

Full Length Research Paper

Oxytocic and anti-implantation activities of the leaf extracts of *Graptophyllum pictum* (Linn.) Griff. (Acanthaceae)

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This study was aimed at evaluating *Graptophyllum pictum* aqueous extract (GPAE) and ethanol extract (GPEE) *in vitro* for oxytocic and *in vivo* for anti-implantation activities. The oxytocic screening of the extracts was carried out on the isolated strip of gravid rat uterus in mid pregnancy and was compared with the activity of an agonist drug, oxytocin. GPEE exhibited oxytocic activity which is comparable to oxytocin while GPAE was found to reduce the normal contraction of the uterine strip. The anti-implantation investigation was done using three groups of eight week old virgin female Sprague-Dawley albino rats (eight rats/group). A selected dose (400 mg/kg) of GPEE was orally administered to a group of the rats. The same dose of GPAE was similarly administered to another group while the vehicle of administration (distilled water) was similarly administered to the third group as control. All administrations started on day one of pregnancy and were given daily for seven days. The rats were sacrificed on day 10 of pregnancy. Presence of foetus, implantation sites and number of corporal lutea in the autopsied rats were recorded and used to calculate the percentage anti-implantation effect. GPEE, GPAE and distilled water have percentage anti-implantation value of 93.8 ± 9.1 , 16.8 ± 8.5 and 3.9 ± 5.4 , respectively. The results support the use of this plant in folkloric medicine as a delivery aid and also suggest that the plant can be used very early in pregnancy as a contraceptive.

Key words: Anti-implantation, oxytocic, *Graptophyllum pictum*, contraceptive.

INTRODUCTION

Numerous herbs have been reportedly used historically by women to aid child delivery, stimulate menstrual flow or reduce fertility (Bodhankar et al., 1974; Farnsworth et al., 1975). Modern scientific studies in experimental animals have confirmed the effects of some of these herbs in the reproductive system (Prakash et al., 1985; Desta, 1994; Uguru et al., 1998; Badami et al., 2003).

Herbal contraceptives offer alternatives for women who have problems with or lack access to modern contracep-

tives options particularly women living in the rural areas in developing nations with very high population like India, China, Africa (Nigeria) and Bangladesh (World population Data sheet, 2008). Studying the potency and toxicity of local plants that are reputed for birth control in the folkloric medicine of these countries may generate greater confidence in and wider acceptance of herbal contraceptives.

Graptophyllum pictum is commonly called caricature plant or Joseph's coat (due to its bicolour which makes it attractive). Though foreign to Nigeria *G. pictum* grows profusely during the rainy season in the country and can easily be cultivated by vegetative propagation at this period. It is mainly used as ornamental plant to adorn the

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home gardens in Nigeria. Elsewhere it was reportedly used in folkloric medicine as poultice on cuts, wounds and all kinds of swellings and for the treatment of ulcer, abscess, haemorrhoids etc (Perry, 1980; Kasahara and Mangunkawatjia, 1986).

Although some pharmacological studies have been carried out on this plant (Ozaki et al., 1989; Kusumawat et al., 2002), there is no report of study on its effect in the reproductive system. Incidental observation (by this principal author) of safe delivery of a pregnant goat shortly after ingestion of *G. pictum* leaves formed the basis of this research. Also the reported research findings of Elujoba et al. (1985) that some oxytocic agents can as well serve as anti-implantation agent when administered early in pregnancy formed the basis for the anti-implantation study.

MATERIALS AND METHODS

Plant material and preparation of extracts of *G. pictum*

The leaves of *G. pictum* were collected in the early hours of the morning from a residential area in Isolo a suburb of Lagos state, Nigeria, in August 2007. They were identified by Mr. Odewo, Forestry Research Institute of Nigeria (FRIN), Ibadan, Nigeria. A voucher specimen (PCLACAN01) was deposited in the Institute herbarium. The leaves were dried at a temperature of 40°C for 18 h in an oven, 250 g of the coarse powder was extracted in acidic water (pH 4.2) or 70% ethanol (pH 6.0) by heating at 40°C for 30 min. The plant is basic in nature so optimum extraction was achieved in an acidic medium. The extracts were filtered through pre-pleated filter paper (Schleicher and Schuell USA, No 560) and concentrated to dryness at 40°C in a rotary evaporator. Both extracts were then suspended in water and defatted by partitioning with n-hexane, the aqueous residue from each extract was freeze dried and kept in the refrigerator at 4-10°C until needed. The aqueous extract (GPAE) yielded 75.6 g (30.2%) while the ethanol extract (GPÉE) yielded 52.8 g (21.1%).

