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Pharmacological reactivity of isolated guinea pig ileum to ethanol leaf extracts of *Amaranthus caudatus* and *Solanum melongena*

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Summary: The pharmacological reactivity of guinea pig ileum to ethanol leaf extract of *Amaranthus caudatus* and *Solanum melongena* were determined *in vitro*. Parameters evaluated include the threshold value and the concentration ratio (CR). The potency of the plant extracts as expressed by EC_{50}, the E_{max} (maximum response) and its corresponding concentration were determined from the concentration response curve in the absence or presence of $2X10^{-7}$ M atropine or $2X10^{-7}$ M mepyramine. The study showed that the extract of *Amaranthus caudatus* or *Solanum melongena* produced a dose-dependent contraction of the smooth muscle of the guinea pig ileum with threshold values at 80 or 100mg/ml respectively. $2X10^{-7}$ M atropine or $2X10^{-7}$ M mepyramine individually caused a right shift on the cumulative concentration-response curve for each plant extract. The potencies of the plant extracts were significantly ($p<0.05$) decreased, and the concentration producing E_{max} was significantly ($p<0.05$) increased in the presence of the antagonists. The ileal contraction produced by *A. caudatus* was more sensitive to mepyramine antagonism. The EC_{50} (373.80±51.56mg/ml) and the concentration producing E_{max} (855.00±75.00mg/ml) for *A. caudatus* extract increased significantly ($p<0.05$) to 849.00±29.16 mg/ml and 875.00±25 respectively in the presence of atropine, indicating that the extract interacted with muscarinic receptors. The mean EC_{50} and the concentration eliciting the E_{max} for *S. melongena* extract increased significantly ($p<0.05$) from 288.91±32.46mg/ml and 600.00±22.00mg/ml to 385.21±19.20mg/ml and 800±0.00 mg/ml respectively in the presence of mepyramine thus indicating stimulation of the histaminergic H_1 receptors of the gastrointestinal tract. Taken together, this study demonstrated that *A. caudatus* predominantly stimulates muscarinic receptors to produce contraction of the gastrointestinal smooth muscle, while *S. melongena* predominantly stimulates histaminergic H_1 receptors.

Keywords*: A. caudatus, S. melongena*, Ileal contraction, M- receptors, H_1- receptors

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INTRODUCTION

Vegetables are part of food taken daily in the world over. They are a rich source of both fat and water-soluble vitamins, especially the anti-oxidant vitamins A and C, β carotene, as well as soluble and insoluble fibers. Some vegetables are used for ornamental or medicinal purposes apart from dietary reason. *Amaranthus caudatus* of the family *Amaranthaceae* is one of the most popular, domesticated amaranths. It is cultivated as an ornamental plant and a pseudocereal (Costea and DeMason, 2001; Costea *et al.*, 2006). The origin of *A. caudatus* remains uncertain and has a distribution that includes Africa, India and South America. The local name of *A. caudatus* is *tete* in Yoruba (Odukoya *et al.*, 2007), its other common names include love-lies-bleeding, Inca wheat, velvet flower or amaranth (Marx, 1977). *A. caudatus* was a staple grain for the Incas and Aztecs, and was nearly as widespread in Tropical America as was maize (Marx, 1977). It is a common vegetable in West Africa and goes with all Nigerian carbohydrate dishes. Amaranth seeds and leaves are a very good source of vitamins, including vitamin A, vitamin K, vitamin B_6, vitamin C, riboflavin, folate and dietary minerals including calcium, iron, magnesium, phosphorus, potassium, zinc, copper, and manganese. Both the seeds and leaf have high protein levels, including the amino acid lysine, usually deficient in vegetables (Juan *et al.*, 2007).

There are documented ethnomedicinal uses of *A. caudatus* such as for treatment of hypertension and other cardiovascular diseases (Czerwiński *et al.*, 2004; Gonor *et al.*, 2006; Martirosyan *et al.*, 2007). Regular consumption of *A. caudatus* has been shown to reduce blood pressure and cholesterol levels, while improving antioxidant status and some immune parameters. The active ingredient in amaranth such as fibre, plant stanols and squalene appear to lower cholesterol. Other species of *Amaranthus* has also

been reported to lower serum cholesterol and blood glucose levels (Chaturvedi *et al.*, 1993; Chaturvedi *et al.*, 1997).

