

OXYTOMIC PROPERTIES OF THE AQUEOUS EXTRACT OF *GLOBIMETULA BRAUNII* (LORANTHACEAE)

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ABSTRACT

The leaves of *Globimetula braunii* are used in ethnomedicine to augment labour. The crude aqueous extract of the plant was tested on isolated rat uteri.

The agonistic activity of the aqueous extract was compared with that of a standard uterine stimulant oxytocin and uterine smooth muscle antagonists atropine and salbutamol. The extract was found to exhibit oxytomic activity on the uterine smooth muscle which was less potent than that of oxytocin. The force of contraction (3.60 ± 0.25 g) elicited by the highest concentration of the extract (640 mg/ml) was similar to that of 2.7×10^{-4} mg/ml of oxytocin (3.61 ± 0.17 g). Oxytocin was also observed to significantly ($p < 0.05$) enhance the uterine contractile effect of the extract. The oxytomic effects of the extract was significantly ($p < 0.05$) inhibited by both atropine and salbutamol. The oxytomic effects indicate the presence of active principle(s) which would explain the use of the leaves of *Globimetula braunii* to "hasten delivery" in traditional medicine practice.

Keywords: *Globimetula braunii*; isolated rat uteri; oxytomic effects.

INTRODUCTION

Globimetula braunii (Engler) Van Tieghem (Loranthaceae) is a semi-parasitic shrub on trees with woody stem. The leaves are simple, leathery and evergreen. They are attached to the host by woody suckers often regarded as adventitious roots. It is distributed mainly in the tropics, but also found in the temperate regions (Shahina-Ghazanfar, 1989). The plant is a common sight in many parts of West Africa, particularly in plantation of cocoa (*Theobroma cacao*) and kola (*Cola acuminata*) where wide branches are often covered with this pest (Irvine, 1958).

The plant represents a family of plants commonly called mistletoe that possesses several local uses. Other members of the mistletoe family with reported biological activities include the European mistletoe (*Viscum album*) and *Psittacanthus calyculatus*. (Gray and Flatt, 1999 and Ohiri *et al.*, 2003, Rodriguez-Cruz, 2003). The leaves of the mistletoe (*Globimetula braunii*) are boiled as soup and used in folk medicine for the treatment of a variety of ailments including diarrhoea, hypertension, diabetes and to assist women who are about to deliver or have just put to birth (Elizabeth Ajayi, Personal communication). Although there is a paucity of information on the biological activities of this species, the hypoglycaemic property of normal saline leaf extract of *Globimetula braunii* in alloxanized diabetic albino rats has been reported (Olagunju *et al.*, 1999).

The present study examined the uterine activity of the

water extract of the leaves of the mistletoe *Globimetula braunii* on the isolated uterus of rats.

MATERIALS AND METHODS

Plant material

The leaves of *Globimetula braunii* parasitic on *Citrus sinensis* were collected in June 2006, at the fields of the University of Benin, Benin City, Nigeria. The plant was identified by Dr.E.I. Aigbokhan of the Department of Botany, University of Benin and authenticated at the Forestry Research Institute of Nigeria, Ibadan, where a herbarium voucher specimen (FHI No.107155) was deposited. The plant material was air dried on the laboratory bench for 5 days and then ground to powder using an electric mill. The powder was kept in an air-tight container, until ready for use for both the phytochemical and pharmacological studies.

Preparation of the extract

250 g of the powdered leaves of the plant material was boiled with 2400 ml of distilled water for 30 minutes. The extract was filtered and concentrated to dryness over a water bath to yield 52.50 g (21 % w/w) of the crude water extract. The residues were re-dissolved in distilled water to the required concentrations.

Phytochemical screening

Preliminary qualitative phytochemical tests were carried out on the powdered plant material employing standard phytochemical procedures (Evans, 2002; Sofowora, 1982).

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Animals

Female non-pregnant Wistar albino rats (200-260 g) bred in the animal house of the Department of Pharmacology, Faculty of Pharmacy, University of Benin, Benin City, Nigeria were used for the test. The animals were kept in multiple cages at room temperature and maintained on standard animal pellets (Pfizer, Nigeria) and water *ad libitum* for 5 days before the start of the experiment.

