RESEARCH ARTICLE

Sokoto Journal of Veterinary Sciences
(P-ISSN 1595-093X/ E-ISSN 2315-6201)
Oridupa & Saba /*Sokoto Journal of Veterinary Sciences* (2013) 11(2): 21-27.
http://dx.doi.org/10.4314/sokjvs.v11i2.4

Relaxant effect of *Lagenaria breviflora* Roberty fruit pulp and seeds on isolated rabbit ileum

OA Oridupa & AB Saba

Faculty of Veterinary Medicine
University of Ibadan

*Correspondence: Tel.: 2348056666304, E-mail: oa.oridupa@mail.ui.edu.ng

Abstract

Lagenaria breviflora roberty is used in West Africa as traditional remedy for treatment of gastrointestinal disorders, particularly diarrhoea. This study was aimed at assessing the effect of the pulp and seed extract of *L. breviflora* on the contractile activity of isolated rabbit ileum. Rabbits of both sexes were used for the study and a rabbit was sacrificed for each experiment. The ileum was located and taken out into a petri dish containing Tyrode solution constantly aerated by an air pump. The organ was cut into strips of about 2cm long and mounted in an organ bath. The contractile responses were recorded on a kymograph. The maximum response (E_{max}), concentration producing 50% of the response (EC_{50}) and affinity (pA_2) were determined from repeated experiments conducted. Values were expressed as mean±SEM and significant differences were determined using one way ANOVA ($p<0.05$). The extracts caused a dose-dependent relaxation of the ileum and the relaxant effect was antagonized by prazosin and phenoxybenzamine (α-adrenergic antagonists) with a right shift in concentration response curves (CRCs) obtained. Propranolol (β adrenergic antagonist) caused a left shift in the CRCs obtained. E_{max} and EC_{50} for the extracts reduced, while pA_2 increased. The extracts produced α-adrenergic receptor-mediated gastrointestinal smooth muscle relaxation which explains the rationale for the use of *L. breviflora* fruit for treatment of diarrhoea.

Keywords: Adrenergic, Antagonists, *Breviflora*, Contractility, Lagenaria.
Received: 09-05-2013 Accepted: 05-06-2013

Introduction

Gastrointestinal (GI) tract is a specialized hollow tube in the body which moves food and water ingested orally through the body and towards the anus. It has specialized segments which digest food, absorb nutrients and water, and prepare the waste as well as other metabolic waste products for expulsion from the body. The GI tract contains smooth muscle cells arranged longitudinally and horizontally which produces the characteristic peristaltic wave or movement that propels the GI content towards the anus. Typically, smooth muscle cells lack the striations characteristic of cardiac and skeletal muscle. Layers of smooth muscle cells line the walls of various organs and tubes in the body, and the contractile function of smooth muscle is not under voluntary control (Webb, 2003).

In the intact body, the process of smooth muscle cell contraction or relaxation is regulated principally by receptors and mechanical (stretch) activation of the contractile proteins myosin and actin (Fukata *et al.*, 2001; Jin *et al.*, 2003). Stimulation of adrenergic receptors result in relaxation of the gastrointestinal smooth muscle cells and constipation or ruminal stasis occurs during increased stimulation of adrenergic receptors (Exton, 1985; Meylan *et al.*, 2004). Stimulation of cholinergic, histaminergic or serotonergic receptors on the other hand cause contraction of the GI smooth muscle. Increased stimulation of cholinergic, histaminergic or serotonergic receptors in the GI tract causes hypermotility which presents clinically as diarrhoea (Hill *et al.*, 1997; Burkhalter *et al.*, 1998; Kennedy *et al.*, 2012). Increased activity of these smooth muscles result in hypermotility, reduced retention time and ultimately diarrhoea. Diarrhoea is sometimes a defensive mechanism by which the

body forcefully expels irritants, toxicants or any assaulting substance from the gastrointestinal tract (Schiller, 2007).

The fruit of *L. breviflora* Roberty is used in folkloric medicine as herbal remedy for the treatment of digestive disorders, and as wound antiseptics (e.g. umbilical incision wound) in man, and Newcastle disease and coccidiosis in various animal species, especially poultry (Sonaiya, 1999; Tomori *et al.*, 2007). The focus of this study is to explore the precise effect of *L. breviflora* on gastrointestinal smooth muscle as it relates to the use of *L. breviflora* for treatment of digestive disorders, particularly diarrhoea.

Thus, the effect of the extracts of the fruit pulp and seeds of *L. breviflora* will be evaluated on the basis of the contractile activity of the ileal smooth muscle of the rabbit. This study will explain the probable mechanism of action of *L. breviflora* on the gastrointestinal tract in its use for ethnomedicinal treatment of ailments with digestive disorders, particularly diarrhoea.

