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Effect of *Annona senegalensis* rootbark extracts on *Naja nigricotlis nigricotlis* venom in rats

B. Adzu[a,*], M.S. Abubakar[b], K.S. Izebe[c], D.D. Akumka[a], K.S. Gamaniel[a]

[a] *Department of Pharmacology and Toxicology, National Institute for Pharmaceutical Research and Development (NIPRD), PMB 21 Abuja, Nigeria*
[b] *Department of Pharmacognosy and Drug Development, Ahmadu Bello University, Zaria, Nigeria*
[c] *Department of Pharmaceutical Microbiology, Human Virology and Biotechnology, NIPRD, PMB 21 Abuja, Nigeria*

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Abstract

Annona senegalensis Pers (family: Annonaceae) is used traditionally in Nigeria to treat victims of snakebite. The potency of the methanol extract of the root bark of the plant was tested against cobra (*Naja nigricotlis nigricotlis* Wetch) venom in rats. The extract was also tested on brine shrimp (*Artemia saline* Leach). The activity of the extract against the venom induced mortality, occurrence of toxic signs, activity on liver enzymes as well as its ability to reverse experimentally induced increase in body temperature were evaluated. Results indicated that the extract caused reduction in the induced hyperthermia and directly detoxified the snake venom used by 16–33%. It, however, failed to restore the biochemical functions (sGOT and sGPT) of the liver. The extract exhibited an LC_{50} of 232.7 μg/ml in the brine shrimp test.

Keywords: *Annona senegalensis*; *Naja nigricotlis nigricotlis*; Antisnake venom; Brine shrimp

1. Introduction

In Africa, majority of the rural population use traditional medicines for their healthcare needs. This usage is attributed to its relatively easy access and affordability to the people who live mostly in poor rural communities. Such traditional medicines often contain herbs and other plant materials whose efficacy and safety have not been determined. Interest in evaluating snake venom antidotes from plant sources has increased recently due to certain drawbacks in the applications of the current remedies that consist almost entirely of serum therapy, coupled with poor availability, storage and allergic reaction problems (Pereira et al., 1995). Some of these plants have been reputed to be active and have been used against snake venom (Rizzini et al., 1988). Their active components bind to the venom proteins, in the process detoxifying the venom (Otero et al., 2000). These plants include: *Diospyros kaki* (Okonogi et al., 1979), *Hemidesmus indicus* (Alam et al., 1994), *Eclipta prostrate* (Melo et al., 1994; Pithayanuku et al., 2004), *Brownea rosademonte* (Otero et al., 2000), *Bauhinia cumanensis*, *Cecropia peltata*, *Aristolochia rugusa*, *Pithocellobium unguis-cati*, *Cola nitida*, *Renealmia alpine* (Lans et al., 2001), *Alocasia cucullata* (Wang, 1986), *Cissus assamica*, *Aristolochia fordiana* (Wang et al., 1997), *Marsypianthes chamaedrys* (Castro et al., 2003), *Guiera senegalensis* (Abubakar et al., 2000) and *Harpalyce brasiliana* (Silva et al., 1997).

Annona senegalensis Pers (Annonaceae), which grows wild in tropical Africa, is the plant of interest in this study. It has aromatic flowers, which are used to flavour food. The fruit, yellow in colour when ripe, has a pleasant smell with sweet edible jelly. The plant is reputed to be of great medicinal value and is used in native medicine (Dalziel, 1937) for

* Corresponding author. Tel.: +234 803 6077356.
E-mail address: bulusadzu@yahoo.com (B. Adzu).

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chest pain, coughs, anaemia, urinary tract infection (Burkill, 1985; Muanza et al., 1994), cancer treatment (Durodola, 1975; Graham et al., 2000), diarrhoea, dysentry (Muanza et al., 1994; Ekpendu et al., 1998; Kudi and Myint, 1999), anthritis and rheumatism (Audu, 1989). Among its several other documented uses are: intestinal and guinea worms (Watt and Breyer-Brandwick, 1962; Alawa et al., 2003), venereal diseases (Durodola, 1975; Bhat et al., 1990; Tabuti et al., 2003), head and body ache (Arnold and Gulumian, 1984; Chhabra et al., 1987), against leishmaniasis (Akendengue et al., 1999) and typanasomiasis (Atawodi et al., 2003), lice treatment (Hirschmann and Rojas De Arias, 1990), eyelid swelling (Klaus and Adala, 1994), bloody stool (Hedberg et al., 1983), and treatment of snakebites (Durodola, 1975; Kela, 1990; Selvanayahgam et al., 1994). *Annona senegalensis* is widely used in both western and northern Nigeria. Despite the widespread use of this plant, little is known of the scientific basis for its use in treating victims of snakebites.