S. melongena is a member of the plant family Solanaceae (Nightshade family). Common names of this plant include *igba* in Yoruba (Odukoya *et al.*, 2007), aubergine, egg plant and apple of love (Van Eck and Snyder, 2006). It is a small tropical perennial plant with purple flowers, native to Africa and Asia; growing up to a height of 55 inches. Wild types can grow much larger, up to 85 inches. The leaves are pubescent and sometimes spiny. Extracts of *S. melongena* have been reported to be effective in the treatment of hemorrhage (Diab *et al*., 2011), asthma (Bello *et al*., 2005), dysentery (Mans *et al*., 2004) and hypercholesterolemia (Guimaraes *et al*., 2003; Sudheesh *et al*., 1997).

It has been a long held traditional opinion that leafy vegetables act only as bulk purgative solely because of its large content of cellulose. Recent findings have shown that some of these vegetables have phytoconstituents that are capable of stimulating the mural cholinergic or histaminergic receptors (Arowolo *et* al., 1989; Dina *et* al., 2001; Saba *et al*., 2003). Stimulation of cholinergic and histaminergic receptors in the gastrointestinal tract produces contraction and increased gastric motility which shortens transit time. This mural stimulation has also been linked to the lipid lowering effect of *A. cruentus* or A. *esculentus* specifically (Ikeda *et al.*, 1989; Chaturvedi *et al.*, 1993; Escudero *et al.*, 2004, 2006; Kim *et al.*, 2006; Anderson *et al.*, 2009). Kabiri *et al* (2010) reported decreased serum lipoproteins, apo-lipoprotein B and oxidized low density lipo-protein thus strategically placing Amaranthus vegetable as a good naturally potent diet for lowering the risk factors of cardiovascular and inflammatory diseases.

This study was designed, using isolated guinea pig ileum, to investigate the effect of *Amaranthus caudatus* and *Solanum melongena* on the gastrointestinal smooth muscle and characterize the probable mediating receptors.

MATERIALS AND METHODS

Preparation of Plant Extract

Fresh leaves of *Amaranthus caudatus* and *Solanum melongena* were obtained from University of Ibadan Farm and were identified at the Department of Botany. The samples were air-dried at room temperature and crushed into a coarse powder with a crucible. The powder was weighed and cold extraction was done with 96% ethanol as the solvent. The ethanol was drained and replaced with a fresh batch every 3 days. The extraction process was completed in a week. The drained ethanol was stored in plastic containers and refrigerated at 4^0C. The extract obtained was clarified by filtration through celite on water pump and was then concentrated *in vacuo* using a rotary evaporator at low temperatures. The remaining moisture was finally removed by placing small volumes in porcelain dishes in the oven set at 40^0C. A stock solution was afterward prepared by dissolving 100g of the extract in 100ml of distilled water, from which serial dilutions were made as required in the course of the experiment.

Experimental Animals

Twenty four adult guinea pigs of both sexes were used in this study. The guinea pigs were housed at the Experimental animal house of the Department of Veterinary Physiology, Biochemistry and Pharmacology, University of Ibadan. They were fed pelletized rodent ration (Guinea Feeds Nig. Ltd) and allowed access to water *ad libitum*. The animals were maintained in 12 hour light: dark period and allowed to stabilize for two weeks before commencement of the experiment. For each experiment, a guinea pig was fasted overnight and humanely sacrificed by cervical dislocation. It was immediately split open from the neck down to the pelvic region with a pair of scissors and the ileum was located and removed. It was immediately placed in a petri dish containing Tyrode's solution constantly aerated by an air pump.

Experimental procedure

Clean ileal segments of 2-3cm long were prepared from the ileum cut out. One end was fixed to a pin attached to the glass aerating tube and the other to a writing level which was adjusted for tension (0.5g) and for recording (six time magnification) on Kymograph drum (Biosciences, Kent, England). The whole preparation was set up in a 20 ml organ bath containing aerated Tyrode solution (NaCl: 1.0; $CaCl_2$: 0.2; KCl: 0.2; $MgCl_2.6H_2O$:0.2; $NaHPO_4$: 0.05; $NaHCO_3$: 1 and glucose 2.0g/dl) and maintained at a temperature of 37^0C.

The tissue was washed at least twice and allowed to equilibrate in the organ bath for 30 minutes. Graded concentrations of each extract from 10mg/ml with a graduation of 5mg/ml were prepared up to 800mg/ml of extract. These were added cumulatively into the organ bath to determine the concentration at which the minimum ileal contractile response (threshold value) was elicited and the concentration at which further increases in the concentration did not elicit a higher magnitude of response (maximal response). Concentrations within the range of the threshold and maximal response levels were used for the agonist and agonist-antagonist study.