Animals

Virgin female and proven male Sprague-Dawley rats (weight, 120-130 g) obtained from NAFDAC animal centre (Yaba-Lagos) was used. The animals were kept in a well-ventilated room in the Laboratory Animal Centre of the College of Medicine, University of Lagos, Lagos, Nigeria. They received standard rat chow (Pfizer Feeds Nigeria, Plc) and water *ad libitum*.

Chemicals and reagents

De-Jalons solution (NaCl 9.0 g, 10% KCl-4.2 ml, 1 M CaCl₂-0.27 ml, glucose 0.5 g, NaHCO₃ 0.5 g - all made to 1 liter solution in distilled water), Oxytocin (Sandoz, Brazil).

Oxytocic screening

A female rat in mid-pregnancy (12 days after conception) was

obtained from the animal centre of the College of Medicine, University of Lagos, Nigeria. It was anaesthetized with 20% pentobarbitone (Mayer and Baker Nig. PLC) administered intramuscularly at 0.2 g/kg and the uterus was rapidly removed by midline incision into the lower abdominal cavity. The screening was performed by suspending 3 pieces of 2 mm longitudinal cut segment of the uterus in 3 different organ baths, each containing 20 ml of De-Jalon solution maintained at 37°C. The lower end of the muscle was fixed to a glass capillary tube with continuous supply of oxygenated gas (95% O₂ and 5% CO₂) to keep the muscle alive while the upper end was suspended by a thread attached to a Grass polygraph (model 7D) with Force Transducer which transformed the muscle contraction into a proportional electrical signal. This signal was recorded using an electrically driven Grass polygraph recording chart at a speed of 5 mm/min. Oxytocin was used as the standard drug at 0.1 – 10 i.u/ml and the extracts at 25-80 mg/ml. The oxytocin and the extracts (GPAE and GPÉE) dissolved in distilled water at known concentrations were dispensed into the 3 organ baths respectively using Eppendorf pipettes (Figure 3).

Evaluation of anti-implantation activity

Virgin female rats exhibiting normal oestrus cycle were selected for this study. They were randomly put into 3 groups of 8 members each. The vaginal smear of each member of the group was daily examined for proestrus. Any member in which this was established was then removed from the others and caged overnight with a proven male for mating. The presence of spermatozoa in the vaginal smear on the following day is taken as evidence of mating and the day is recorded as day 1 of pregnancy. An effective dose of 400 mg/kg of GPÉE selected according to Elujoba et al., (1985), an equal dose of GPAE and distilled water (vehicle of administration) were orally administered daily to members of the 3 groups as shown in Table 1 for 7 days starting on day 1 of pregnancy. On day 10 of pregnancy, the animals were laparotomised under light ether anaesthesia and sterile conditions. Both uterine horns were examined for number of implants, aborted implants and corpora lutea; which were recorded.

Data were expressed as a mean ± SD (standard deviation). Statistical analysis was performed with Student's t-test.

Preliminary phytochemical studies

Phytochemical screening was performed on both GPAE and GPÉE for the presence of secondary metabolites using the following test methods; alkaloids -, with Mayer's and Dragendorff's reagents (Farnsworth, 1966; Harborne, 1998), tannins - with 1% gelatine and NaCl solutions; flavonoids-with the use of Mg and HCl (Silva et al., 1993; Houghton and Raman, 1998) and saponin with the ability to produce suds and haemolyse red blood cells (Houghton and Raman, 1998).