Drugs

The drugs used were Oxytocin (Rotex Medical Germany), Salbutamol (Glaxo Smithkline), and Diethylstilboestrol (Sigma). Atropine sulphate (BDH).

Uterine muscle experiment

Female non-pregnant wistar albino rats (200-260 g) were injected with 0.2 mg/kg diethylstilboesterol 2 days prior to the start of the experiments in order to induce oestrus in the uterus. The rats were killed by cervical dislocation. The uterus was isolated and longitudinal strips (2 cm) of rat uterine smooth muscle was threaded and mounted up in a 50 ml organ bath containing aerated De jalon's solution (composition NaCl 9 g/L NaHCO₃ 0.5 g/L, D-glucose 0.5 g/L, KCL 0.42 g/L, CaCl₂.2H₂O 0.08 g/L maintained at a temperature of 37°C. It was aerated with 95 % O₂, 5 % CO₂. One end of the thread was attached to a tissue holder and the other end to a smooth muscle transducer connected to a unirecorder model 7050 (Ugo Basil, Italy). The transducer was previously calibrated to establish a relationship between the force applied to the transducer and the gauge deflection with a 1 g corresponding weight. The preparation was allowed to stabilize for 30 min. A 5 min dose cycle was used consisting of 1 min contact time, 1 min for rinsing and 3 min recovery time. All drugs were applied in a 50 ml organ bath (Uguru *et al.*, 1995).

Direct effects of oxytocin and the aqueous extract of *Globimetula braunii* on the isolated uterus

Oxytocin 1.3×10^{-4} - 1.1×10^{-3} mg/ml were added to the organ bath. Then the aqueous extract of *Globimetula braunii* 40 mg/ml - 640 mg/ml were added to the organ bath until a muscle response (increase or reduction in baseline contractions) was observed, in order to determine if the plant had a direct effect on uterine muscle contraction.

Effects of the aqueous extract of *Globimetula braunii* and reference drug (salbutamol) on the responses of the agonist (oxytocin) on the uterus

- (i) Responses to different concentrations i.e. 1.3×10^{-4} - 1.1×10^{-3} mg/ml of oxytocin were obtained. This was repeated in the presence of a submaximal dose (80 mg/ml) of the aqueous extract of *Globimetula braunii*.
- (ii) The effect of the aqueous extract of *Globimetula braunii* on oxytocin-induced contractions was also

compared with the effect of salbutamol on oxytocin-induced contractions.

The inhibitory effects of salbutamol and atropine on the contractile effects induced by the aqueous extract of *Globimetula braunii*

Responses to different concentrations i.e. 40 mg/ml -640 mg/ml of the aqueous extract of *Globimetula braunii* were obtained. This was repeated after pretreatment with 5µg/ml of salbutamol. The same procedure was repeated with 5µg/ml of atropine.

STATISTICAL ANALYSIS

Where applicable, data obtained were analysed statistically by means of Student's t-test and the analysis of variance (ANOVA) using Graph pad instant version 2.05a. The level of significance was $p < 0.05$.

RESULTS

Phytochemical tests

Preliminary phytochemical tests showed the presence of flavonoids, tannins and saponin glycosides as the chemical class of compounds present in the crude extract (table 1).

Table 1: Phytochemical analysis of leaves of *Globimetula braunii*

Components	<i>Globimetula braunii</i> (Leaves)
Flavonoids	+++
Saponins	+++
Tannins	++
Alkaloids	-
Anthraquinone glycosides	-
Cardiac glycosides	-
Cyanogenic glycosides	-

Parenthesis = Plant part
 +++ = Appreciable amount
 ++ = Moderate amount
 + = Trace amount
 - = Completely absent

Uterine muscle experiment

In the test for uterine activity both oxytocin and the aqueous extracts of *Globimetula braunii* induced a concentration dependent oxytocic effect on the isolated rat uterus. Oxytocin 61.3×10^{-4} mg/ml produced a force of contraction of 3.0 ± 0.13 g. The maximum force of contraction of 3.80 ± 0.22 g was caused by 1.1×10^{-3} mg/ml of oxytocin.

