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Original Research Article

Effect of Aqueous Extract of *Persea Americana Mill (Lauraceae)* Seeds on Non-Pregnant Rat Uterus

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Abstract

Purpose: To verify the folklore use of *Persea americana* Mill (Lauraceae) for the management and control of preterm labour.
Methods: The contraction of an isolated rat uterus was measured following treatment with acetylcholine (0.006-26.27 mg/ml), oxytocin (0.00067-0.016 iu), ergometrin (0.006-20.0 mg/ml) and potassium chloride (80 mM) in the presence and absence of the extract of *P. americana* (0.83-3.33 mg/ml). Effect of the extract was compared with salbutamol (1 µg/ml) and atropine (1 µg/ml) against oxytocin and acetylcholine induced contractions respectively. Data collected were compared using Student's T-test.
Results: The extract significantly ($p<0.05$) inhibited the contractions produced by oxytocin, acetylcholine, ergometrine, and potassium chloride. Inhibitory effects of the extract was more prominent on acetylcholine induced contractions ($p<0.001$) than on oxytocin induced contractions. On potassium chloride induced contraction, the extract also produced significant ($p<0.05$) reduction in the amplitude of contractions. These inhibitory effects were comparable to those of salbutamol and atropine on oxytocin and acetylcholine induced contractions, respectively.
Conclusion: Aqueous extract of *Persea americana* seeds possesses relaxant effect on the contractility of uterine smooth muscles possibly because of its inhibitory action on extracellular calcium and muscarinic receptors. This justifies the use of the aqueous extract of *Persea americana* seeds for the management of pre-term birth.

Keywords: Uterine contraction, *Persea americana*, Potassium chloride, Oxytocin

Indexing: Index Copernicus, African Index Medicus

Introduction

The use of herbs to treat disease is almost universal among non-industrialized societies and is often affordable than purchasing expensive orthodox pharmaceuticals. Medicinal plants have continued to attract attention in the global search for effective methods of using medicinal plant parts (e.g. seeds, stems, leaves, roots, and barks etc) for treatment of many diseases [1]. World Health Organization estimates that 4 billion people (80 % of the world population) presently use herbal medicines for some aspect of primary health care [2].

Persea americana is known as avocado, alligator pear or butter pear. Its flowers are scented and the green skinned fruits are well known and appreciated all over the world [3]. The seed is used in ethnomedicine to treat intestinal parasite, dysentery, and diarrhea. The plant is acclaimed to have uterine relaxant effect; the oil from the seed is also used as a weight suppressant [4].

Aqueous leaf extract of *P. americana* has been showed to possess hypoglycemic effects in normal rats, hepatoprotective action, significant and dose dependent inhibition of writhes and oedema in analgesic and anti-inflammatory studies respectively [5, 6]. Other ethnomedicinal uses is that of the oil derived from the plant which includes wound healing [7] and hepatoprotective properties [8].

Figure 1: *Persea americana* fruit and cross section

Scientific data on the use of the seeds in the management of preterm-labour, spontaneous abortion are inadequate. Therefore this study is geared at screening the aqueous extract of the seed for tocolytic activity on the uterine smooth muscles.

Experimental

Plant Material

Fresh seeds of *Persea americana* were collected in Oraifite, Anambra State in the month of June, 2012 and was authenticated by Prof BA Ayinde of the Department of Pharmacognosy, University of Benin, Benin City. Specimens with voucher No FHI – 108336 was deposited with the Forest Research Institute of Nigeria. The seeds were chopped into little chips and oven dried at a temperature of 30 oC using a thermostat oven and the dried seeds were reduced to powder using a milling machine.

Drug and Chemicals

These include oxytocin (Greenfield Pharmaceuticals, China), ergometrine (Rotex Medica, Germany), salbutamol (Glasgo Smithkline, UK), diethyl-stilbesterol (BDH chemicals, UK), chloroform (Sigma-Aldrich, UK), absolute ethanol (BDH, Chemicals, UK) and atropine (Martindale, England). The stock solutions of these drugs were made using physiological salt solutions (De jalon's solution) prior to use in *in vitro* experiments.