Earlier scientific investigation of the plant has revealed its activity against malaria (Balansard and Timon-David, 1985). The plant has also been shown to be effective as an antiulcer/antacid, smooth muscle relaxant (Langason et al., 1994), antibacterial (Muanza et al., 1994), antiprotozoan (Igweii and Onabanjo, 1989), molluscicidal (Sofowora and Adewunmi, 1980), antitumor agent (Fatope et al., 1993; Sahpaz et al., 1994) and hormonal activities (Jacobson et al., 1975). More recently, our own studies on the plant (Adzu et al., 2003a) revealed that the methanol extract of the root bark of this plant has analgesic and anti-inflammatory activities that might be exerted through peripheral mechanisms and phytochemical tests indicating the presence of saponins, tannins and resins. The plant has been reported to contain in addition, wax, alkaloids (Philipov et al., 1995; You et al., 1995), proteins, amino acids, antraquinones (Bamba et al., 1984; Burkill, 1985; Ekpendu et al., 1998), sterols, glycosides, flavonal terpenoids (Mackie and Misra, 1956; Mackie and Ghatce, 1958; Adesogan and Durodola, 1976; Fatope et al., 1996; Sahpaz et al., 1996) and terpenes (Ekundayo and Oguntimein, 1986).

The traditional healers often administer the medicinal preparation by pounding the fresh roots into a paste, and applying the mashed product over incisions made at the point of bite holding it into place with a bandage. Some herbalists prefer to administer the prepared extracts orally. We, therefore, investigated activities of the methanol extract of the root bark against cobra (*Naja nigricotlis nigricotlis* Wetch) venom. We decided on cobra venom because it (cobra) was reported to be the major cause of snakebite injury in Nigeria (Houghton and Harvey, 1989). The detoxifying effects of the extract were investigated and activities against liver enzymes assessed by looking at the serum glutamate-oxalate-transminase (sGOT) and serum-pyruvate-transaminase (sGPT) both of which indicate the condition of liver function (Hsu et al., 1998). The ability of the extract to reverse yeast-induced pyrexia as well as the toxic activity (cytotoxic) on zoologic system was also tested using brine shrimp lethality test.

2. Materials and methods

2.1. Plant sample

Fresh roots of *Annona senegalensis* were collected from Midlu, Adamawa State in April 2003. The plant material was authenticated at the Department of Medicinal Plant Research and Traditional Medicine, National Institute for Pharmaceutical Research and Development (NIPRD), Abuja, Nigeria. A voucher specimen (no. 5481) was deposited at the herbarium of the institute for future reference.

The root bark was removed, cleaned and air-dried. The dried material (500 g) was added to 1 l of methanol (M&B, England) in a flask followed by occasional shaking using flask shaker (GFL 3017, Germany) and extracted using soxhlet extractor (Quickeit, England) for 24 h. Thereafter, the solvent was evaporated under reduced pressure using rotary evaporator. This gave a yield of 26.4 g (5.28%, w/w).

2.2. Venom sample

The venom sample was obtained by M.S. Abubakar using the milking method (Markfarlane, 1967) from locally caught cobra (*Naja nigricotlis nigricotlis* Wetch), kept at the Herpetarium Unit, Department of Pharmacognosy and Drug Development, Ahmadu Bello University, Zaria, Nigeria. The pooled venom was lyophilized and stored. The LD_{99} of the venom was established to be 9.55 mg/kg, i.p., in mice (Abubakar et al., 2000).

2.3. Animals

Adult Wistar rats (*Mou norvegicus albinus*) obtained from the Animal Facility Centre, NIPRD, were used for the study. They were kept in plastic cages with sawdust as bedding under conditions of 12:12 h light and dark cycle and fed with standard diet. Equal numbers of male and female rats were used in each experimental group, keeping their mean weight as near as possible. Their usage was according to the standard protocol of 'principles of laboratory animals' care' NIH publication N0. 85-23 revised (1978).

2.4. Brine shrimp lethality test

The brine shrimp lethality test was used to test the activity of the extract and to estimate its toxicity against zoologic systems (Meyer et al., 1982). The test was performed according to the method described by Mackean et al. (2000) but slightly modified to suit our local laboratory settings. Briefly, 50 mg of brine shrimp (*Artemia saline* Leach) eggs (HOBBY®, Germany) were sprinkled into a 50 ml beaker containing natural seawater (collected at Bar Beach, Victoria Island, Lagos, Nigeria) and placed in a secure place for 48 h to hatch. The phototropic nauplii of the hatched shrimps were harvested with plastic pipette by covering three-fourths of the beaker with black carbon paper, as they move towards a torchlight