Addition of varying concentrations of the aqueous extract of *Globimetula braunii* caused a concentration dependent increase in contractions, the highest effect was obtained

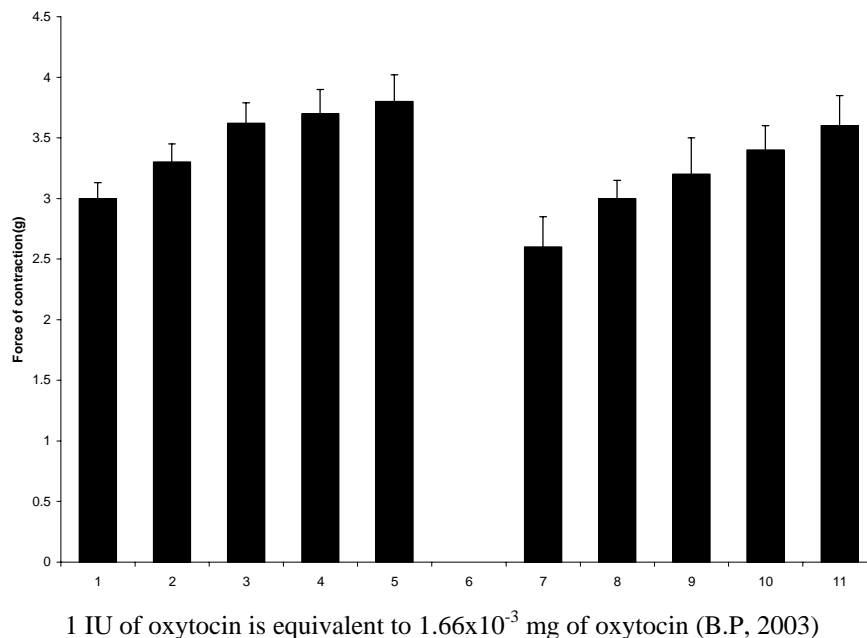


Fig. 1: The uterotonic effects of oxytocin and the aqueous extract of *Globimetula braunii*.

Key:	A-Oxytocin	6.6×10^{-5} mg/ml	F-Extract	40 mg/ml
	B-Oxytocin	1.3×10^{-4} mg/ml	G-Extract	80 mg/ml
	C-Oxytocin	2.7×10^{-4} mg/ml	H-Extract	160 mg/ml
	D-Oxytocin	5.3×10^{-4} mg/ml	I-Extract	320 mg/ml
	E-Oxytocin	1.1×10^{-3} mg/ml	J-Extract	640 mg/ml

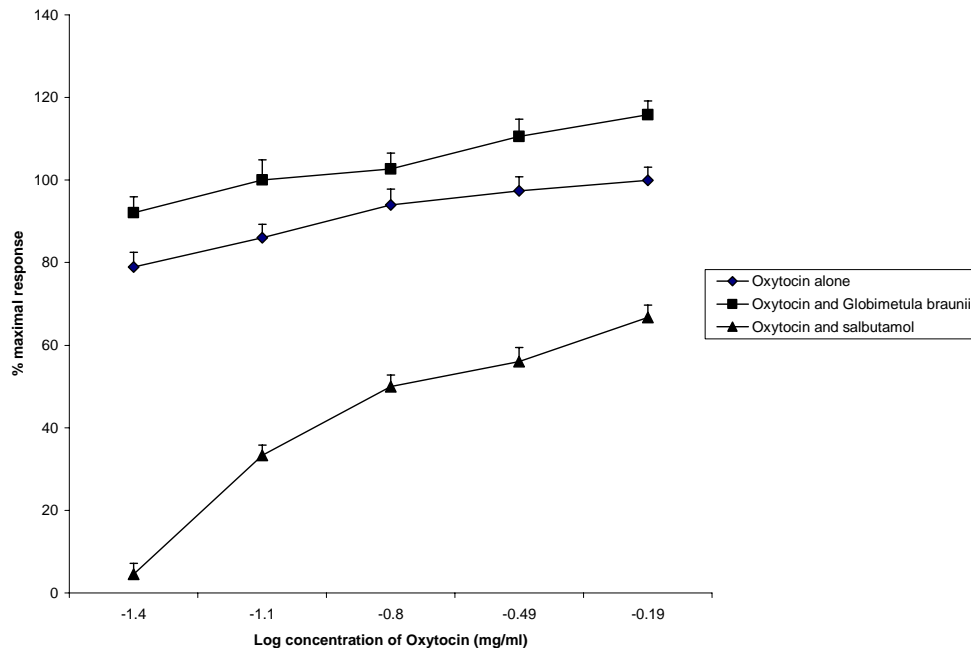


Fig. 2: Log. Concentration response curve for oxytocin alone and after pretreatment with the aqueous extract of *Globimetula braunii* and salbutamol.