Materials and methods

Preparation of extract

Fresh fruits of *Lagenaria breviflora* Roberty were obtained from the Teaching and Research Farm of University of Ibadan and identified at the Department of Botany and Microbiology, University of Ibadan, Nigeria. Fresh fruits were washed and the bark was dehulled from the pulp and seeds were separated. The fruit parts were soaked in ethanol and the filtrate obtained were clarified by filtration through celite on water pump and were then concentrated *in vacuo* using a rotary evaporator (Rotavapor R-210, Switzerland) at low temperatures. The remaining moisture was finally removed by freeze-drying.

Experimental animals

Adult rabbits of both sexes were used for this experiment. They were housed in the Experimental Animal House of the Department of Veterinary Physiology, Biochemistry & Pharmacology, University of Ibadan. The rabbits were fed with compounded standard rabbit feed and allowed access to water *ad libitum*.

Preparation of isolated illeal smooth muscle segments

At the beginning of each experiment, an animal was stunned and sacrificed by cervical dislocation of the neck. Immediately, it was split open from the neck down to the vent with a pair of scissors to allow access to the abdominal cavity. The ileum was located, cut and taken out into a petri dish containing Tyrode solution (NaCl 8.0g, KCl 0.2g, $MgCl_2$ 0.01g, NaH_2PO_4 0.05g, $NaHCO_3$ 1.0g, $CaCl_2$ 0.2g, Glucose 1.0g) constantly aerated by an air pump. The organ was dissected inside the petri dish containing aerated Tyrode solution and cut into strips of about 2cm long. The lower end of the tissue was attached to the aerating tube inside the organ bath while the upper end was attached to a simple isotonic lever counterbalanced to provide a load of 2g on the tissue. This frontal writing lever was aligned on a kymograph drum. Each strip of the tissue was allowed to equilibrate for at least 15 minutes before experimentation (Christ *et al.*, 1990, Dina & Arowolo, 1990).

Sensitivity study of ileal smooth muscle

After equilibration, concentration response data was obtained by cumulative additions of each extract, initially sensitivity study was undertaken for each extract in order to determine the minimum (i.e. threshold) and maximum concentrations at which responses were initiated or completed respectively. This was done by exposing the tissue to the lowest concentration of the ten-fold serially diluted extract. Stronger concentrations were then applied consecutively until a threshold response was obtained. The maximum response was ascertained when increases in the strength of the extract applied did not produce a corresponding increase on the magnitude of the tissue response.

Extract-antagonist Study of Ileal smooth muscle

After the minimum and maximum responses of the tissues to the extracts were determined, extract-antagonist studies were carried out. Adrenergic antagonists namely; propranolol, prazosin and phenoxybenzamine were used for this study. The tissue was first pre-incubated with the antagonist for at least 10 minutes to ensure adequate contact of the antagonist with the tissue. Without washing out the antagonist, extract concentration response curves (CRC_S) was determined by adding the respective extract to the bath at concentrations corresponding to those in the preceding extract study. The same tissue strip was used for the extract study and its corresponding antagonist experiment.

Evaluation of extracts

Determination of EC_{50} and Affinity (pA_2): Extract sensitivity was measured by the effective concentration of the extract that induced 50 per cent (EC_{50}) of the maximum response (E_{max}) and this was determined from CRCs. Single CRCs were plotted for each extract experiment. The EC_{50} values are the molar concentration of the extract where the curves intersected the response at 50 percent level. The mean EC_{50} (+S.E.M) was calculated from the pooled data. The affinity (pA_2) was determined as the inverse of EC_{50} (1/ pA_2). The EC_{50} and pA_2 obtained were expressed as control (+S.E.M.) values on the tables of results.

Determination of the extract maximum response (E_{max}): The maximum response of the tissue was calculated for each of the extract studied. The E_{max} was obtained by finding the percentage maximal contraction to extract from individual experiments. The mean (+S.E.M) was determined from pooled data and tabulated as control (c) E_{max} in the table of results.

Results

The fruit pulp and seed extracts of *L. breviflora* caused relaxation of isolated rabbit ileum. The threshold response was obtained at the concentration of 10^{-1}mg/ml for the pulp and 10^{-2}mg/ml for seed extract. Maximum response was obtained at $4X10^{1}$ and $4X10^{1}$mg/ml concentrations for the fruit pulp and seed extract, respectively.