Animals

Female Wistar albino rats (180- 250 g) were obtained from the animal house of the Department of Pharmacology University of Benin, Benin City, Nigeria. They were housed in a single large cage in an environmentally controlled room provided with a 12:12 hours light and a dark cycle for each 24 hours period at a temperature of 26 ± 1 ^{o}C in the animal house of the Department of Pharmacology, University of Benin, Benin City, Nigeria for at least two weeks prior to the experiment. The rats had free access to water and food.

All experiments were carried out in a quiet laboratory setting with ambient illumination and temperature close to the animal house. Carcasses of the killed animals were properly disposed off.

Ethical approval

Approval for the use of the animals was obtained from the Ethical Committee on the Use of Animals, Faculty of Pharmacy, University of Benin, Benin City, Nigeria.

Extraction of Plant Material

The seed powder was separated from the chaff using a sieve, weighed and 968.9g was macerated in 2 litres of distilled water for 24 hours. Resultant aqueous extract was separated from the mixture using a white sieve and concentrated on a water bath. The dried plant extract (55.6g) was obtained giving a percentage yield of 8.7% w/w. Fresh stock solution of the dried extract and subsequent dilutions were made in distilled water daily during the experiments.

In vitro assay for Uterotonic activity

Effect of the aqueous extract of Persea americana seeds on OXY, ACH, and ERG induced contractions

Diethylstilbesterol (0.2 mg/kg) constituted in 1:1 ethanol/water solution was administered intraperi-toneally as a pre-treatment to the rats to induce oestrus 24 hours prior to experiment. On the day of experiment, the rats were anaesthetized by chloroform inhalation in a gas chamber and sacrificed. The lower abdomen was dissected and the two horns of the uterus were cut out and transferred into a Petri dish containing aerated De-jalon's solution comprising NaCl 154.1 mM, $NaHCO_3$ 5.95 mM, d-glucose 2.75 mM, KCl 5.36 mM and $CaCl_2$.2H2O 0.055 mM [9]. The horns were separated from the fats and adhering blood vessels. Uterine segment measuring about 1.5 cm in length was cut out and threaded. A loop was then formed at one end of the tissue and attached to the tissue holder. The other end was connected to a previously calibrated isometric transducer connected to the unirecorder model 7050 (Ugobasile, Italy) and the tissue was mounted in a 30 ml organ bath (temperature maintained at 32 oC) containing continuously aerated De-jalon's solution and allowed to equilibrate for 30 minutes.

A preliminary test was done to ascertain the effect of the aqueous extract of *Persea americana* extract on the uterus and based on this study, the doses of extract to administer were selected (0.83 mg/ml, 1.67 mg/ml, and 3.33 mg/ml). A time cycle of 1 minute 45 seconds was allowed (45 seconds of contact time and 1 minute of relaxation time). The effects of antagonists (salbutamol and atropine sulphate) and the extract were investigated against the concentration response curves for ACH, OXY and ERG respectively. These antagonists were administered separately, each allowed an equilibration time of 10 minutes before subsequent administration of the agonist.

Effect of the aqueous extract of Persea americana seeds on KCl induced contractions

The effect of the extract/salbutamol was also investigated against KCl induced contractions. KCl (80 mM) was introduced into the organ bath, having

obtained a sustained contraction; the extract at varying doses (0.83 mg/ml, 1.67 mg/ml, 3.33 mg/ml)/ salbutamol (0.5-2 µg/ml) was introduced [10].

Data analysis

Data are presented as mean ± standard error of mean (SEM). The baseline obtained during equilibrium of 7 N tensions was taken as zero centimeters, and the tension increase after the addition of the drugs was calculated with the run-up distance of the zero centimeters baseline. Concentration response curves were plotted for all the different treatment procedures. Statistical evaluation of the data was done by Students' t-test. A $P<0.05$ was considered to be significant.

Results

Effect of PA on OXY-induced contraction

Ooxytocin produced a dose dependent contraction of the uterus; this contraction was significantly inhibited by the extract (Figure 2). This inhibition was dose dependent and significant at 0.83 mg/ml, 1.67 mg/ml, and 3.33 mg/ml ($p<0.05$). The inhibitory effect of salbutamol (63.74 ± 6.4 g) at 0.027 iu on oxytocin was not as significant when compared with the effect of the extract (31.52±10.43 g) at same dose.