with 640 mg/ml of the aqueous extract (fig. 1). The force of contraction (3.60 ± 0.25 g) elicited by the highest concentration of the extract (640 mg/ml) was similar to that of 2.7×10^{-4} mg/ml of oxytocin (3.61 ± 0.17 g).

Addition of increasing concentrations of oxytocin in the presence of 80 mg/ml of the aqueous extract of *Globimetula braunii* resulted in a significant ($p < 0.05$) parallel shift to the left of the log concentration-response

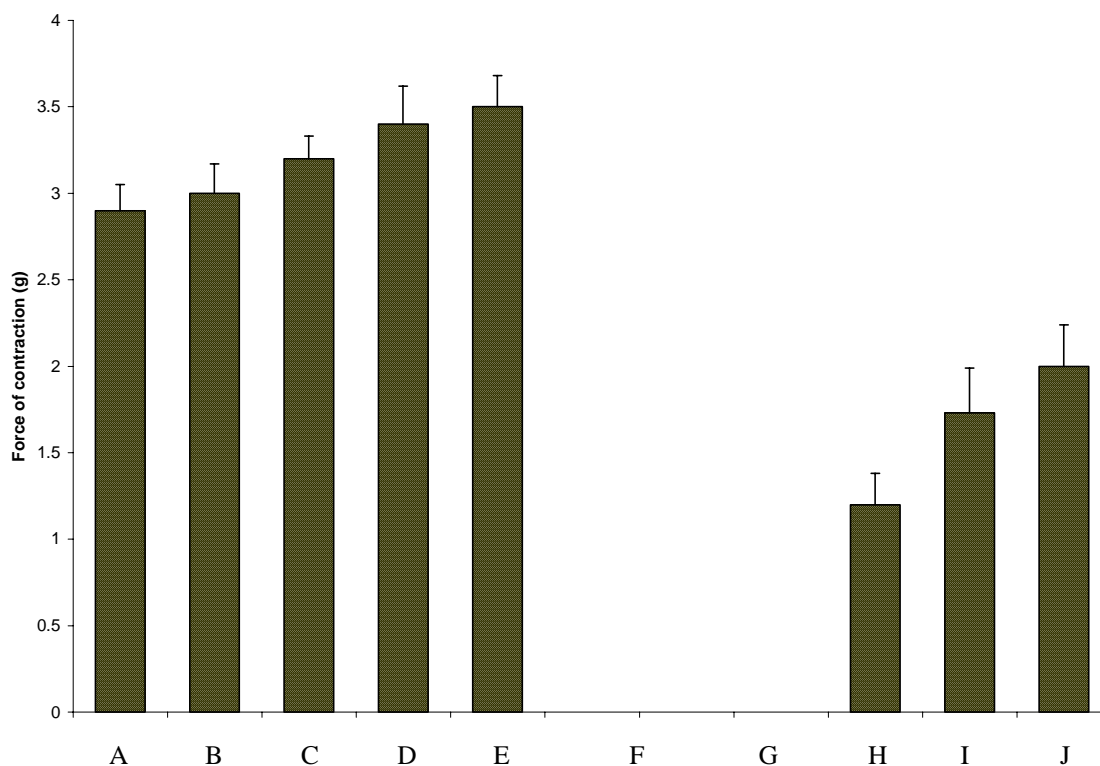


Fig. 3: Inhibitory effects of salbutamol on the contractile effects of the aqueous extract of *Globimetula braunii*.

