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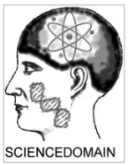

Phytochemical Analysis and Anticholinergic Properties of Methanol Leaf Extract of *Arachis hypogea* on Isolated Rabbit Jejunum

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Authors' Contributions

This work was carried out in collaboration between all authors. Author RCI designed experimental protocol. Author GSA read and corrected manuscript draft. Authors GCI, UIE and FOAI performed plant sampling, extraction and phytochemical analysis. Authors SNI, EUE and CJN performed animal studies. All authors read and approved the final version of manuscript.

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ABSTRACT

The acute toxicity and parasympatholytic properties of methanol extract of *Arachis hypogea* leaves were studied *in vitro* and *in vivo*. Qualitative phytochemical screening revealed the presence of alkaloids, glycosides, fats and oils, phenols and lignins. Acute toxicity test showed that the extract was not toxic up to 5000 mg/kg body weight. Tonicity studies using rabbit jejunum showed a significant ($p<0.05$) relaxation effect on that smooth muscle. At 14.28 µg/ml, 29 µg/ml and 57.14 µg/ml, the extract inhibited *in vitro* acetylcholine-induced contraction of the rabbit jejunum by 84.21%, 86.84% and 89.47% respectively, which compared closely with the effect of atropine (90.21% at 0.28 µg/ml). In conclusion, the results of this study strongly indicated that the extract of

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Arachis hypogea leaves is safe at the tested dose and possess smooth muscle relaxant properties which may be of value in the management of diseases associated with excess activity of the parasympathetic arm of the autonomic nervous system.

Keywords: Acetylcholine; Arachis hypogea; parasympatholytic; relaxant; smooth muscle; T/oxicity.

1. INTRODUCTION

Herbal medicine in Nigeria is current enjoying a boost. This may be because the nation is host to hundreds of thousands of plants species, many of which have medicinal values [1]. Many of these plants have been exploited while a host of others remain uninvestigated. Researchers have continued to explore the systemic effects of these plant preparations with the intention to discover new drugs and or increase the potency of existing ones. *Arachis hypogea* is one of such medicinal plants that are being used to treat various ailments. According to Guyton and Hall, [2], Parasympatholytic agents are substances that block the action of neurotransmitter acetylcholine in the parasympathetic outflow and thereby inhibit cholinergic nerve impulses by selectively occupying the nic receptors to which acetylcholine molecules should bind to. This interaction between parasympatholytics and muscarinic receptors is the basis for the use of parasympatholytic agents to manage disorders caused by over activity of the parasympathetic innervation including diarrhea, incontinence, gastrointestinal cramps, gastritis, peptic ulcers, motion sickness with vomiting etc. The widespread use of the leaf extract of the plant for the management of diseases in ethnomedicine coupled with its anti-epileptic potential spurred this study designed to evaluate the tonic properties of methanol extract of *Arachis hypogea* leaves on rabbit jejunum.

2. MATERIALS AND METHODS

2.1 Plant Material

The leaves of *Arachis hypogea L*. *(peanut)* were collected in June 2016 from Umudike in Ikwuano Local Government Area of Abia State. Nigeria. A taxonomist, Prof. I.C.Okwulehie of Plant Science and Biotechnology. Michael Okpara University of Agriculture, Umudike. Taxonomically identified the plant, where a herbarium voucher specimen of *Arachis hypogea* leaves was deposited.

2.2 Animals

Three adult rabbits and 18 mice was used for the study. The rabbits were obtained from the Animal Houses of the College of Veterinary Medicine. Michael Okpara University of Agriculture, Umudike, Abia state Nigeria. The animals were acclimatized to laboratory conditions for two weeks, under laboratory conditions and had free access to food and water until the end of the experiments.