RESULTS AND DISCUSSION

The alcoholic extract GPÉE showed agnostic effect which was rapid in onset as shown in Figures 2 and 4; but the aqueous extract GPAE, suppressed the normal uterine contraction (Figure 1). The preliminary Phytochemical screening showed the presence of saponin, tannin and

Table 1. Anti-implantation screening for the 400 mg/kg of both alcoholic and aqueous extracts of *G. pictum*.

Group	Mean wt (g) on day 1 of pregnancy	(F) No of foetus	(A) No. of aborted Implantation sites	(C) Total conception (F+A)	(L) No of Corpora lutea	Mean Corpora lutea	No of death	% Anti-implantation L-C/L x100	Mean % Anti-implantation
GPEE (alcoholic extract)	127.4 ± 2.7	0, 2, 0, 0, 1, 0, 0, 0.	0, 0, 0, 0, 0, 0, 1, 0	0, 2, 0, 0, 1, 0, 1, 0	8, 9, 7, 8, 6, 8, 9, 8.	7.9 ± 1.0	None	100, 78, 100, 100 83, 100, 89, 100	93.8 ± 9.1
GPAE (aqueous extract)	125.8 ± 4.1	6, 6, 8, 5, 7, 6, 8, 6.	1, 0, 0, 3, 0, 0, 0, 0	7, 6, 8, 8, 7, 6, 8, 6	8, 8, 9, 10, 9, 8, 8, 7.	8.4 ± 0.9	None	12.5, 25, 11, 20, 22, 25, 0, 14.3	16.8 ± 8.5
Control (distilled water)	128.0 ± 3.0	10, 9, 9, 8, 9, 10, 8, 9.	0, 0, 0, 0, 0, 0, 0, 0	10, 9, 9, 8, 9, 10, 8, 9	10, 9, 10, 9 9, 10, 8, 10	9.4 ± 0.7	1	0, 0, 10, 11, 0, 0, 0, 10	3.9 ± 5.4

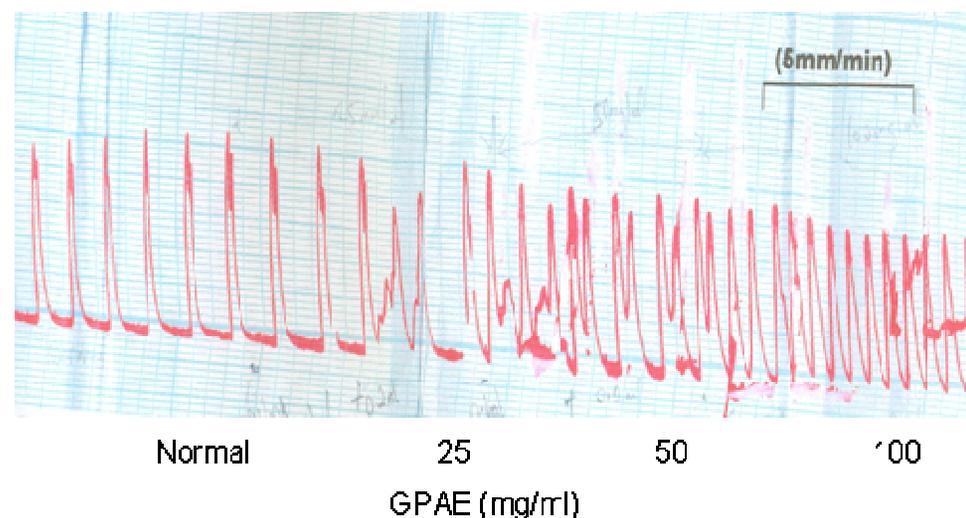


Figure 1. Effect of aqueous extract of *G. pictum* (GPAE) on the gravid rat uterus.

flavonoids in glycosidic forms in both extracts while the water extract showed the presence of alkaloid-like substance in addition.