The presence of propranolol in the isolated rabbit ileum produced a right shift in concentration-response curve (CRC) obtained for the pulp extract (Fig 1). EC_{50} was significantly ($p<0.05$) reduced with a significant ($p<0.05$) increase in the affinity (pA_2) from 20.00±0.01 to 100.00±0.01. Maximum response (E_{max}) was significantly reduced from 23.33±6.67 mg/ml to 13.67±8.57mg/ml of the bark extract (Table 1). In the presence of prazosin or phenoxybenzamine, the CRC shifted to the right (Fig 2&3) with non-significant ($p>0.05$) increases in EC_{50} but significant ($p<0.05$) decreases in pA_2 in the presence of prazosin (20.00±0.73 to 14.29±0.05). E_{max} was also non-significantly ($p>0.05$) unchanged for both antagonists (Table 1).

The CRC of the seed extract in the presence of propranolol shifted initially to the left, but then to the right before E_{max} was attained (Fig 4). The EC_{50} reduced from 0.007±0.001mg/ml to 0.006±0.001mg/ml of the seed extract in the presence of propranolol. pA_2 significantly ($p<0.05$) increased from 142.86±0.12 to 166.67±0.27, while E_{max} significantly ($p<0.05$) reduced from 13.34±6.66 mg/ml to 3.67±3.18 mg/ml of the seed extract (Table 2). In the presence of prazosin or phenoxybenzamine, the CRC shifted to the right with a wide margin in the CRCs obtained in the absence and presence of phenoxybenzamine (Fig 5&6). EC_{50} was significantly unchanged in the presence of prazosin, but this was significantly increased in the presence of phenoxybenzamine (0.008±0.001 to 0.010±0.007mg/ml) and its pA_2 was consequently significantly reduced. E_{max} was reduced for both of the alpha adrenergic antagonists (Table 2).

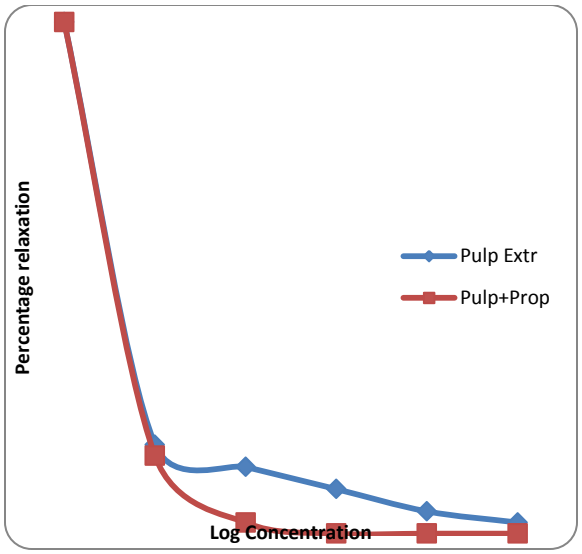

Figure 1: Effect of the pulp extract on isolated rabbit ileum smooth muscle in the absence and presence of propranolol

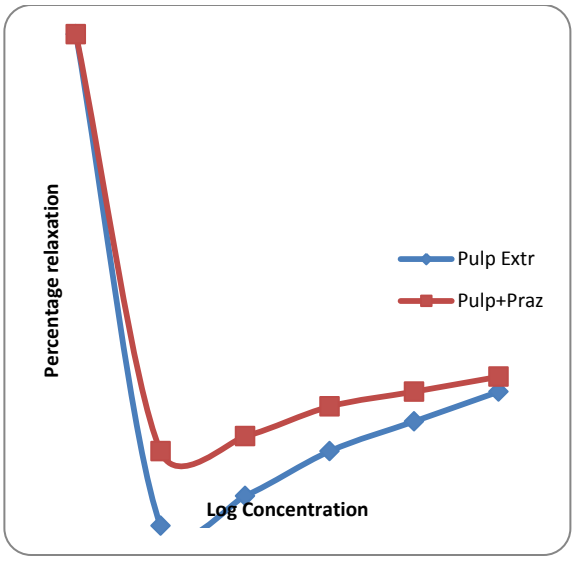

Figure 2: Effect of the pulp extract on isolated rabbit ileum smooth muscle in the absence and presence of prazosin

