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The anti-snake venom activities of the methanolic extract of the bulb of *Crinum jagus* (Amaryllidaceae)

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Abstract

The anti-snake venom activities of the methanolic extract of the bulb of *Crinum jagus* plant (Amaryllidaceae) were investigated in vitro and in vivo against the venoms of three notable snake species: *Echis ocellatus*, *Bitis arietans* and *Naja nigricollis*. The extract was prepared by cold marceration in 50% methanol at 37 °C with intermittent shaking for 48 h. An yield of 12.8% w/w dry extract was obtained. Oral administration of *C. jagus* extract (1000 mg/kg) protected 50% of mice, while injection of a 30 min pre-incubated mixture of the same dose of extract and venom gave 100% protection against the lethal effects of *E. ocellatus* venom (10 mg/kg, i.m.). The intraperitoneal administration of the extract at 250 mg/kg, 30 min before the injection of *E. ocellatus* venom (10 mg/kg, i.m.), significantly ($p<0.05$) prolonged the death time of poisoned mice. *C. jagus* extract (500 mg/kg, *per os*), gave 50% protection against *B. arietans* venom (9.5 mg/kg, i.m.) in mice while the pre-incubation of a mixture of the same dose of venom and extract (500 mg/kg), prior to injection (i.p.) of the mixture, gave only 33.3% protection. The pre-incubation of 500 mg/kg of *C. jagus* extract with *N. nigricollis* venom (6 mg/kg) prior to i.p. injection of the mixture protected 50% of the treated mice. There were generally no significant differences in the death times of mice that were given the same dose of the extract orally 30 min before injection of the venoms and those administered with the pre-incubated mixtures of venom and extract. The pre-incubation of the extract and *E. ocellatus* venom (5 mg/kg) for 30 min, before the i.m. injection of the mixture, significantly reduced infiltration of inflammatory cells to the site of injection 4 h post treatment. The concentrations of plasma creatine kinase in poisoned mice were significantly ($p<0.01$ or $p<0.05$) reduced after the injection (i.p.) of *C. jagus* extract (1000 mg/kg) pre-incubated with *E. ocellatus* (5 mg/kg) or *B. arietans* (7 mg/kg) venom, respectively. The bulb extract of *C. jagus* blocked the haemorrhagic activity of a standard haemorrhagic dose (2.8 mg/ml) of *E. ocellatus* venom at various concentrations (1.7, 3.3 and 6.7 mg/ml). The methanolic bulb extract of *C. jagus* was therefore able to significantly protect mice from death, myonecrosis and haemorrhage induced by the lethal effects of venoms of notable snake species in Nigeria.

Keywords: *Crinum jagus*; *Echis ocellatus*; *Bitis arietans*; *Naja nigricollis*; Antihaemorrhagic activity; Creatine kinase activity

1. Introduction

In Nigeria, snakebites cause significant deaths in human and animal species. Unfortunately, conventional antivenoms currently available are not only expensive, but do not effectively neutralize venom induced haemorrhage, myonecrosis and nephrotoxicity. Some of the antivenoms cause allergic reaction in patients (Grant et al., 2000; Gutierrez et al., 1980; Ferreira et al., 1992). In humans, treatment of snakebite is normally continued until the clinical

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signs of envenomation disappear (Viejeth et al., 2000); the prolonged regime of injections imposes a lot of stress on the victims. Plants have reportedly been used locally to treat diverse cases of snakebites (Akunyili and Akubue, 1987; Wang et al., 1997; Borges et al., 2000; Asuzu and Harvey, 2003; Yang et al., 1998) but many of the studies lack systematic scientific procedures, which are necessary for the development of an antivenom agent from plants (Martz, 1992). The bulb of *Crinum jagus* plant is used traditionally for the treatment of various cases of snakebite by the *Igede* speaking tribe of Oju Local Government Area in Benue State and the *Fulani* nomads from Northern Nigeria living among them. It is an acclaimed snake venom antidote, which is effective even at advanced stages of envenomation.

C. jagus commonly called Harmattan lily, belongs to Amaryllidaceae, a heterogenous family of 86 genera and about 1310 species (Lawrence, 1951) which are widely distributed throughout the world. *C. jagus* is popularly known in *Igede* as 'Aru inyi' (Elephant's knee) or 'Okonkilo inyi' (Elephant's potato) and as gadali among the *Fulani* and *Hausa* in Northern Nigeria (Dalziel, 1937). All the species are of ornamental value. In Sierra leone, a cold infusion of the fresh leaves is used to bathe young children suffering from general body debility, rickets, etc. (Dalziel, 1937). In Gold Coast, a decoction is given as a vermifuge. In Lagos, the bulbs of several species are sold for various medicinal purposes. In East Africa, the decoction of *Crinum* is used for the treatment of sores (Kokwaro, 1976).

The present study investigates the acclaimed antivenom activities of the bulb of *C. jagus* using venoms from three notable snake species found in Nigeria, namely, *Echis ocellatus*, *Bitis arietans* and *Naja nigricollis*. There was until now, no systematic investigation of the antivenom effect of this plant using various pharmacological models. The extract of *C. jagus* will be administered through various routes so as to ascertain the most effective route of administration.

2. Materials and methods

2.1. Solutions, reagents and chemicals

Freshly prepared solutions and analytical grade chemicals were used in all the experiments. Creatine kinase kit (Quimica Clinica Applicada, Spain), freeze dried *E. ocellatus*, *B. arietans* and *N. nigricollis* venoms (Liverpool School of Tropical Medicine, UK), spectrophotometer (Spectrolab, USA) and a locally fabricated incubator were used.

2.2. Animals

Inbred Wistar mice of both sexes with average weight of 26 g were used as test animals. The animals, which were obtained from the laboratory animal facility of the Faculty of Veterinary Medicine, University of Nigeria, Nsukka, were housed in stainless steel cages at room temperature of 28–32 °C and under a light period of 16–18 h daily. They were fed on standard commercial feed (Topfeeds®, Nigeria).

2.3. Preparation of plant material

Fresh bulbs of the plant were collected in April 2004 from farm locations in Ochimode village, Oju Local Government Area in Benue State, Nigeria. The plant was duly identified as *C. jagus* by Mr. Ozioko, a taxonomist with the University of Nigeria, Nsukka. The bulbs (underground stems) were cut into small pieces with a knife, dried under mild sunlight and pulverized into powder with a laboratory mill. The powder (500 g) was exhaustively extracted with 31 of 50% methanol. The extraction was by cold maceration at 37 °C with intermittent shaking for 48 h. The extract was concentrated by vacuum rotary evaporation and stored in a refrigerator at 4 °C. The concentration of the extract was determined and the percentage yield was calculated.

2.4. Acute toxicity study

Five groups of Wistar mice of both sexes with each group containing five mice were used. Four of the groups were treated orally with varying doses of *C. jagus* extract at 250, 500, 1000 and 2000 mg/kg, respectively. The fifth group was given an equivalent volume of distilled water to serve as control. The animals were observed for toxic signs like excitability, dullness, diarrhoea, inappetence and death over 24 h.

The rest of the in vivo experiments were performed separately with 24 adult Wistar mice of both sexes randomly allocated to 4 groups with each group having 6 mice. At the end of each of the experiments, the number of mice that were