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Inhibitory role of *Dalbergia saxatilis* on β-adrenergic actions of salbutamol in isolated rat uterine strips

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Abstract

Objective: To evaluate the biological activity of aqueous root extract of *Dalbergia saxatilis* on isolated uterine muscle strips of the rat. Materials and methods: The plant material was subjected to cold extraction, initially in petroleum ether (60°-80°C) and subsequently in water. The dried aqueous extract was redissolved in water and tested on isolated uterine muscle strips of the rat mounted in a thermostatically controlled organ bath (37°C) containing physiological solution. Results : The extract (0.58-2.32 mg/ml) had no effect on spontaneous or carbachol (1.97 µmol) induced myometrial contractions but potentiated contractions initiated by phenylephrine (3.5 µmol). Salbutamol (0.15 µmol) abolished both spontaneous contractions and uterine motility stimulated by carbachol (1.97 µmol). These inhibitory effects of salbutamol on contractility were reversed by the extract (1.16 mg/ml). Conclusion : The aqueous root extract of *D. saxatilis* contains biologically active principle that acted as a competitive inhibitor of the β_2 - selective adrenoreceptor agonist and enhanced α - adrenoreceptor function.

Key words: *Dalbergia saxatilis*, uterine muscle, contractility, adrenoreceptor.

1. Introduction

Dalbergia saxatilis, a member of the subfamily Leguminosae, is a local African shrub noted for producing termite resistant and durable ornamental wood. Decoction from the root is believed to be a cure for topical skin lesions, small pox and toothache by the natives in southern Nigeria [1] while other parts of the plant have been widely reported to contain other biologically active phytochemicals [2]. The spasmogenic activity of the aqueous ethanolic root extract of the plant on the myometrium and the partial chemical characterization of the active contractile principle have been described previously [3.4]. The effect of aqueous root extract of this medicinal plant on *in vitro* myometrial contractions is reported in this communication.

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2. Materials and methods

2.1 Plant materials

The plant was collected from Umuahia, Abia state, Nigeria in June, 1994 and was certified as authentic *Dalbergia saxatilis* by Mr. A.O. Ozioko of the Department of Botany, University of Nigeria, Nsukka where voucher herbarium specimen has been deposited.

2.2 Extraction

The air-dried root was pulverised in a size 8" laboratory hammer mill with a sieve diameter of 1 mm. About 290 g of the dry shavings was subjected to cold extraction initially with 2.6 l of petroleum ether (60°-80°C) for 48 h, and subsequently with water (2 liters) with shaking for 72 h. The aqueous extract was concentrated and evaporated to dryness in petroleum ether (60°-80°C). The yield was 11.73 g (4.04%). The dry extract was reconstituted in water and tested for biological activity using isolated uterine muscle strips of the rat.

2.3 Drugs and chemicals

Carbachol (May & Baker, England); phenylephrine (Boots Pharmaceutical, England); tolazoline, atropine sulphate, acetylcholine, EGTA, (Sigma, U.S.A.); propranolol, stilboestrol (Antigen Pharmaceuticals, Ireland); salbutamol (Allen & Hanburys, England).

2.4 In-vitro biological assay

Non-pregnant female Wistar rats weighing 200-250 g were used for the in-vitro bioassay experiments. Twenty four hours prior to the experiments, the rats were given 0.1 mg/kg stilboestrol in paraffin oil subcutaneously. The animals were killed by stunning and decapitation and the uterine horns exteriorized and trimmed free of fat.

For isotonic contraction studies, about 12 mm segment was removed and attached by ligatures at one end to a specimen holder and at the other to a writting lever connected to the smoked drum of a kymograph. The preparation was suspended vertically in a thermostatically controlled organ bath (37°C) containing physiological solution comprising (mmol): KCl (4.7), NaCl (118), $CaCl_2$ (2.5), KH_2PO_4 (1.2), $NaHCO_3$ (2.5), $MgSO_4$ (1.2), and glucose (11), perfused continously with 95% O_2 and 5% CO_2 gas mixtures.

The tissues were allowed to be fully relaxed, with a resting tension of 0.2 g to allow adequate assessment of the effect of the extract on excitatory stimulus induced by standard drugs. The effect of salbutamol was studied in equilibrated tissues undergoing spontaneous contractions either with or without agonists.