The effect of PA on ACH-induced contraction

The effect of acetylcholine was significantly inhibited by the extract at 0.83 mg/ml ($p>0.05$), 1.67 mg/ml ($p<0.01$) and 3.33 mg/ml ($p<0.001$). More inhibitory effect on acetylcholine was observed than on oxytocin. At concentration of 26.66 mg/ml, the inhibitory effect of the extract (20.10 ± 10.94) was more when compared with that of atropine (38.09 ± 11.25) at the same concentration (Figure 3).

Effect of PA on ERG-induced contraction

The presence of the extract at all doses significantly ($p<0.001$) inhibited the effect of ergometrine induced contractions (Figure 4).

Effect of PA on KCl induced contraction

The presence of the extract and salbutamol significantly ($p<0.001$) inhibited the sustained induced contractions of potasium chloride (Figure 5).

Discussion

Persea americana significantly inhibited the effects of all the oxytocic agents used in this study. This relaxant/tocolytic effect of the extract may have been mediated by various mechanisms such as potassium channel opening or calcium blockade, or by receptor antagonism such as antimuscarinic, oxytocic or via attenuation of adrenoceptors [11]. Tocolysis which is the relaxation of the contracting pregnant uterus or undialated cervix needed to postpone parturition was seen with the aqueous extract.

Oxytocin binds to specific receptors to increase intracellular calcium (Ca^{2+}) by inhibiting calcium extrusion and suppression of calcium ATPase through the opening of calcium channels and stimulation of inositol -1, 4, 5- triphosphate to release internally stored calcium [12,13]. The action of oxytocin is to raise intracellular calcium level by facilitating the activation of voltage gated ion

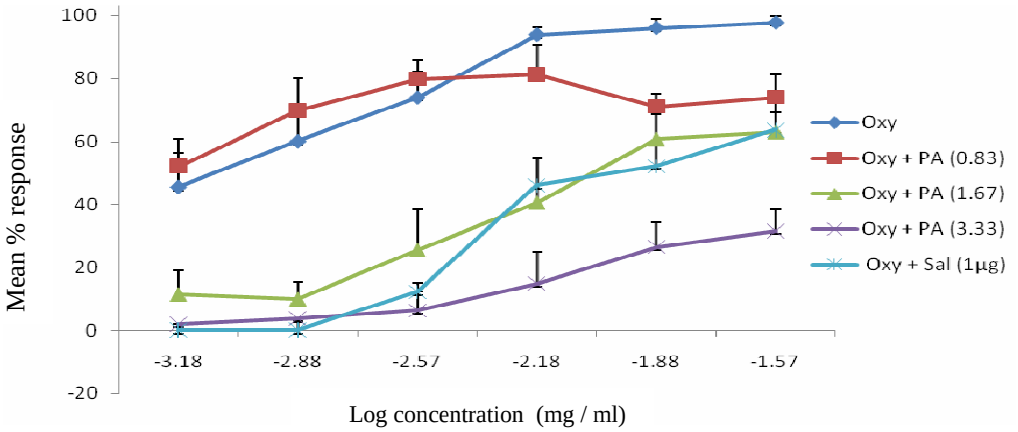

Figure 2: The effect of the aqueous extract of *Persea americana* seeds and salbutamol on oxytocin-induced contractions of rat uterus. Values are mean ± SEM. (n = 5 per experiment). **P<0.05, *P<0.0001 significantly different from oxytocin induced contractions alone for PA (1.67 and 3.33) and salbutamol respectively
Oxy – oxytocin alone
Oxy + PA (3.33) - Oxytocin + 3.33 mg/ml of extract
Oxy + PA (1.67) – Oxytocin +1.67 mg/ml of extract
Oxy + PA (0.83) – Oxytocin + 0.83 mg/ml of extract
Oxy + Sal (1µg) – Oxytocin + Salbutamol (1µg)

