

Effect Of Irvingia Gabonensis Leaf Extracts On Non Pregnant Rat Uterus

C Nosiri, I Hussaini, I Abdu-Aguye

Citation

C Nosiri, I Hussaini, I Abdu-Aguye. *Effect Of Irvingia Gabonensis Leaf Extracts On Non Pregnant Rat Uterus*. The Internet Journal of Alternative Medicine. 2009 Volume 8 Number 2.

Abstract

The effects of ethanol and water extracts of the leaves of *Irvingia gabonensis* on isolated non-pregnant rat uterus and ileum have been investigated. The isolated uterine horns and ileum each were mounted into organ baths containing different physiological solution connected to a recording microdynamometer to measure the contractions. The standard drugs Acetylcholine (Ach), Atropine, pirenzepine and verapamil and the extracts were injected into the organ bath to study their pharmacological effects on the uterine smooth muscles. Ethanol extract (25 - 400µg/ml) contracted the rat uterus while the water extract (0.025 – 20mg/ml) had no effect. Both extracts had no observable contractile effect on rat ileum. The ethanol extract was less potent than acetylcholine in contracting the tissue. The response of the uterus to the ethanol extract was reduced by atropine (14nM); Pirenzepine (0.1µM) and completely blocked by Verapamil (0.1µM). This result indicates that the ethanol extract of *Irvingia gabonensis* may produce a Ca⁺⁺ dependent muscarinic receptor – mediated contraction (extracellular calcium dependent) of the non pregnant rat uterus.

INTRODUCTION

Irvingia gabonensis (O'Rorke) baill Var. *excelsa* (Keay, 1989) is a tropical forest tree mostly found in southern Nigeria, Sierra Leone and Equatorial Africa.. It is a species from the family of Irvingiaceae.

The inedible fruit pulp is bitter and acrid although it can be eaten and has a turpentine flavor (Udeala et al, 1980) It acts as a source of human food and commonly known as Ogbono in Igbo land of Nigeria where it is used as a soup thickener. The seeds are rich in oil (54 – 67%) calculated on dry kernel. This is known as "dika" fat, which has been evaluated and used now as a tablet lubricant (Udeala et al, 1980). It has been reported that the seeds reduces fasting blood glucose levels in obese subjects (Ngondi et al, 2005). The leaf extract has also been reported to increase urine output and electrolytes in adult wistar rats (Nosiri et al, 2010). A decoction of the stem bark of *Kigelia africana* and leaves of *Irvingia gabonensis* is used to cure spleen infection (Sofowora 1986). The leaves are used in the treatment of dysentery and wound dressing (Okafor and Okolo, 1974). The aqueous leaf extract of *Irvingia gabonensis* has been found to cause a significant dose-dependent decrease of gastrointestinal motility in mice (Gamaniel, 2004). The stem bark has been reported to have analgesic properties (Okolo et al, 1995).

Preliminary phytochemical screening of the aqueous leaf extract of *Irvingia gabonensis* revealed the presence of Saponins, tannins, phenols and phlobatins. *Irvingia gabonensis* is largely used in both traditional and modern medicine for the treatment of several illnesses (Lowe et al, 2000). In this study the effects of *Irvingia gabonensis* extracts on isolated non-pregnant rat uterus and rat Ileum were examined and the mechanisms of action investigated.

MATERIALS AND METHODS

COLLECTION AND PREPARATION OF PLANT MATERIAL

The leaves of *Irvingia gabonensis* were collected from the Herbarium Keeper of the Forestry Research Institute, Ibadan, Oyo State, Nigeria. A voucher specimen of the plant has been deposited with the Institute's Herbarium under voucher number FHI 103947.

The plant leaves were obtained in large quantities and left to dry at room temperature for two days after which they were dried in an oven at 35 – 40°C for 36 hrs. After that some of the leaves were ground into a coarse powder. The powdered leaves were kept in an air-tight glass container and stored in a dry place. 50g of the powder was extracted using Soxhlet apparatus for continuous extraction. The extracts (ethanol and water) were concentrated and dried under vacuum. The

percent yield was 8.4% for ethanol extract and 0.82% for cold water extract.

EXPERIMENTAL ANIMALS

Adult female wistar rats weighing 200-270g were obtained from the animal house of the department of Pharmacology/ Clinical Pharmacy, Faculty of Pharmaceutical Sciences, Ahmadu Bello University Zaria, Nigeria. They were maintained on Master feeds and water ad libitum. All experimental protocols were approved by the university animal ethic committee.