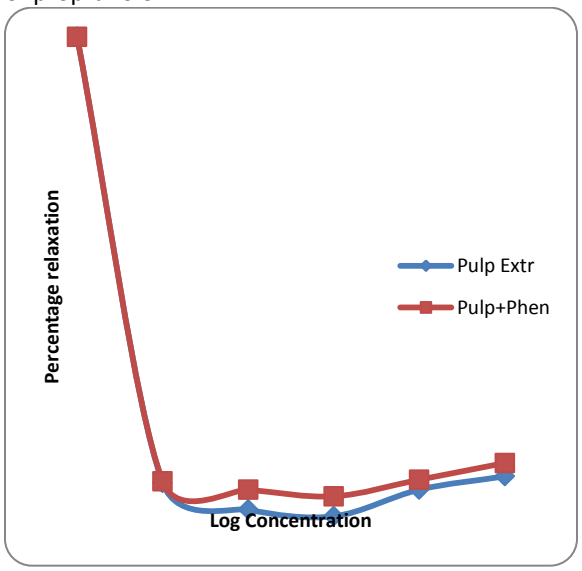

Figure 3: Effect of the pulp extract on isolated rabbit ileum smooth muscle in the absence and presence of phenoxybenzamine

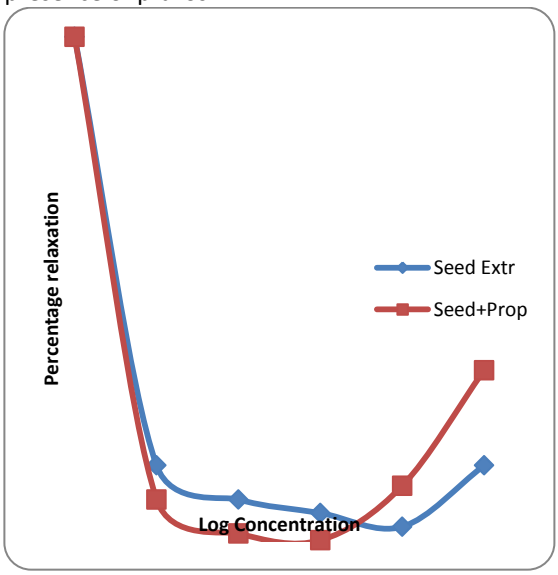

Figure 4: Effect of the seed extract on isolated rabbit ileum smooth muscle in the absence and presence of propranolol

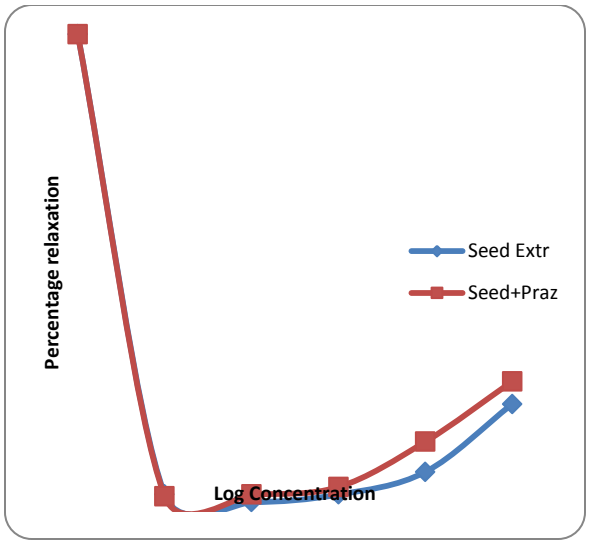

Figure 5: Effect of the seed extract on isolated rabbit ileum smooth muscle in the absence and presence of prazosin

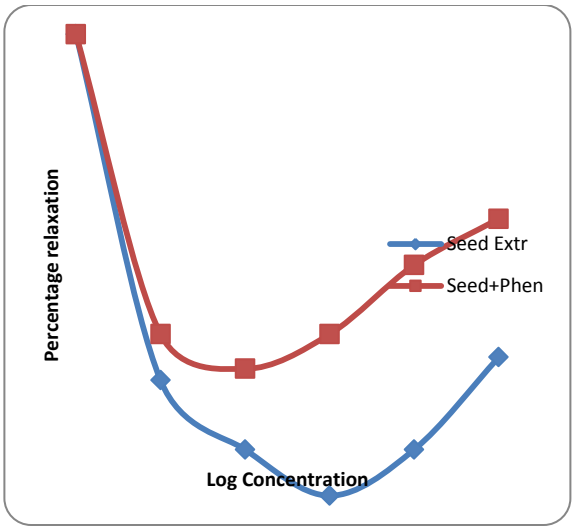

Figure 6: Effect of the seed extract on isolated rabbit ileum smooth muscle in the absence and presence of phenoxybenzamine

Table 1: Pharmacodynamic values obtained for the effect of the pulp extract of *Lagenaria breviflora* on isolated rabbit ileum in the presence and absence of adrenergic antagonists