Responses were recorded by a Kymograph (Harvard Student Kymograph, UK). After a concentration-response curve for the extract had been established, the same preparation was in turn exposed to predetermined concentrations of $2X10^{-7}$ M atropine sulphate (Nutritional Biochemical Corporation, Cleveland, Ohio, USA) or $2X10^{-7}$ M mepyramine maleate (Sigma Chemical Company, St. Louis,

Missouri, USA), for a period of 10 minutes to re-establish another concentration-response curve for the extract in the presence of each antagonist. The procedure was repeated five times for agonist and agonist-antagonist interaction using new ileal strips from different guinea pigs.

Determination of Parameters

The heights of contractions were determined and concentration-response curves were plotted for all the recordings. The pooled data collected from concentration-response curves were plotted, for the agonist alone and the agonist in the presence of antagonist. These parameters include the potency of the extract as measured by EC_{50}, the affinity of the extract (pA_2 = $1/EC_{50}$) and the extracts' efficacy (E_{max}) for the extract alone (Control) and the same parameters for the extract in the presence of the antagonists (Test) (Oriowo and Bevan, 1987; Saba and Arowolo, 2006; Saba *et al*., 2006). The effectiveness of antagonism by atropine or mepyramine was determined by the concentration ratio (CR). The CR is an indication of the relative effectiveness and specificity of the antagonists. It is evaluated as the ratio of agonist concentration giving equivalent responses in the presence and absence of antagonist.

It is expressed by the formula:

CR=AB/AO

Where AB and AO represent agonist EC_{50} with or without antagonist respectively

Statistical analysis

All the values were recorded as mean ± standard error of mean (S.E.M.) and the data was analyzed using Student's t-test (Steel and Torrie, 1996) with the differences of the means considered significant at $p<0.05$.

RESULTS

Response of isolated guinea pig ileum extract

The plant extracts individually elicited dose-dependent contractions of the guinea pig ileum. The threshold response of leaf extract of *A. caudatus* or *Solanum melongena* was observed at concentration of 80 or 100 mg/ml and the maximal contractile response were obtained at 900 (Table 1) or 800mg/ml (Table 2) of the extract respectively . The concentration-response curve (CRC) obtained for the contractile effect of the extract of *A. caudatus* or *Solanum*

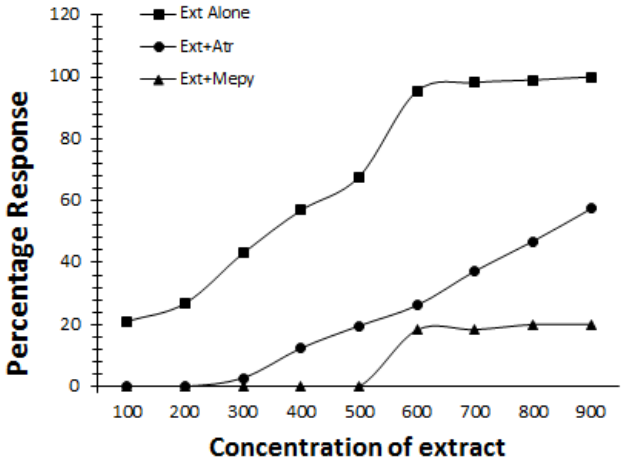

Figure 1. Dose-response curve of isolated guinea pig ileum to graded concentration of ethanol leaf extract of *Amaranthus caudatus*. Ext= extract., Atr = Atropine ($2x10^{-7}$M)., Mepy = Mepyramine ($2x10^{-7}$M)

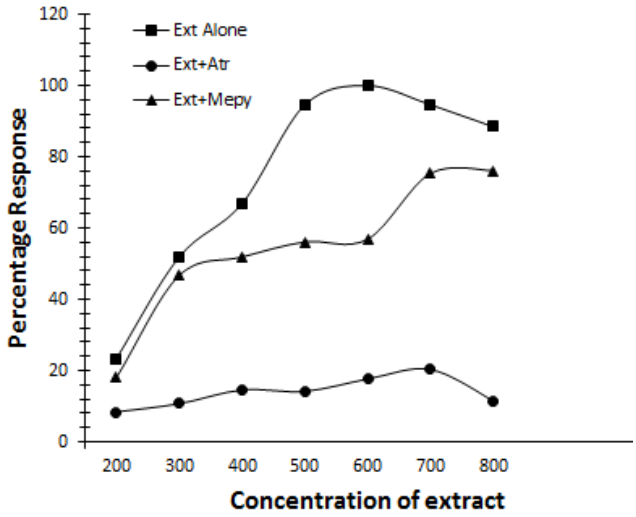

Figure 2. Dose-response curve of isolated guinea pig ileum to graded concentrations of ethanol leaf extract of *Solanum melongena*. Ext= extract., Atr = Atropine ($2x10^{-7}$M)., Mepy = Mepyramine ($2x10^{-7}$M)