PREPARATION OF ISOLATED UTERINE HORN

The adult female wistar rats weighing 200-250g were used for the study. They were injected intraperitoneally with diethylstilboesterol (1mg/kg) in order to induce a state of oestrous. This was confirmed by histological examination of the vaginal smear. The rats were killed by a blow on the head and exsanguinated. The abdomen was opened and the uterine horns with attached fat and mesentery were surgically removed from both the right and left sides of the animal and placed in a Petri dish containing De Jallon's solution of the following composition in millimole (mM): NaCl, 15.4; KCl, 5.63; CaCl₂, 0.41; NaHCO₃, 5.95 and glucose 2.25.

The fat and mesentery were detached from the uterus and the latter was cut into left and right horns. Each uterine horn was mounted into an organ bath (20ml) under initial tension of 2g and contractions were measured isometrically using transducer (Ugo Basile) connected to a recording microdynamometer (7050). The preparation was allowed to equilibrate for 1hr before drugs were added.

EFFECT OF EXTRACT ON THE RAT UTERINE SMOOTH MUSCLE

Standard drugs (Ach, (5µg/ml), atropine (14nM), pirenzepine (0.1µM), and verapamil (0.1µM) and extracts were injected into the organ bath with syringe and needle to study their pharmacological effects. After the administration of each drug and/or extract, the tissues were washed three times, allowed to rest for two minutes before the addition of another drug or extract. To investigate the mechanism involved in the contraction of the uterus induced by the ethanol extract, pirenzepine an M₁ – muscarinic receptor antagonist, atropine (a non – specific muscarinic receptor antagonist) and verapamil (calcium antagonist) were used in the study. The latter was used to examine the involvement of extracellular calcium, added to the De Jallon solution and the

tissue challenged with ethanol extract and acetylcholine.

TEST ON RAT ILEUM

The ileum of both male and female wistar rats was set up the same way except that the physiological solution used was Tyrode solution of the following composition in millimole (mM): NaCl, 137, KCl, 2.7; CaCl₂, 1.8; MgCl₂, 1.0; NaHCO₃, 11.0 and glucose 5.6. The rats were starved for 24hrs before the experiment commenced.

STATISTICAL ANALYSIS

All values were expressed as mean ±SEM and results analysed using student's t-test. P values less than 0.5% were taken to be statistically significant.

RESULTS

EFFECT OF ETHANOL EXTRACT AND ACH ON THE ISOLATED RAT UTERINE SMOOTH MUSCLE

Fig. 1. Effect of Ach and Ethanol Extract of *Irvingia gabonensis* on non-pregnant rat uterus. Vertical bars represent Standard error of mean ±SEM

Figure 1

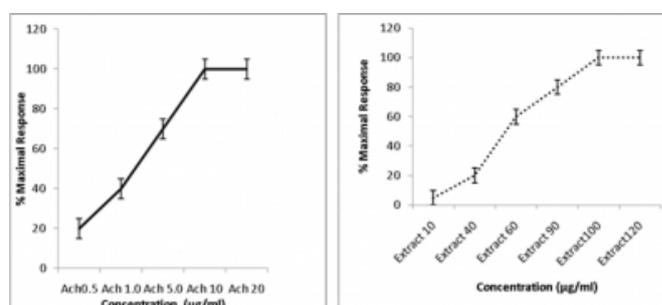


Fig. 1 shows the contractile effects of Acetylcholine (Ach) (0.5 – 20 µg/ml) and ethanol extract of *Irvingia gabonensis* (25 - 100µg/ml) on the rat uterus. The contraction exhibited by the ethanol extract is similar to the agonistic effect of Ach on the rat uterine smooth muscle. Maximum contraction was obtained with 100µg/ml of the extract. The cold water extract showed no contractile effect on the uterus.

EFFECT OF EXTRACT AND ACH IN THE ABSENCE AND PRESENCE OF PIRENZEPINE, ATROPINE AND VERAPAMIL ON RAT UTERINE MUSCLE

When the contractile effects of Ach and ethanol extract of *I. gabonensis* were challenged in the absence and presence of pirenzepine, atropine and verapamil, there was reduction in their effects. Atropine (14nM), a calcium channel blocker

blocked the effect of the extract on the uterus by 90%.

