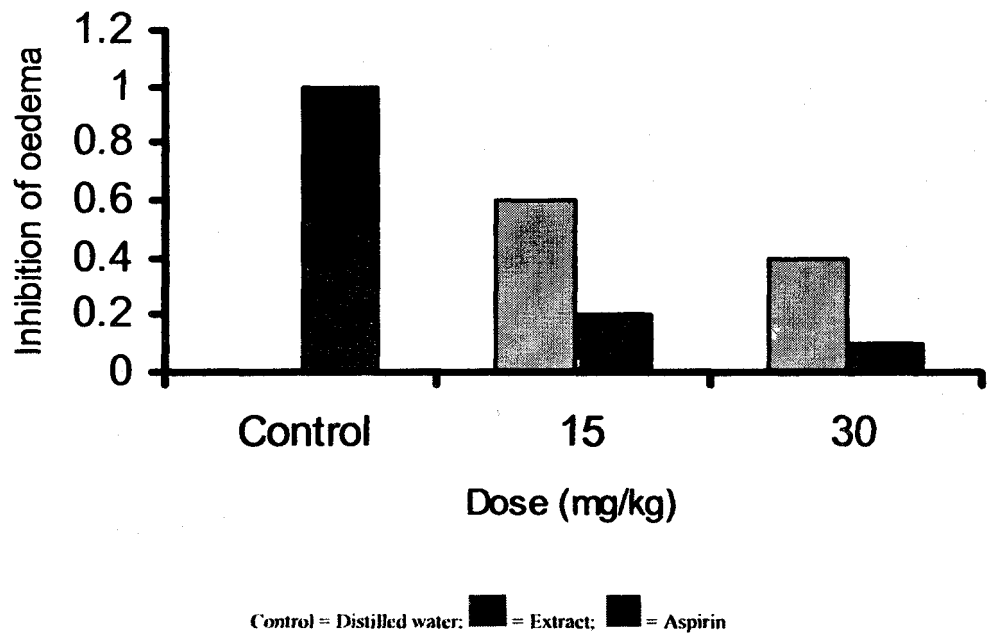
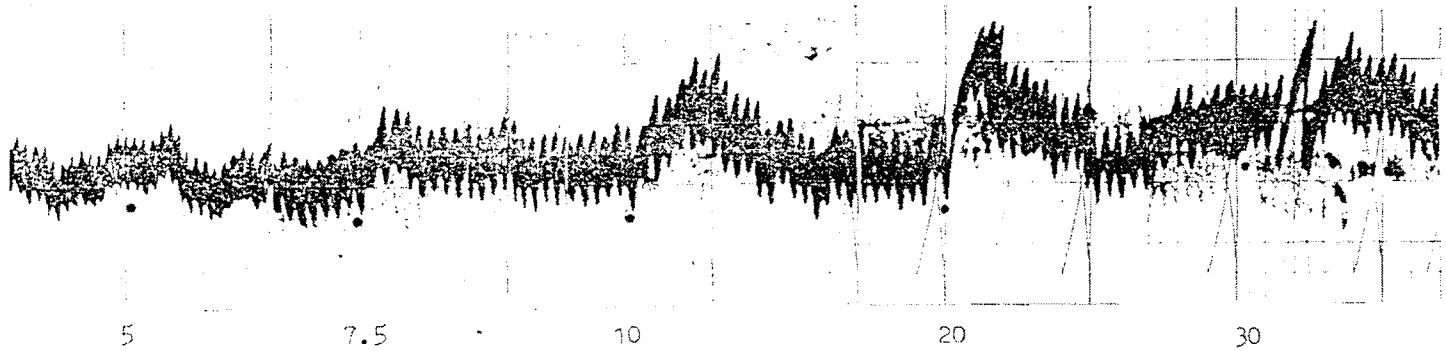
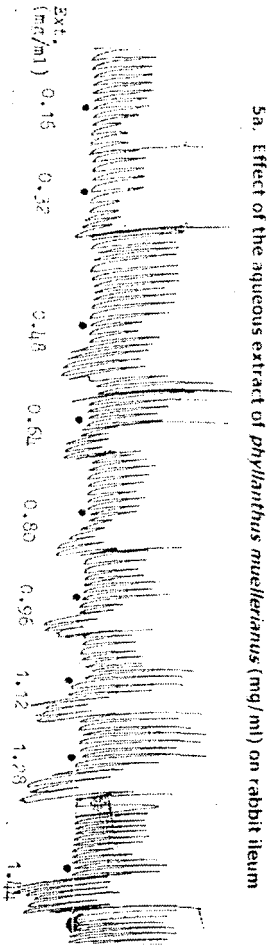


Fig. 3: Effect of aqueous extract of *Phyllanthus muellerianus* on egg albumin induced paw oedema in rats

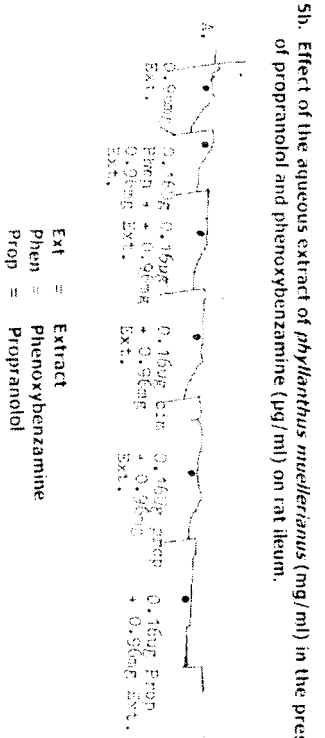
Fig. 4 Effect of the aqueous extract of *phyllanthus muellerianus* (mg/ml) on cat Blood Pressure



Ext = Extract
Phen = Phenoxybenzamine
Prop = Propranolol



5a. Effect of the aqueous extract of *phyllanthus muellerianus* (mg/ml) on rabbit ileum



5b. Effect of the aqueous extract of *phyllanthus muellerianus* (mg/ml) in the presence of propranolol and phenoxybenzamine (µg/ml) on rat ileum.

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