Table 1: Response of isolated guinea pig ileum to graded concentration of ethanol leaf extract of *Amaranthus caudatus*

Concentration (mg/ml)	Extract Alone (n=5)	Extract+Atropine (n=5)	Extract+Mepyramine (n=5)
100	21.03± 3.50	0.00±0.00	0.00±0.00
200	26.80± 5.97	0.00±0.00	0.00±0.00
300	42.99± 8.49[a]	2.75±2.76[a]	0.00±0.00
400	56.87±10.11[a]	12.36±0.60[a]	0.00±0.00
500	67.67± 7.05[a]	19.56±1.04[a]	0.00±0.00
600	95.37± 4.63[ab]	26.20±0.33[a]	18.25±5.10[b]
700	98.15± 1.85[ab]	37.23±3.91[a]	18.60±8.09[b]
800	99.00± 0.93[ab]	46.87±6.12[a]	20.00±2.06[b]
900	100.00± 0.00[ab]	57.35±7.37[a]	20.00±2.02[b]

Same superscripts in a row are statistically significant ($P<0.05$)

Table 2: Response of isolated guinea pig ileum to graded concentrations of ethanol leaf extract of *Solanum melongena*

Concentration (mg/ml)	Extract Alone (n=5)	Extract+Atropine (n=5)	Extract+Mepyramine (n=5)
200	23.46±10.61	8.46±18.49	18.08±9.33
300	51.92±18.18	10.77±26.63	46.75±26.63[a]
400	66.92±12.04	14.62±25.09	51.92±25.71
500	94.62± 7.52	14.23±16.30	56.15±19.13
600	100.00±5.45	17.69±18.1	56.92±5.51
700	94.62±2.52	20.38±4.65	75.38±0.0
800	88.46±5.49	11.54±1.99	76.15±2.31

Same superscripts on a row are statistically (P<0.05) significant

Table 3: Pharmacodynamic values obtained for the effect of the extracts of *A. caudatus* and *S. melongena* on isolated guinea pig ileum in the presence and absence of atropine and mepyramine

Parameter	Ac Alone (n=5)	Ac+Atrop (n=5)	Ac+Mepy (n=5)	Sm Alone (n=5)	Sm+Atrop (n=5)	Sm+Mepy (n=5)
Potency (EC_{50})(mg/ml)	373.80±51.56[a]	849.00±29.16[ab]	NA	288.91±32.46[bc]	NA	385.21±19.20[c]
Efficacy (E_{max})(%)	98.12±4.32[a] (675.00±75.00)	57.89±3.91[abc] (875.00±25.02)	20.05±1.11[bd] (800.00±40.82)	100.00±0.01[ce] (600.00±22.00)	21.10±1.35[d] (700.00±25.12)	76.78±4.20[e] (800.01±2.00)
Affinity (pA_2)	0.0112±0.003	0.0098±0.001	NA	0.02418±0.006	NA	0.0208±0.003
CR	NA	2.27±0.31	NA	NA	NA	1.33±0.69

Ac= *A. caudatus*., Atrop= Atropine., Sm=*Solanum melongena*., Mepy=Mepyramine, NA=Not applicable

shifted to the right in the presence of $2X10^{-7}$ M atropine or $2X10^{-7}$ M mepyramine respectively (Figs 1 & 2).

The potency of the extracts

The potency of *A. caudatus* or *Solanum melongena* on the isolated guinea pig ileum decreased when the tissue was pre-treated with $2X10^{-7}$ M atropine or $2X10^{-7}$ M mepyramine. There was significant ($p<0.05$) increase in the mean EC_{50} obtained for the extract of *A. caudatus* alone (373.8±51.56 mg/ml) compared to the value obtained in the presence of $2X10^{-7}$ M atropine (849.00±29.16 mg/ml) (Table 3). The EC_{50} of *S. melongena* also increased from 88.91±32.46 mg/ml without mepyramine to 385.21±19.20 mg/ml in the presence of mepyramine (Table 3).

Affinity of the extracts in the presence and absence of antagonists

The affinity of *A. caudatus* for the muscarinic receptors non-significantly decreased from 0.0112±0.003 to 0.0098±0.001 in the presence of atropine. The affinity of *S. melongena* non-significantly decreased from 0.02418±0.006) to 0.0208±0.003 in the presence of mepyramine (Table 3).