2.3 Preparation of Plant Material

The leaves of *Arachis hypogea* were collected in June 2016, washed with distilled water and dried at room temperature for 4 weeks. They were then pulverized into powder with a creston high speed milling machine. The powdered sample (500 g) was macerated in 2.5 litres absolute methanol for 48hr with regular shaking. After that, the resulting extract was wrung out with muslin cloth before filtering through Whatman no. 1 filter paper. The resulting filtrate was concentrated to dryness using rotary evaporator set at 40℃. The dry extract was stored in a refrigerator until used.

3. RESULTS

3.1 Qualitative Phytochemical Analyses of the Extract

The phytochemical screenings of the extract were done to detect the presence or otherwise of secondary metabolites using the standard methods described by trease and evans [3].

3.2 Acute Toxicity and Lethal Dose Test (LD_{50})

It was carried out with modification according to the method of Lorke [4]. A total of 18 mice was used. They were divided into two stages: I and II. In stage one (phase 1), the animals were placed three in (3) groups of three mice each, and were orally administered 10 mg/kg, 100 mg/kg and 1000 mg/kg body weight (b.wt) of the extract respectively. The stage (phase II) animals were given 160, 2900 and 5000 mg/kg b.wt. Orally. The mice were observed for signs of toxicity hourly in the first 12 hr and then daily for 7 days. They were observed for toxicity, signs of calmness and quietness, licking of forelimbs, passive movements, clustering together, prostration and death.

3.3 Preparation of Intestinal Smooth Tissue for *in vitro* Isometric Contraction Effect of Methanol Extract of *Arachis hypogea Leaves*

The method of Uchendu [5] was adopted. The rabbits were killed by stunning and decapitation. The abdomen was cut open and the jejunum carefully isolated and transferred into tyrode solution (pH7.4) that was continuously bubbled with air and maintained at 37℃. The jejunum, about 2-3 cm in length, was cut out and suspended vertically in a 35 ml organ bath by means of ligatures attached at one end to a tissue holder, and at the other end to an isometric force displacement transducer connected to a digital physiological recorder (Medicaid Physiopac) and computer screen for displaying isometric contractions. Resting tension in the muscle strip was readjusted, just sufficient to remove the slack. The preparation was allowed to equilibrate for 30 minutes after mounting. After regular rhythmic contractions were recorded, dose-response relationships were established for acetylcholine, atropine and *Arachis hypogea* methanol extract (AHME). Effective concentration (EC_{50}) values of the drugs were also administered in the presence of the antagonist, atropine for acetylcholine. EC_{50} doses of acetylcholine and atropine were also administered in the presence of AHME. For all administrations, a minimum time of 2 minutes was allowed for individual tissue responses before being washed 3 times with Tyrode solution. The concentrations of the tested substances presented in the text are all final bath concentrations (FBC), except otherwise indicated.

4. DISCUSSION

The leaf extracts of the *A. hypogea* analyzed are rich in phytochemicals. The results of the preliminary phytochemical screening of the leaves studied clearly showed that the leaves are nutritious and contained some phytochemicals such as alkaloids, glycosides, tannins, sterols, fats, oils, phenols and lignins. All these phytochemicals present in these leaves compared favorably with those reported from some medicinal plants found in eastern Nigeria [6].

Table 1. *In vitro* effect of acetylcholine on an isolated rabbit jejunum

FBC (µg/ml)	MBA (mm)	Amplitude In response to Ach(mm)	% rise in amplitude
0.0286	9.0± 0.13	19.00±0.02	52.63
0.057	8.90± 0.01	21.00±0.03	57
0.086	9.0± 0.02	22.00±0.02	59.09
0.11	9.0± 0.12	24.00±0.01	62.5