Pulp		Propranolol	Prazosin	Phenoxybenzamine
	Control	0.05±0.001[a]	0.05±0.002	0.06±0.003
EC_{50} (mg/ml)	Test	0.01±0.001[a]	0.07±0.03	0.07±0.01
	Control	20.00±0.01[b]	20.00±0.73[a]	16.67±0.02
Affinity (pA_2)	Test	100.00±0.01[b]	14.29±0.05[a]	14.29±0.06
	Control	23.33±6.67[c]	0.01±0.001	6.67±3.33
Efficacy(E_{max}) (mg/ml)	Test	13.67±8.57[c]	0.01±0.001	7.00±6.51

Same superscript on a column are statistically ($p<0.05$) significant (significant difference between test and control)

Table 2: Pharmacodynamic values obtained for the effect of the seed extract of Lagenaria breviflora on isolated rabbit ileum in the presence and absence of adrenergic antagonists

Seed		Propranolol	Prazosin	Phenoxybenzamine
	Control	0.007±0.001	0.007±0.001	0.008±0.001[a]
EC_{50} (mg/ml)	Test	0.006±0.001	0.007±0.002	0.01±0.007[a]
	Control	142.86±0.12[a]	142.86±0.01	125.00±0.05[b]
Affinity (pA_2)	Test	166.67±0.27[a]	142.86±0.15	100.00±0.22[b]
	Control	13.34±6.66[b]	3.34±3.33	4.00±7.003
Efficacy(E_{max}) (mg/ml)	Test	3.67±3.18[b]	1.34±0.33	3.00±6.51

Same superscript on a column are statistically ($p<0.05$) significant (significant difference between test and control)

Discussion

Contractility study of isolated rabbit ileum showed that the fruit pulp and seed extracts of *L. breviflora* individually caused a dose-dependent relaxation of the gastrointestinal smooth muscle. The seed produced the least threshold response at the concentration of $1X10^{-2}$mg/ml, while the pulp produced its threshold response at $1X10^{-1}$mg/ml. Relaxation of gastrointestinal smooth muscle is largely mediated through stimulation of alpha and beta adrenergic receptors (Rang *et al.*, 2007), and this was the rationale for antagonising the relaxant effect of this fruit with known alpha and beta receptor antagonists. The relaxant effect of these extracts was competitively inhibited by prazosin

and phenoxybenzamine (alpha adrenergic antagonists) with a right shift in the concentration response curve (CRC) obtained for the fruit extracts. CRCs obtained shifted to the left in the presence of propranolol (beta adrenergic antagonist), indicating that the relaxant effect of the fruit extract could also be mediated via non-adrenergic receptors which the scope of this study could not unravel.

The potency, affinity and the concentration producing the maximum effect (E_{max}) of these extracts decreased in the presence of prazosin and phenoxybenzamine, but increased in the presence of propranolol. The fruit extracts of *L. breviflora* can be said to predominately stimulate alpha adrenergic receptors to produced smooth muscle relaxation. Stimulation of alpha 1 adrenergic receptors is known to cause relaxation of GI smooth muscle and alpha 2 also cause contraction of GI sphinters, thereby preventing GI hypermotility (Sagrada *et al.*, 1987; Chen-Izu *et al.*, 2000) and eventually diarrhoea. One of the traditional uses of this fruit is for the treatment of gastrointestinal ailments, especially diarrhoea (Sonaiya, 1999). This study explains one of the probable mechanisms of inhibition of diarrhoea by this fruit extract.

These fruit extracts may also interact with other non-adrenergic receptors that cause relaxation of the gastrointestinal (GI) smooth muscle, but were not included in this study. A family of peptides known as endothelins (ET) cause smooth muscle relaxation in the oesophagus, stomach and ileum through interaction with ET(A) or ET(B) receptors (Huang, 2005). Neurotransmitters such as adenosine, adenosine monophosphate (AMP), adenosine diphosphate (ADP), adenosine triphosphate (ATP) and other derivatives of these neurotransmitters, and nitric oxide are known to cause GI smooth muscle relaxation by stimulating purinoceptors (Burnstock, 2001; Giaroni *et al.*, 2005; McDonnell *et al.*, 2008).

Findings from this study imply that consumption of this fruit extract will reduce or inhibit gastric motility and invariably increase transit time. This may have beneficial impact on food absorption, thus a nutritive effect, but caution must however be taken in patients prone to constipation. Also, this fruit stimulates alpha adrenergic receptors which causes vasoconstriction and has positive isotropic effect on blood vessels (Elliot, 1997; Rang *et al.*, 2003). Cautious use is thereby recommended in hypertensive patients. Further studies are also warranted to isolate and characterise the bioactive principle in the fruit responsible for stimulation of alpha-adrenergic receptors.

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