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<b>Author 1</b>	<b>UCHENDU, C.N.</b>
<b>Author 2</b>	<b>LEEK B. F.</b>
<b>Author 3</b>	
<b>Title</b>	<b>Uterine Muscle Contractant from the Root of Dalbergia Saxatilis</b>
<b>Keywords</b>	<b>Dalbergia Saxatilis; Saponins; Uterine Muscle Contractant Activity</b>
<b>Description</b>	<b>Uterine Muscle Contractant from the Root of Dalbergia Saxatilis</b>
<b>Category</b>	<b>Veterinary Physiology &amp; Pharmacology</b>
<b>Publisher</b>	<b>Elsevier Scientific Publishers Ireland Ltd</b>
<b>Publication Date</b>	<b>October, 1998</b>
<b>0Signature</b>	



## Uterine muscle contractant from the root of *Dalbergia saxatilis*

C.N. Uchendu<sup>a,\*</sup>, B.F. Leek<sup>b</sup>

<sup>a</sup>Department of Veterinary Physiology and Pharmacology, University of Nigeria, Nsukka, Nigeria

<sup>b</sup>Department of Veterinary Physiology and Biochemistry, University College Dublin, Ballsbridge, Dublin 4, Ireland

Received 15 December 1997; accepted 24 October 1998

### Abstract

Contractile responses of uterine muscle strips to a saponin (DSS) isolated from the root of *Dalbergia saxatilis* was investigated in the rat. Uterine muscle response to the glycoside was characterized by a single but transient contraction that was concentration-dependent, with an  $ED_{50}$  of 0.13 mg/ml and 0.04 mg/ml as the lowest active concentration. Atropine sulphate (0.69  $\mu$ mol) abolished uterine muscle responses induced by acetylcholine (1.82  $\mu$ mol) but failed to block responses to submaximal concentration of DSS (0.24 mg/ml), suggesting that responses to DSS were not mediated by cell membrane cholinergic activation. © 1999 Elsevier Science B.V. All rights reserved.

**Keywords:** *Dalbergia saxatilis*; Saponins; Uterine muscle contractant activity

### 1. Introduction

*Dalbergia saxatilis* L. (Fabaceae), known as 'Ola Nkita' (Dog's neck collar) by the 'Ibos' of south-eastern Nigeria is a local African shrub, producing termite resistant and durable ornamental wood. Various parts of the plant are claimed to possess medicinal values in different parts of Africa and extensively used by traditional healers as remedies for various ailments such as skin lesions, smallpox and toothache [1–3].

\* Corresponding author.

In south-eastern Nigeria, decoction from the root is used to accelerate birth and to expel the placenta. In a preliminary investigation (unpublished results), the crude 70% ethanol root extract was found to exhibit spasmogenic activity in rat uterine muscle preparations in vitro. The present study was aimed to isolate, by a bioassay guided extraction, and biologically characterize the utero-active principle contained in the root extract of this medicinal plant.

## 2. Experimental

### 2.1. Plant material

*D. saxatilis* fresh roots were collected from Umuahia, Abia State, Nigeria in June 1994 and identified by Mr A. Ozioko of the Department of Botany, University of Nigeria, Nsukka where a voucher specimen was deposited.

### 2.2. Extraction and isolation

Air-dried roots (500 g) were defatted with petrol (60–80°C) and subsequently Soxhlet-extracted with 10 l of 70% EtOH. The crude extract (yield: 6.41%) was partitioned between  $\text{CHCl}_3$  and water; *n*-BuOH extraction of the aqueous phase afforded a milky powder which was decolorized with charcoal and subjected to preparative TLC (cellulose PSC-Fertigplatten Art 15375; 40 × 20 cm; upper phase of *n*-BuOH–AcOH water 4:1:5 as developing solvent). Two distinct yellow bands ( $R_f = 0.28$  and 0.34) were observed under a 360-nm UV light. Elution with MeOH of the main (90%) band at  $R_f = 0.34$  afforded a white fluffy solid which recrystallized from MeOH as colourless rhomboidal crystals (yield: 5.7% of 70% EtOH extract). The product (hereinafter referred to as DSS) gave a positive Liebermann–Burchard reaction for triterpenoids [4], yielded fructose upon 6% HCl hydrolysis and proved to be a saponin by sheep red blood cell haemolysis [5].

### 2.3. Animals

Non-pregnant female Wistar rats, weighing 200–250 g, were used. They were supplied by the Faculty of Agriculture, University College Dublin and reared under specific pathogen-free (SPF) conditions with a controlled ambient temperature of  $20 \pm 1^\circ\text{C}$ ,  $68 \pm 2\%$  relative humidity and a 14-h/10-h light/dark schedule. Standard rat chow and tap water were supplied ad libitum. Each rat received 0.1 mg/kg of oestradiol benzoate in paraffin subcutaneously 24 h prior to the experiments.