Key: A-Extract 40 mg/ml
 B-Extract 80 mg/ml
 C-Extract 160 mg/ml
 D-Extract 320 mg/ml
 E-Extract 640 mg/ml
 F-Extract 40 mg/ml and salbutamol 5 µg/ml
 G-Extract 80 mg/ml and salbutamol 5 µg/ml
 H-Extract 160 mg/ml and salbutamol 5 µg/ml
 I-Extract 320 mg/ml and salbutamol 5 µg/ml
 J-Extract 640 mg/ml and salbutamol 5 µg/ml

curve for oxytocin alone and an elevation of the maximal response (fig. 2).

Addition of 40 mg/ml and 80 mg/ml of the aqueous extract of *Globimetula braunii* after pretreatment with 5 µg/ml of salbutamol caused the contractile effects of aqueous extract of *Globimetula braunii* to be the abolished completely (fig. 3).

5 µg/ml of salbutamol reduced the contractile effects of the aqueous extract of *Globimetula braunii* from 3.2 ± 0.13 g and 3.4 ± 0.22 g (elicited by 160 mg/ml and 320 mg/ml of the aqueous extract) to 1.2 ± 0.25 g and 1.7 ± 0.20 g respectively i.e. 62.5 % and 50.0 % inhibition. 5 µg/ml of salbutamol also caused a 42.86 % inhibition of contractions elicited by the highest concentration (640 mg/ml) of the extract. The percentage inhibition however reduced with increase in the concentration of the extract (fig. 3).

Contractions elicited by the addition of 40 mg/ml and 80 mg/ml of the water extract were abolished completely by 5 µg/ml of atropine. 5 µg/ml of atropine also inhibited the contractile effect of 160 mg/ml and 320 mg/ml of the aqueous extract of *Globimetula braunii* by 54.29% and

28.6 % respectively. The maximal response was however retained (fig. 4).

DISCUSSION AND CONCLUSION

In the study of the uterine effects of *Globimetula braunii*, both oxytocin and the water extract showed a concentration dependent increase in the spontaneous contraction of the isolated rat uteri. Polyphenolic compounds such as the flavonoids rutin and epicatechin have been isolated from *Muehlenbeckia hastulata* (Polygonaceae), a plant with potent oxytocic activity (Silvia *et al.*,2002).

The uterotonic effect caused by the pure compound oxytocin (the standard agonist) was more potent than that of the crude water extract of *Globimetula braunii*. This may be due to the fact that oxytocin, the most potent of the endogenous uterotonic agents has a dual action in the uterus. It acts on one subtype of oxytocin receptors to cause contraction of myometrial cells and on another subtype of receptor to promote synthesis and release of prostaglandins (Chan *et al.*, 1993). Further more oxytocin is a pure compound while the extract is crude.

The response of the tissue to increasing concentrations of oxytocin in the presence of 80 mg/ml the crude extract was higher than the response to oxytocin alone indicating that the crude extract has a potentiation effect. This was supported by the fact that the crude extract had a direct effect of on the rat uterus which was agonistic.

The uterotonic effect of the crude extract was inhibited by both salbutamol and atropine. Both alpha (excitatory) and beta (inhibitory) receptors are demonstrable in the myometrium of mammals (Rall and Schleifer, 1985) Goodman and Gilman, 1985). The standard β_2 adrenergic agonist salbutamol inhibited the uterotonic effect of the crude extract in a more pronounced fashion than atropine and it depressed the maximal response. This may be a physiological antagonism. Atropine inhibited the contractile effect of the crude extract but maintained the maximal response which is typical of competitive antagonism. This inhibition of the contractile effects by atropine- a non specific muscarinic antagonist suggests that the active principles of the extract may stimulate the muscarinic receptors. This effect is similar to that of some crude plant extracts e.g. the extracts from the leaves of *Agapanthus africanus* and *Monechma ciliatum* whose contractile effects have been shown to be significantly inhibited by the muscarinic antagonist atropine (Veale *et al.*, 1999; Uguru *et al.*, 1998).

This study has shown the oxytocic effect of the water extract of *Globimetula braunii* on the isolated rat uterus and that the active principles may possess agonistic action on the muscarinic receptors. The oxytocic effect would explain the use of this plant to "hasten delivery" in traditional medicine practice.

The determination of the oxytocic activity of the crude extract of plants provides the starting point towards the determination of their potential use in humans to induce uterine contractions. Further study is on to elucidate the exact mechanism of the oxytocic effect and to isolate and characterize the active principles responsible for the effect.

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