After each experiment, the strip was washed 2 to 3 times with the physiological solution and allowed to recover from the effect of each extract or drug administration. In experiments involving Ca^{2+}-free physiological solution, 2.0 mmol EGTA was used to replace $CaCl_2$ in the perfusate.

2.5 Statistics

Values are expressed as means ± S.E.M. Statistical analysis of differences between means was carried out using paired Student's *t* - test. P values less than 0.05 were considered significant.

3. Results

Submaximal concentration of carbachol (1.97 µmol) contracted the resting uterine muscle preparation. The pattern of response was an immediate tonic contraction of long duration (about 30 seconds) that decayed to a new baseline, followed by a series of rhythmic contractions of shorter duration (Fig 1).

The aqueous extract of *D. saxatilis* did not evoke any contractile response on the relaxed tissue and also failed to influence contractions

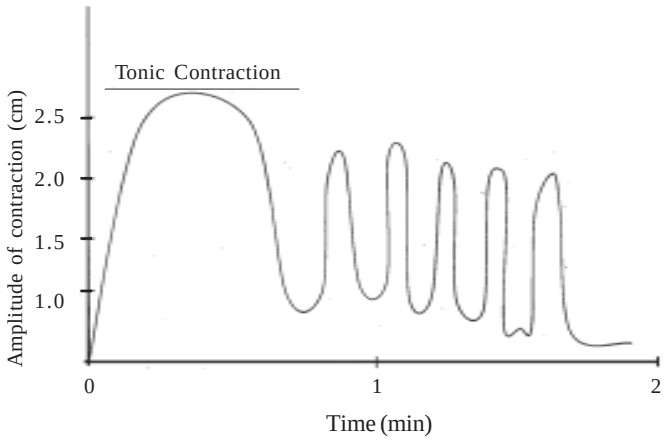

Fig. 1. Effect of carbachol (1.97 µmol) on isolated uterine muscle strips of the rat.

induced by carbachol (3.94 µmol) irrespective of the concentration of the extract used.

However, a graded response to phenylephrine (3.5 µmol) was observed when varying concentrations, in ascending order (0.58-1.16 mg/ml), of the extract was added to the perfusate during 2 min. preceding phenylephrine.

The contraction was transient, of short duration, and was abolished by tolazoline (1.09 µmol) (Fig 2). Propranolol (1 µmol) similarly potentiated the contractile response to phenylephrine (2.45 µmol) by 136%.

The effects of (a) the extract and (b) salbutamol on spontaneous and carbachol induced contractions are presented in Table 1. The extract (0.58-1.16 mg/ml) had no effect on spontaneous uterine motility whereas the β_2-selective adrenoreceptor agonist, salbutamol (0.15 µmol) abolished the spontaneous contractions and contractile responses to carbachol (1.97 µmol). The contractions were restored after repeated washing of the tissue with physiological solution.

However, the extract (1.16 mg/ml) reversed this inhibitory effect of the β_2-selective adrenoreceptor agonist on carbachol (0.4 µmol) induced myometrial contractions. Although the amplitude of contraction was not fully restored,

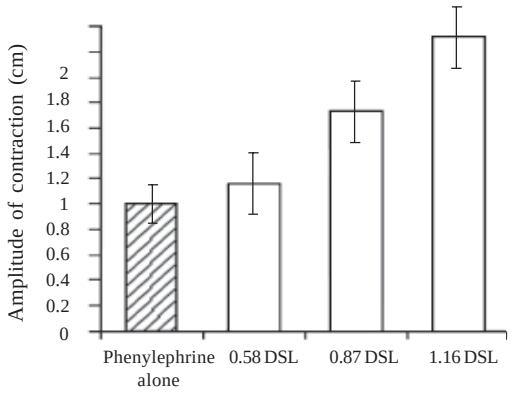

Figure 2. Effect of graded concentrations (0.58 - 1.16 mg/ml) of aqueous extract of *D.saxatilis* (DSL) on uterine muscle responses to phenylephrine (3.5 µmol). Results are the mean ± standard error of the mean (SEM) of 5 replicates. Shaded bar represents effect of phenylephrine (3.5 µmol) alone.