### 2.4. Tissue preparation and isometric contraction studies

Uterine horns from the rats were trimmed free of fat and connective tissue. A uterine segment approximately 12 mm long was cut out and attached by ligatures at one end to a specimen holder and at the other to a myograph attached to a Lectromed Multitrace 2 isometric force displacement transducer/amplifier. The

tissue was suspended vertically in a 30-ml conventional organ bath containing Krebs solution (37°C) administered continuously with an O<sub>2</sub>/CO<sub>2</sub> (95:5) gas mixture. Recordings of uterine contractions were displayed on an LC 475 Macintosh computer screen connected in parallel to a MacLab/2e instrument and to the transducer. All strips used were put under a small amount of resting tension and experiments were started once stable rhythmic spontaneous contractions had developed. Control recordings of the mean amplitude and frequency of contractions were made for 5 min and compared with the mean amplitude and frequency of contractions after exposure to 0.04–0.36 mg/ml of the isolated compound (DSS). The percentage changes were calculated. A dose–response curve was constructed and evaluated alongside similar contractions induced by carbachol (0.3–9.6 μmol). The effects of atropine sulphate (0.69–2.07 μmol) on acetylcholine (1.82 μmol)-induced contractions and on the spasmogenic activity of DSS were also investigated. The concentration of test substances given in the text are all final nutrient bath concentrations.

### 3. Results and discussion

The saponin (DSS) isolated from the 70% EtOH extract of *D. saxatilis* root displayed concentration-dependent contractions of the uterine muscle preparations from the rat. Administration of 0.04 mg/ml of DSS was sufficient to initiate forceful contractions of a quiescent uterine muscle strip, whereas the equivalent concentration of the crude aqueous ethanolic extract was without effect (data not shown). Concentrations in the range 0.08–0.36 mg/ml produced transient single contractions with a progressive increase in the amplitude of contractions and

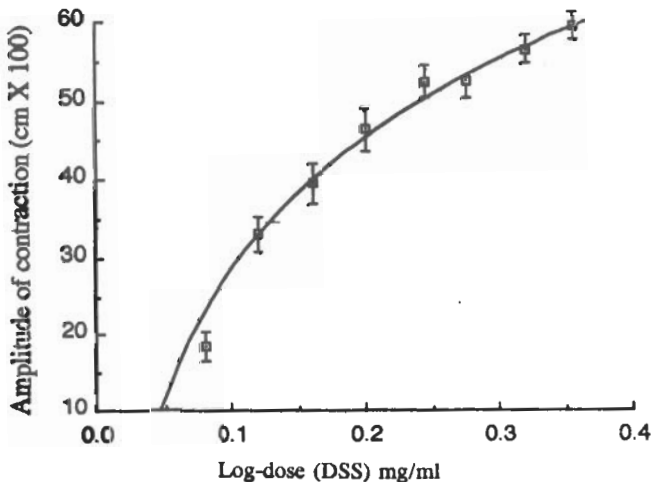


Fig. 1. Log–dose (mg/ml) response curve of saponin from *Dalbergia saxatilis* root extract (DSS) on uterine muscle contraction.

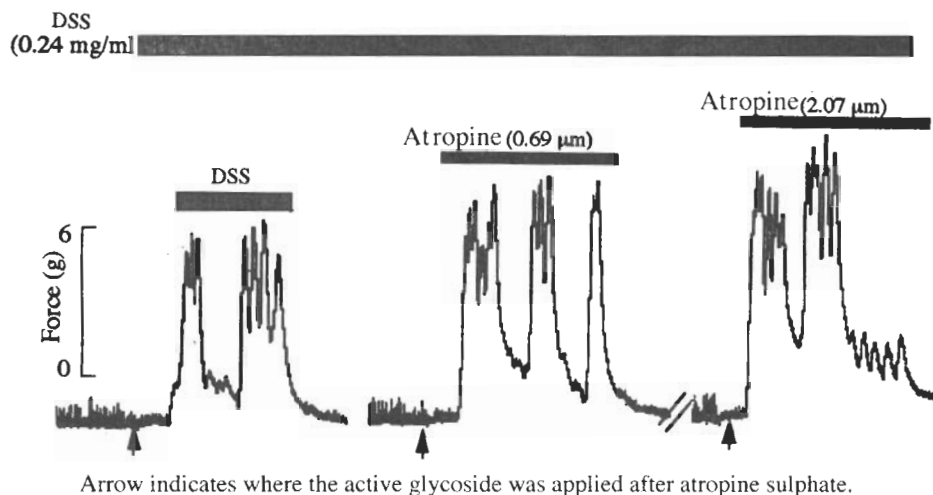


Fig. 2. Effect of atropine sulphate (0.69–2.07  $\mu\text{mol}$ ) on uterine muscle responses to saponin from *D. saxatilis* root extract (DSS).

frequency of spikes within each burst. The  $\text{ED}_{50}$  was 0.13 mg/ml (Fig. 1). Carbachol also caused a dose-dependent increase in uterine muscle contraction ( $\text{ED}_{50} = 2.31 \mu\text{mol}$ ; lowest active concentration: 0.30  $\mu\text{mol}$ ).

Application of acetylcholine (1.82  $\mu\text{mol}$ ) evoked a strong myometrial contraction which was completely abolished by atropine sulphate (0.69  $\mu\text{mol}$ ). In contrast, uterine muscle responses to a submaximal concentration of DSS (0.24 mg/ml) remained unchanged (Fig. 2) in the presence of varying concentrations of atropine sulphate (0.69–2.07  $\mu\text{mol}$ ). This suggests that tissue responses to DSS were mediated by mechanisms other than activation of myometrial cell membrane cholinceptors. Work is in progress to elucidate the structure and the mechanism(s) of action of this active glycoside.

### Acknowledgements

The authors wish to thank the World Bank and the National Universities Commission in Nigeria for financial support.

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