The E_{max} of the isolated guinea pig ileum to the extracts

Isolated guinea pig ileum responded to the contractile effect of *A. caudatus* with mean E_{max} value of 98.12±4.32% at extract mean concentration of 675.00±75.00mg/ml. The tissue attained a mean E_{max} value of 57.89±3.91% at extract mean concentration of 875.00±25.02 with CR value of 2.27±0.31. The E_{max} value in the presence of mepyramine was 20.05±1.11, CR value could not be determined being a noncompetitive antagonism.

The contractile response of ileal smooth muscle to *S. melongena* attained a mean E_{max} value of 100.00% at extract concentration of 600.00±22.00 mg/ml, 21.10±1.35% at extract concentration of 700.00±25.12 mg/ml in the presence of $2X10^{-7}$ M atropine, and 76.78±4.2% at extract concentration of 800.01±2.00 mg/ml in the presence of $2X10^{-7}$ M mepyramine with CR value of 1.33±0.69. The CR value for the response was undeterminable for atropine being a noncompetitive interaction.

DISCUSSION

The results obtained from this study showed that the extract of *Amaranthus caudatus* Linn or *Solanum melongena* Linn produced a dose-dependent contraction of the smooth muscle of the guinea pig ileum. Atropine or mepyramine individually caused a right shift (Figs.1 & 2) on the cummulative dose-response curve for each plant extract, suggesting a competitive antagonism. The potency of the plant extract was significantly decreased, while the concentration producing maximal response was significantly increased in the presence of the antagonists. The ileal contraction produced by *A. caudatus* Linn was blocked competitively by atropine, indicating that the extract interacted predominantly with muscarinic cholinergic receptors of the guinea pig ileum. The reverse was the case for ileal contractions produced by *S. melongena.* The contractions were sensitive competitively to mepyramine antagonism thus indicating stimulation

of the histaminergic H_1 receptors of the gastrointestinal tract. Taken together, this study demonstrates that *S. melongena* predominantly stimulates histaminergic receptors to produce contraction of the gastrointestinal smooth muscle, while *A. caudatus* predominantly stimulates muscarinic receptors.

Reports on the pharmacological reactivity of the gastrointestinal smooth muscle to *A. caudatus* or *Solanum melongena* are very rare in the literature. However, similar stimulatory effect by *S. melongena* on the muscarinic and histaminergic receptors of the respiratory tract have been reported (Mans *et al*., 2004., Bello *et al*., 2005), and products from the grain and leaf of *A. caudatus* have also been proven to lower blood pressure (Zoblkova *et al*., 2004., Martirosyan *et al*., 2005a; 2005b).

Findings from this study on the cholinergic or histaminergic properties of the extracts of the two plants imply that their consumption will often be accompanied by increased contraction produced by stimulation of the gastrointestinal smooth muscle receptors and decreased intra-luminal transit time which correlates very well with decreased absorption of gastrointestinal content (De la Roca-Chiapas and Cordova-Fraga, 2011). This presumably serves a good purpose of control of diet especially in clinical conditions such as diabetes, obesity and heart related conditions where there is a need to achieve a stomach filling effect without necessarily an increase in absorption of nutrients in order to prevent hyperlipidaemia, hyperglycaemia or hypercholestoraemia (Chaturvedi *et al.*, 1993., Kim *et al.*, 2006; Martirosyan *et al*., 2007., Anderson *et al.*, 2009).

Reports show that cholinergic or histaminergic stimulation usually results in vasodilatation (Eglen and Whiting, 1985; Chiba and Tsukeda, 1991; Bedarida *et al.*, 1995). Even though this study did not include the cardiovascular system, it is safe at this point to hypothesize that stimulation of histaminergic or cholinergic receptors in the vascular bed by the phytoconstituents of these vegetables are synergistic to the hypocholesterolaemic action of *S. melongena* or *A. caudatus* (Ikeda *et al.*, 1989; Escudero *et al.*, 2004, 2006; Das *et al.*, 2011) which is thought to be solely responsible for the reported anti-hypertensive effect of these vegetables.

On the other hand, in view of the interactions of these plants with cholinergic and histaminergic receptors in the body, extreme conditions such as gastrointestinal hypermotility, diarrhea, and malabsorption may accompany excessive consumption of *A. caudatus or S. melongena*. It is therefore pertinent to consume these vegetables with caution especially in very sensitive individuals.

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