Clinically, drugs that contract the uterine smooth

muscle are used to induce labour or abortion. Such drugs include oxytocin, ergometrine and quinine (Bowman and Rand, 1980). The oxytocic screening of *G. pictum* showed the aqueous

extract GPAE exhibiting a depressant effect on the normal uterine contraction (Figure 1); while the alcohol extract GPEE exhibited agonistic effect (Figure 2) which was comparable in magni-

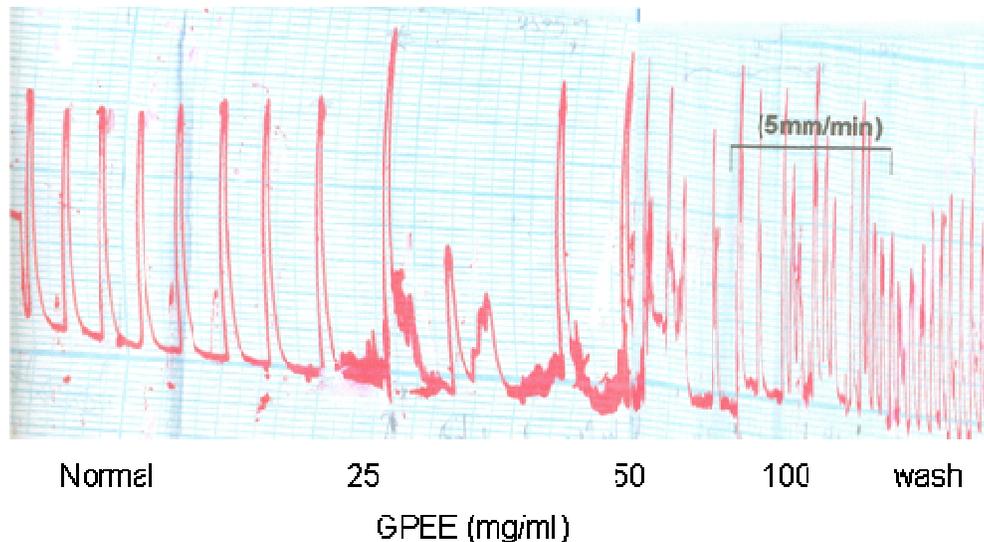


Figure 2. Effect of alcohol extract of *G. pictum* (GPEE) on the gravid rat uterus.

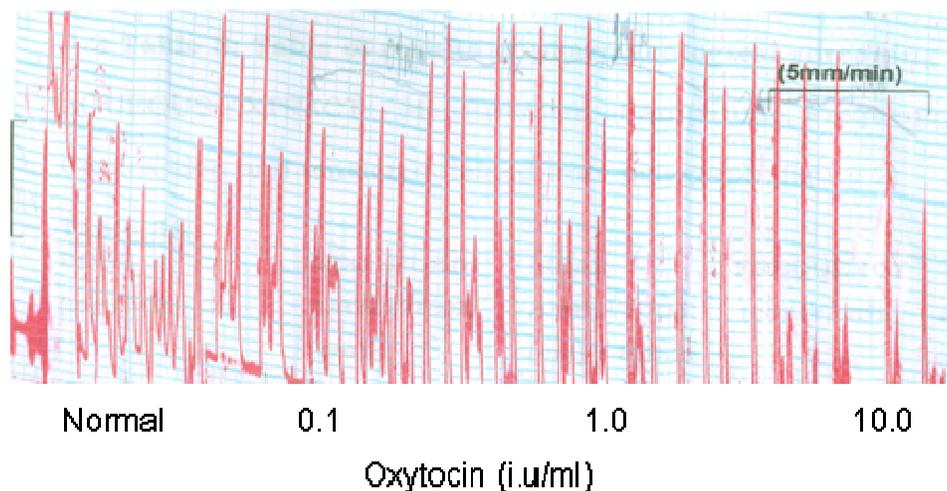


Figure 3. Effect of oxytocin on the gravid rat uterus.

tude with oxytocin. This observed uterus-contracting action of GPEE was fast in onset and could be totally eliminated by washing with the extract-free Dejalon solution. This may suggest the presence of low molecular weight active compound(s) in the extract, which penetrated rapidly to its site of action. While GPEE exhibited a strong and progressive increase in contraction at initial low concentration up to 20 mg/ml (Figure 4), increase in concentration after this, that is, > 20 mg/ml showed a progressive decrease in observed contraction. Whereas the contraction exhibited by oxytocin though not strong at the lower concentration of < 0.1 i.u/ml was stronger after

0.1 i.u/ml with progressive increase in magnitude to 1.0 i.u/ml after which increase in concentration did not produce further increase in magnitude.