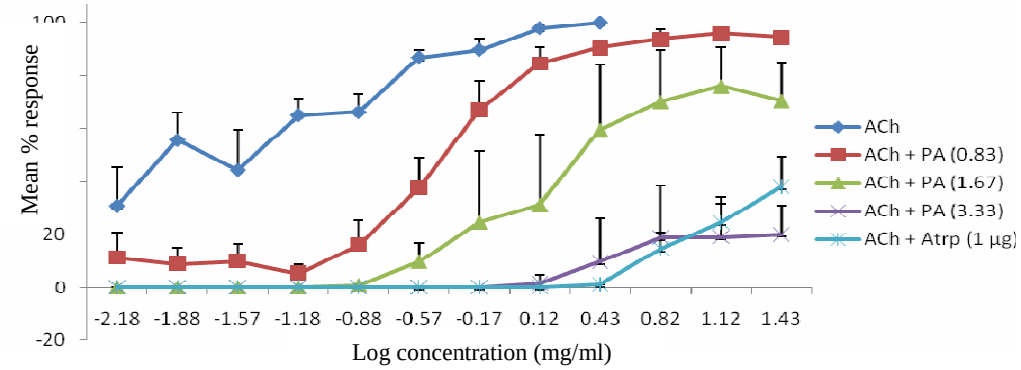

Figure 3: The effect of the aqueous extract of *Persea americana* and atropine on acetylcholine induced contraction of rat uterus. Values are mean ± SEM. (n = 5 experiments). **P<0.05, *P<0.0001 significantly different from acetylcholine induced contractions alone for PA (1.67 and 3.33) and atropine respectively.
ACh – Acetylcholine alone
ACh + PA (0.83) – 0.83 mg/ml of Extract +Acetylcholine
ACh + PA (1.67) – 1.67 mg/ml of Extract + Acetylcholine
ACh + PA (3.33) - 3.33mg/ml of Extract + Acetylcholine
ACh + Atrp (1µg) - 1µg of Atropine + Acetylcholine

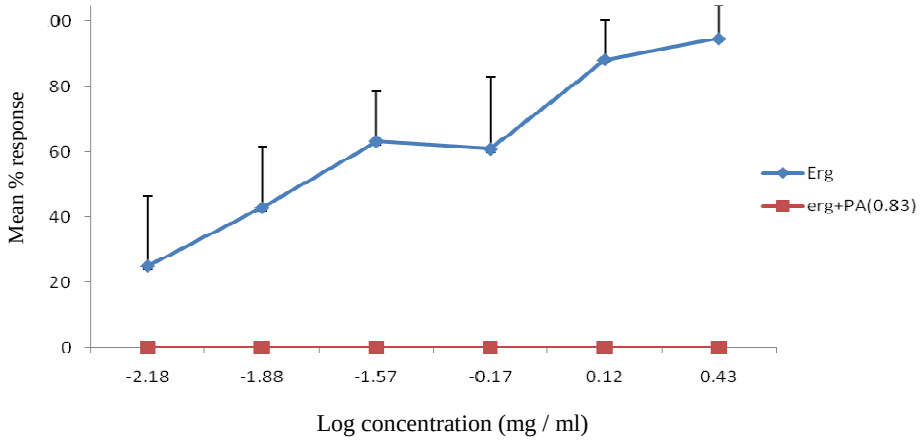

Figure 4: The effect of the aqueous extract of *Persea americana* seed on ergometrine induced contraction of rat uterus. The values are % mean responses ± SEM (n = 5 per experiments). **P<0.05, significantly different from ergometrine induced contractions alone for PA (0.83mg/ml).
Erg- Ergometrine alone
Erg + PA (0.83) – 0.8 mg / ml of extract+ Ergometrine

channels during the process of excitation; this suggests that the extract inhibits oxytocic receptor directly or indirectly and hence cause a decrease of intracellular calcium and blockade of the opening of the calcium channels.

Acetylcholine induced contractions were significantly inhibited by the extract (p<0.001) through muscarinic receptors. Atropine is a muscarinic receptor antagonist that inhibits the muscarinic effect of acetylcholine; this suggests that the inhibitory effect of the extract may possibly be through blockade of muscarinic receptors.

Ergometrine acts partially as an agonist α-adrenergic, dopaminergic, and serotonergic receptors. This could explain the significant dose dependent inhibition of the contraction of ergometrine by the extract was as well. Thus it can be inferred that adrenoceptors, dominergic and serotonergic receptors may have been blocked by the extract.