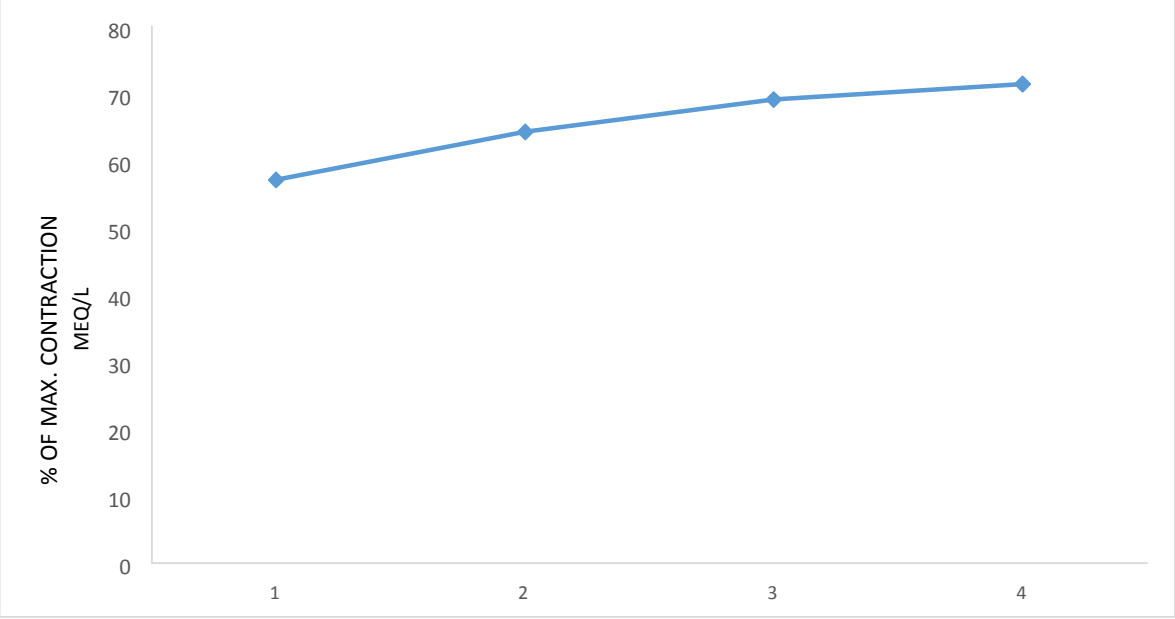


Figure 1. Dose response curve for acetycholine