Table 1.
Effect of aqueous extract of *D. saxatilis* and salbutamol (9.15 µmol) on spontaneous and carbachol (1.97 µmol) induced myometrial contractions. Values are expressed as mean ± standard error of the mean (n=6)

Drugs/Extract	**Uterine muscle responses (cm)**
1. Control contractions	0.95 ± 0.24
2. Extract (0.58 - 1.16 mg/ml)	0.93 ± 0.23
3. Salbutamol	No response
4. Carbachol	2.48 ± 0.29
5. Salbutamol + carbachol	No response
6. Extract (1.16 mg/ml) + salbutamol + carbachol	1.80 ± 0.78*

*P>0.05 when compared with carbachol.

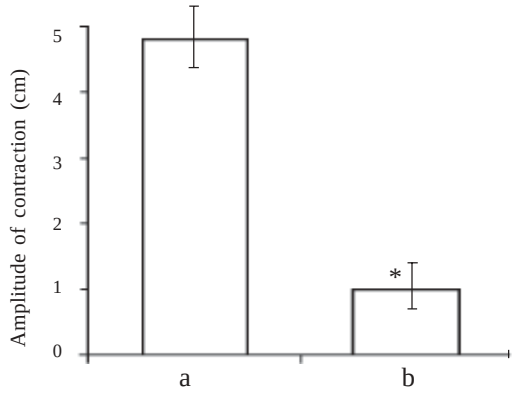

Figure 3. The effect of acetylcholine (3.2 µmol) on uterine force in the (a) presence, and (b) absence of extracellular calcium.(n=5). *P<0.05.

the difference, when compared with carbachol alone was not significant (P>0.05). The effect of salbutamol on spontaneously contracting tissue was similarly reversed by the extract (result not shown).

Acetylcholine (3.2 µmol) contracted the tissue maximally in Ca^{2+}-containing physiological solution. The contraction was phasic and of long duration but was replaced by a single transient contraction of small amplitude (P<0.05) in Ca^{2+}-free medium containing EGTA (Fig 3).

4. Discussion

As with other smooth muscle preparations, carbachol caused contraction of the isolated uterine smooth muscle of the rat (Fig 1). The mechanism of contraction appears to be mediated via increases in the cytoplasmic phosphatidylinositide 4,5-bisphosphate (PiP_2) turnover and the generation of inositol 1, 4, 5-trisphosphate (iP_3) [5, 6]. IP3 interacts with the receptors of the sarcoplasmic reticulum, leading to the release of Ca^{2+} from the intracellular storage site which is critical for sustained myometrial contraction [5,7]. That this contractile activity is dependent on extracellular Ca^{2+} has been reported by other investigators [8, 9, 10] and supported by the results of the present study in which acetylcholine was able to elicit only single transient contraction of lower amplitude in Ca^{2+}-free physiological solution (Fig 3).

In the present study, aqueous extract of *D. saxatilis* alone did not elicit any excitatory or inhibitory effect on the uterine muscle preparation neither did it alter contractions stimulated by the cholinergic receptor agonist, carbachol. However, the selective stimulation of the uterine β_2-adrenoreceptors by salbutamol and the accompanying inhibitory effect on contractility was reversed by the extract (Table 1).

In addition, the extract was able to potentiate contractant signals initiated by phenylephrine, an α_1-adrenoreceptor agonist, in conformity with the known role of other β-adrenoreceptor blockers on uterine muscle function [11,12]. It is known that although the mammalian uterine muscle contains both α and β adrenoreceptors, it is the stimulation of the β_2-receptor subtype that produces relaxation [13].

G-protein coupled receptors is thought to couple β-adrenergic agonists to the heterotrimeric complex $G_{\alpha s}$ [14,15] which activates all isoforms of membrane bound adenylate cyclase to produce elevation of cytoplasmic cAMP content and uterine muscle relaxation [16]. The extract therefore acted as a competitive inhibitor of the β_2-adrenoreceptor function. This represents a novel biological activity and may provide further justification for the known use of the extract in the management of dystocia and related postpuerpural conditions by the traditional healers in southern Nigeria.

Although the mechanism of this β-adrenoreceptor inhibition is unknown, it is proposed that this may involve conformational changes in the receptors which may alter the

kinetics of ion fluxes across the cell membrane, culminating in increases in the sensitivity of the contractile proteins to excitatory stimulus. The nature of such molecular alterations and the role of other mediators in affecting them remain to be explored.

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