Figure 2

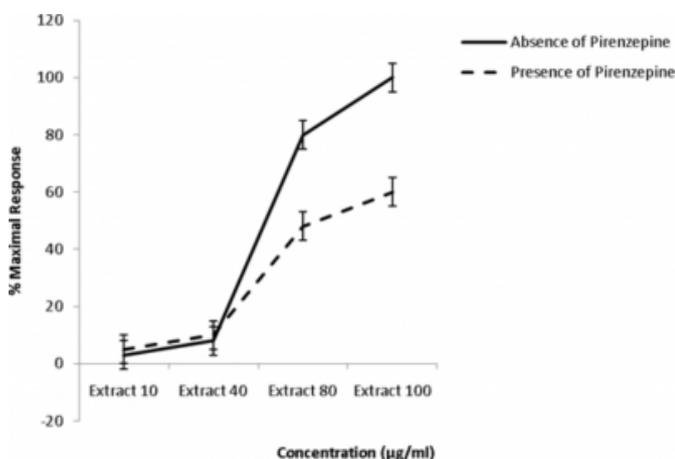


Fig.2 Effect of Ethanol Extract of *Irvingia gabonensis* on the isolated non-pregnant rat uterus in the absence and presence of Pirenzepine(0.1μM). Vertical bars are ±S.E.M n=5

In Fig. 2, Pirenzepine (0.1μM) an M₁-muscarinic receptor antagonist shifted the dose – response curve for the ethanol extract to the right but the maximum response to the extract was reproduced in the presence of this antagonist.

Figure 3

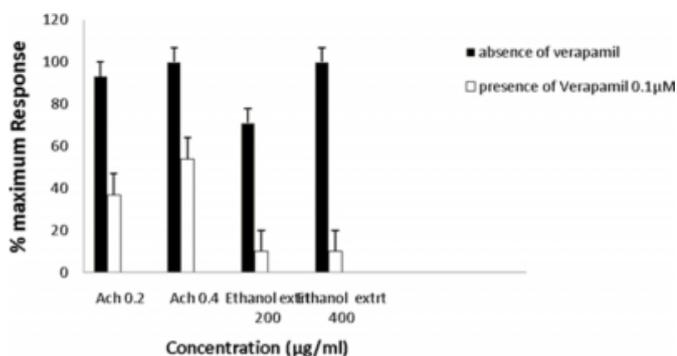


Fig.3 Effect of Ach and ethanol extract of *I. gabonensis* on the isolated non pregnant rat uterus in the absence and presence of verapamil. Vertical bars are ±S.E.M

Fig. 3 shows the effect of verapamil in the absence and presence of verapamil. Verapamil (0.1μM) reduced the contractile effects of both acetylcholine (0.2 and 0.4 μg/ml) and ethanol extract (200 and 400 μg/ml) on the tissue by approximately 46 -56% and 61-90% respectively.

DISCUSSION

Ethanol extract produced a dose – related contraction on the non-pregnant rat uterus and had no effect on rat ileum. Water extract of *Irvingia gabonensis* had no effect on both rat ileum and uterus. Investigating the mechanisms involved in this pharmacological effect induced by the ethanol extract, some standard drugs were employed. Ach, a standard

muscarinic receptor agonist contracted the rat uterus like the agonistic activity of the extract. The antagonism of the extract and acetylcholine-induced contractions of the uterine smooth muscle strips via various antagonists was also investigated in order to find out the muscarinic receptor subtype(s) of uterine smooth muscle. Atropine, a non specific muscarinic receptor antagonist which relaxes smooth muscles (Kurtel et al, 1990, Buch, 2009) reduced this contractile effect of the ethanol extract in the uterus. Challenged by Pirenzepine , a selective M₁ receptor antagonist (Kurtel et al, 1990, Rang et al, 2003) , there was reduction of the agonistic effect of the extract by shifting the dose response curve of the extract to the right. Examining the involvement of extracellular calcium in this contraction, Verapamil, a phenylalkylamine Ca⁺⁺ channel antagonist (Rang et al 2003) blocked the contraction induced by the ethanol extract (200 and 400μg/ml) as well as that of a standard muscarinic-receptor agonist, acetylcholine (0.2 and 0.4 μg/ml) by 61 -90% and 46-56% respectively. These observations suggest that the ethanol extract of *Irvingia gabonensis* may contain a biologically active principle(s) which can exhibit agonistic activity on uterine smooth muscle via the muscarinic receptors since it was antagonised by atropine and pirenzepine or a system coupled to the receptors. This speculated phenomenon may also require the availability of extracellular calcium, since it was inhibited by the presence of verapamil which blocks cellular entry of ca²⁺ through calcium channel.

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Author Information

Chidi Nosiri

Department Of Pharmacology And Clinical Pharmacy, Faculty Of Pharmaceutical Sciences, Ahmadu Bello University

I.M. Hussaini

Department Of Pharmacology And Clinical Pharmacy, Faculty Of Pharmaceutical Sciences, Ahmadu Bello University

I. Abdu-Aguye

Department Of Pharmacology And Clinical Pharmacy, Faculty Of Pharmaceutical Sciences, Ahmadu Bello University