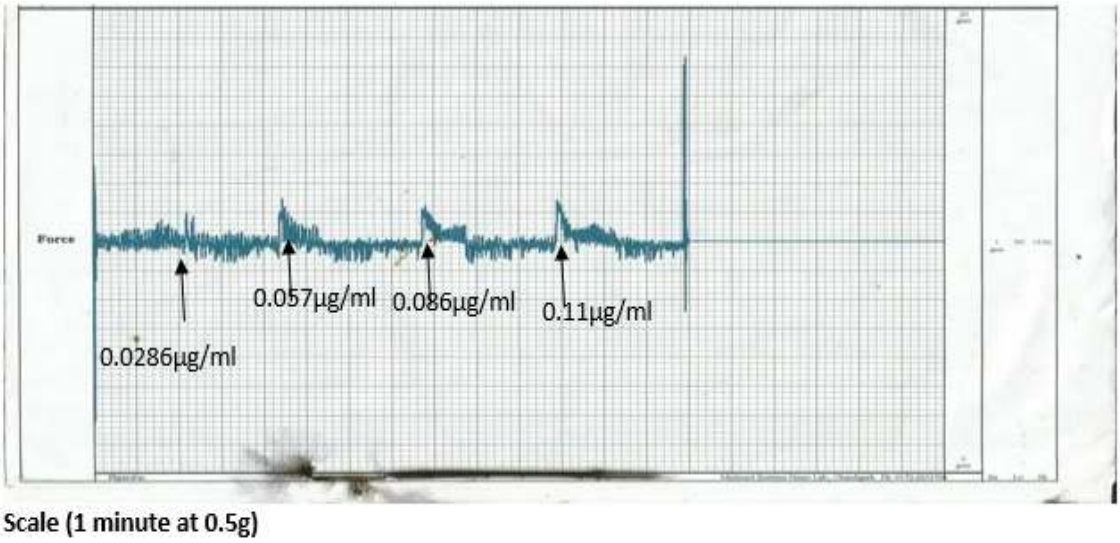
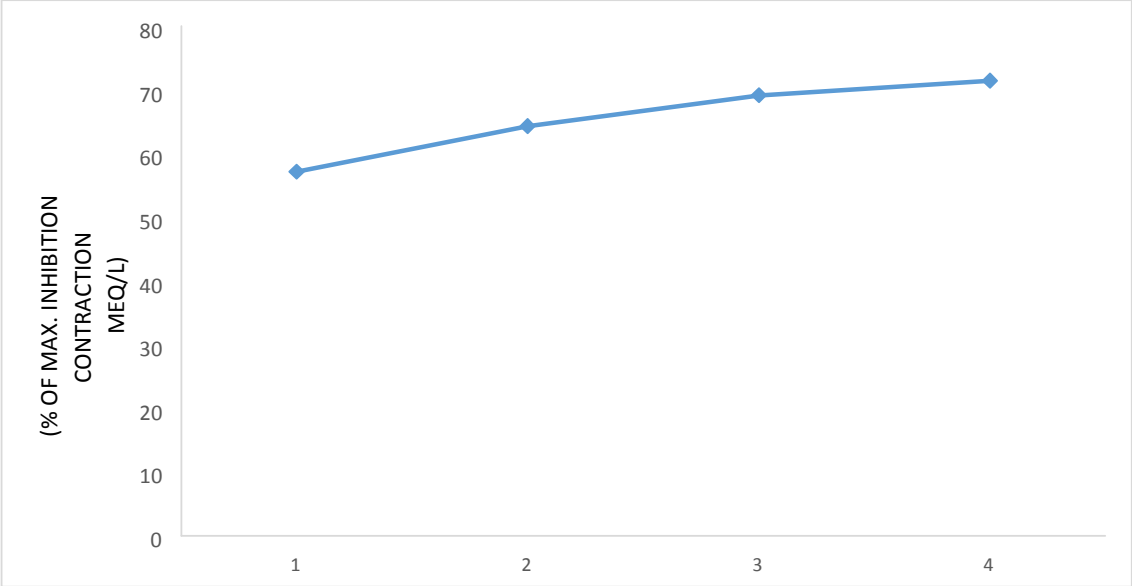