High K^+ in intracellular fluid is known to depolarize muscle membrane through voltage gated operated channel. Muscle contraction by K^+ is directly related to the influx of calcium (Ca^{2+}) into the cell through L-type channel. Thus the inhibition of the sustained contraction of potassium chloride was both by the extract and salbutamol (though more significant) suggesting a possible inhibition of extracellular Ca^{2+} influx [14].

Conclusion

The aqueous extract of *Persea americana* significantly inhibited potassium chloride, oxytocin, acetylcholine, and ergometrine induced contraction of the non pregnant rat uterus. This effect was more

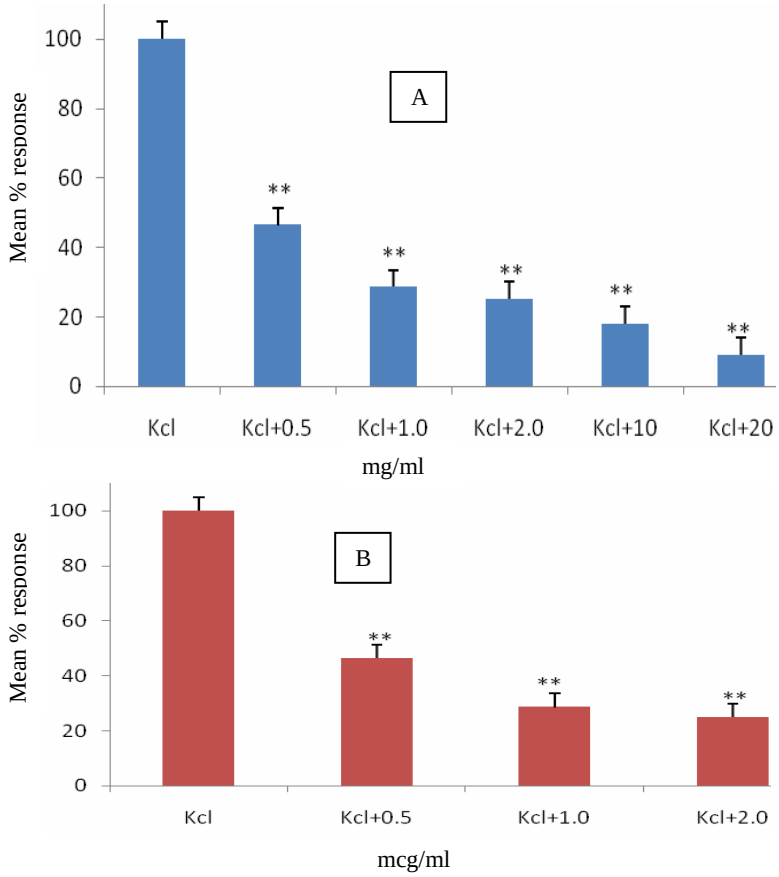

Figure 5: The effect of the (A) aqueous extract of *Persea americana* seed and (B) salbutamol on potassium chloride induced contraction of rat uterus. The values are % mean response ± SEM. (n = 5 per experiment). **P<0.001, significantly different from potassium chloride induced contractions alone for all doses of extract or salbutamol.
KCl – Potassium chloride
KCl + 0.5 - Potassium chloride + 0.5 mg/ml extract
KCl + 1.0 - Potassium chloride + 1.0 mg/ml extract
KCl + 2.0 - Potassium chloride + 2.0 mg/ml extract
KCl + 10 - Potassium chloride + 10 mg/ml extract
KCl + 20 - Potassium chloride + 20 mg/ml extract
KCl + 0.5- Potassium chloride + 0.5 µg/ml salbutamol
KCl + 1.0 - Potassium chloride + 1.0 µg/ml salbutamol
KCl + 2.0 - Potassium chloride + 2.0 µg/ml salbutamol

significant in acetylcholine and ergometrine than on oxytocin induced contractions. Also, the inhibition suggests that the mechanism of action is through muscarinic blockade or could be via non specific receptor antagonism.

These findings justifies that the aqueous extract of *Persea americana* seed has potential therapeutic value for the control or management of preterm labour.

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Conflict of interest

No conflict of interest associated with this work.

Authors' contribution

We declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by the authors. Owolabi and Anaka conceived and designed the study, Owolabi, Anaka and Eboh collected and analysed the data, and Arhewoh assisted in the writing of manuscript.

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