The progressive decrease at higher concentration with time in the oxytocic action of GPEE may be due to metabolic changes in the structure of the active ingredients. The chemical constituent of this extract were identified to be mainly glycosides which may quickly be degraded by hydrolysis, the product of which is usually not as water soluble as the un-degraded parent compound.

The *in vivo* anti-implantation study supported the *in*

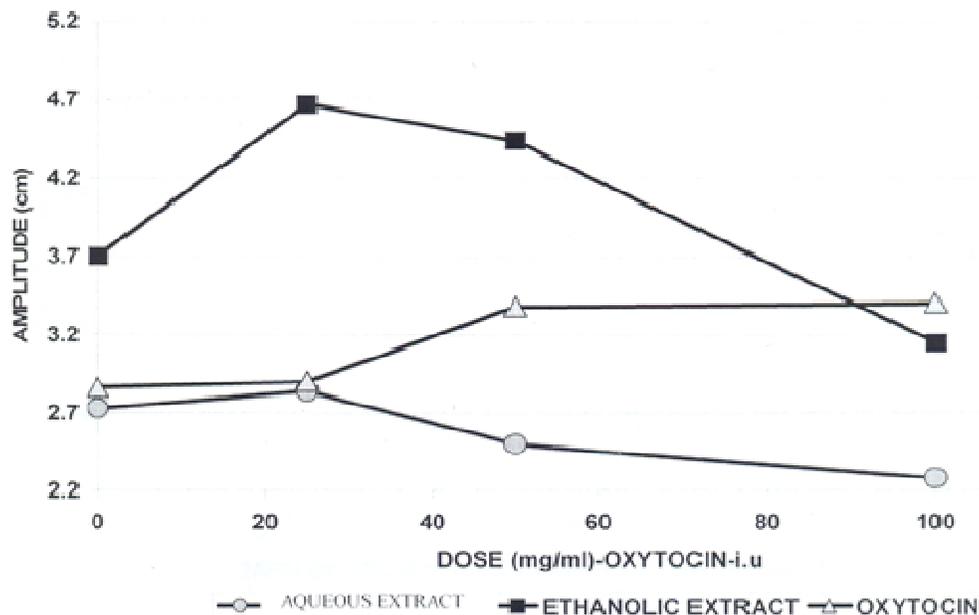


Figure 4. Graph of Amplitude of contraction versus dose (aqueous and alcoholic extracts of *G. pictum* and oxytocin).

vitro oxytocic screening in that the alcohol extract GPEE exhibited high percentage of anti-implantation (93.85%) while the aqueous extract GPAE exhibited very little (16.8%) when compared with the control (3.9%).

The phytochemical screening result showed the two extracts to contain similar constituents except for the prominent presence of alkaloid-like constituent in the aqueous extract which was not detected in the alcohol extract. This alkaloid-like constituent may be responsible for the suppressant effect on the uterine normal contraction and the low anti-implantation activity exhibited by the aqueous extract GPAE.

The statistical evaluation of this work, as recommended by the W.H.O. (1981) indicated that the results obtained from the control animals were significantly different from the anti-implantation effects observed in the treated animals at 99% confidence limit ($p = 0.01$), with a standard deviation of 0.68.

Conclusion

This study has shown the alcoholic extract of *G. pictum* leaf to be a potent uterine stimulant. The result supports the principal author's view that the plant *G. pictum* eaten by the pregnant goat she observed might have aided its delivery. Hence the alcoholic extract of *G. pictum* is here presented as a potent delivery or abortion aid. It could also serve as oral contraceptive through anti-implantation mechanism.

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