Figure 2. *In vitro* effect of acetylcholine on an isolated rabbit jejunum

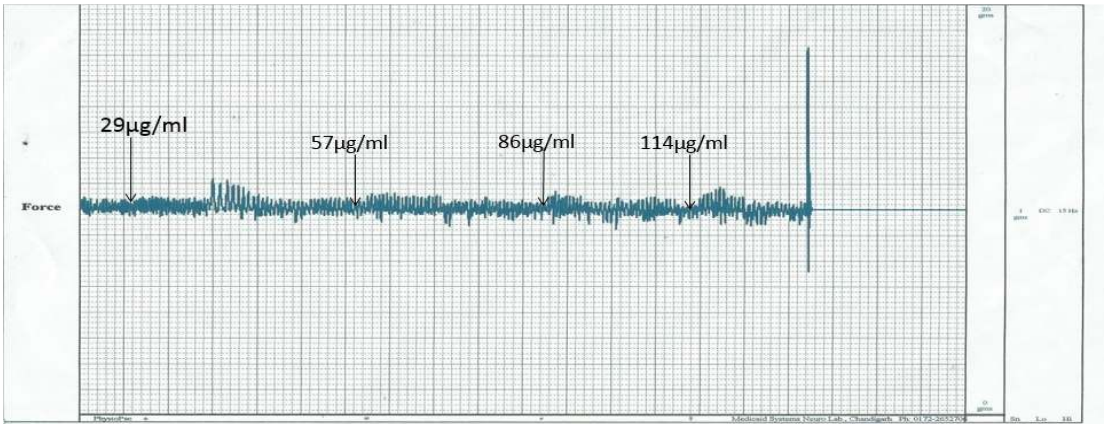


Dose response curve for methanol leaves extract of *Arachis hypogea*

Figure 3. *In vitro* effect of *Arachis hypogea* on an isolated rabbit jejunum

Table 2. *In vitro* effect of *Arachis hypogea* on an isolated rabbit jejunum

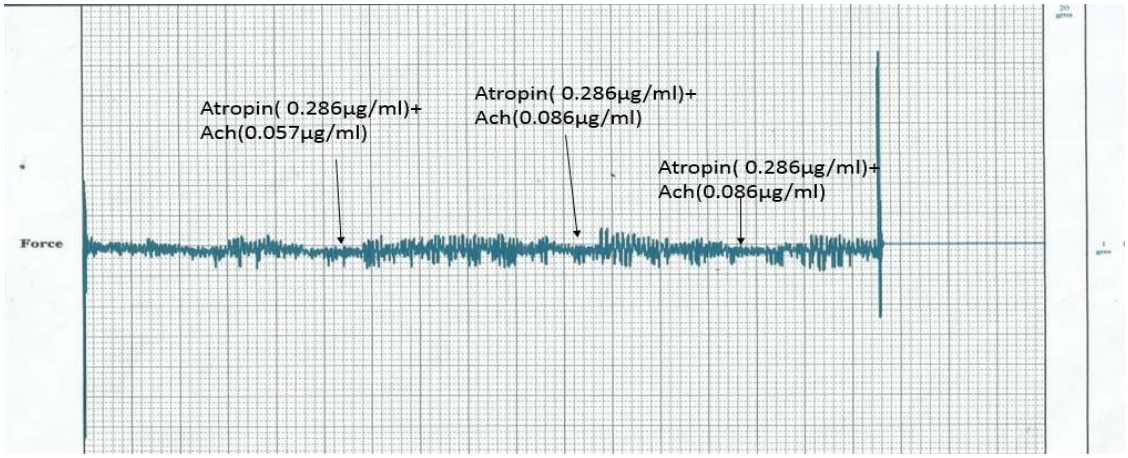
FBC (µg/ml)	MBA (mm)	Amplitude in response to AHME(mm)	% inhibition
29	7.0± 0.13	3.00±0.02	57.14
57	7.05± 0.01	2.50±0.03	64.29
86	7.0± 0.02	2.16±0.02	69.14
114	7.0± 0.12	2.00±0.01	71.43



Scale (1 minute at 0.5g)

Figure 4. *In vitro* effect of *Arachis hypogea* on an isolated rabbit jejunum

From the tracing the extract shows significant (P<0.05) relaxation of the rabbit jejunum at the tested doses



Scale (1 minute at 0.5g)

Figure 5. *In vitro* effect of *atropin*on acetylcholine-induced contractions on the isolated rabbit jejunum

A final bath concentration of 0.029µg/ml of atropine significantly (P<0.05) inhibited the effect of Acetylcholine on the rabbit jejunum

Table 3. Phytochemical profile of methanolic extract of *Arachis hypogea* leaves

Alkaloids	Gragendorff's test	+
	Wagner's test	+
Glycosides	Molisch test	+
	Bromine water test	+
Tannins	$FeCl_3$test	-
	Lead acetate test	-
Flavonoids	Lead acetate test	-
	$FeCl_3$test	-
Sterols	Salkowski's test	-
	LibermannBurchard test	-
Fats & oils	Stains test	+
Phenols	Elagic acid test	+
	$FeCl_3$ test	-
Lignins	Furfuraldehyde test	+
Saponins	Foam test	-

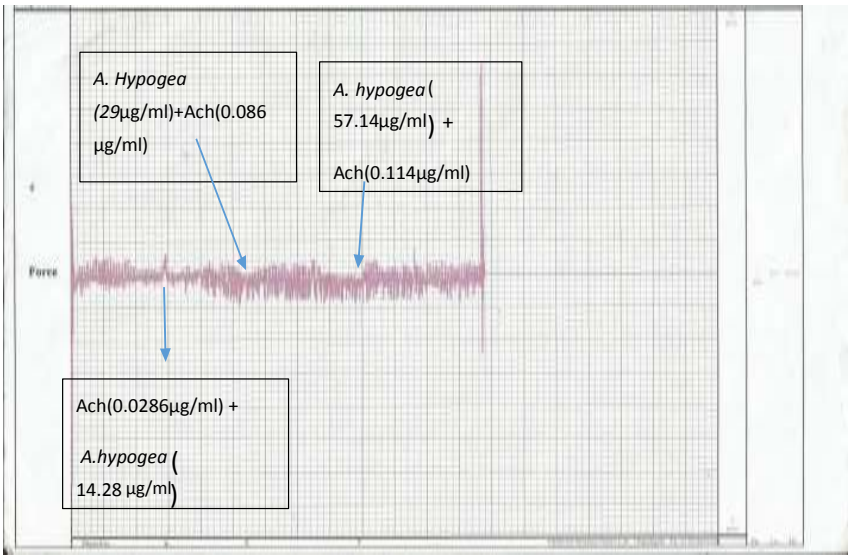


Figure 6. *In vitro* effect of *Arachis hypogea* on acetylcholine-induced contractions on the isolated rabbit jejunum

A final bath concentration of 86µg/ml of AHME significantly (P<0.05) inhibited the effect of Acetylcholine on the rabbit jejunum. The inhibitory effect of AHME compared favorably with that of Atropine (0.029µg/ml), a standard muscarinic receptor blocker

It is established that the smooth muscles of the gastrointestinal tract is host to numerous muscarinic receptors of both M2 and M3 subtypes which play major role in intestinal contractility and peristaltic activity. While the M3 receptors does so by triggering phosphoinositide hydrolysis, Ca^{2+} mobilization and direct contractile response, M2 subtype does same by inhibiting adenylcyclase and Ca2+ activated K+ channels and potentiating Ca2+ dependent, non-selective conductance [7,8,9]. Thus, the administered acetylcholine in the *in vitro* experiment generated inositol,1,4,5-triphosphate (IP3) which evoked Ca2+ release from intracellular storage sites in the rabbit GIT smooth muscle cells and elicited contractions in the isolated tissue [5]. Atropine (1 mg/kg), a standard parasympatholytic agent inhibited the contractions induced by acetylcholine in the experiments conducted by competitively binding to muscarinic receptors [10]. *Arachis hypogea* methanol extract (AHME) exerted a strong inhibitory effect on the rhythmic contractions of the isolated rabbit jejunum in a dose independent fashion and also significantly ($p< 0.05$) blocked acetylcholine induced contractions. The results suggest that AHME may contain parasympathometic agents and may have achieved its effect by antagonizing the activity of acetylcholine via binding to available muscarinic receptors and as a result inhibiting intestinal peristaltic contractions as was observed.

By blocking cholinergic pathway, the extract may display a typical adrenergic property of increasing blood flow to the arteries and arterioles of the penis, thus causing and sustaining erection. It is indeed established that agents which block cholinergic pathway usually raise blood pressure by increasing the force and rate of contraction of heart muscles which usually favors the erection process [10].

5. CONCLUSION

In conclusion, the inhibitory effect of *Arachis hypogea* methanol leaf extract on the rhythmic contraction of the rabbit jejunum coupled with its significant blockade of acetylcholine induced contractions in the *in vitro* experiments suggests that the extract may contain substances with potent anticholinergic properties and may be of value in the management of diseases like diarrhea, Asthma, incontinence, peptic ulcers, muscular spasmsetc and may further become a

template for yet another synthetic anticholinergic agent of clinical significance.

CONSENT

It is not applicable.

ETHICAL APPROVAL

All authors hereby declare that all experiments have been examined and approved by the Institutional ethics committee.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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