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Methanol extract of *Tephrosia vogelii* leaves potentiates the contractile action of acetylcholine on isolated rabbit jejunum

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ABSTRACT

Objective: To investigate the modulating role of methanol extract of *Tephrosia vogelii* leaves on acetylcholine (ACh)-induced contraction of isolated rabbit jejunum.
Methods: Rabbit jejunum segment was removed and placed in an organ bath containing Tyrode's solution, and its contractions were recorded isometrically.
Results: ACh (2.0×10^{-10} g/mL) and the extract (2.0×10^{-4} g/mL) individually increased the frequency of contraction (mean ± SEM) of the isolated smooth muscle tissue by 47.6% ± 9.5% and 77.8% ± 66.5%, respectively. When ACh and the extract were combined, the frequency of contraction of the tissue was increased by 222.2% ± 25.9%, representing a 366.7%-increase ($P < 0.001$) over the effect of ACh alone. Similarly, ACh (2.0×10^{-9} g/mL) and the extract individually increased significantly ($P < 0.001$) the amplitude of contraction of the tissue by 685.7% ± 61.1% and 455.2% ± 38.1%, respectively. When ACh and the extract were combined, the amplitude of contraction of the tissue rose by 1 263.8% ± 69.0%, representing 84.3% increase over the effect of ACh alone.
Conclusions: The findings demonstrate that methanol extract of *Tephrosia vogelii* leaves potentiates the contractile effect of ACh on intestinal smooth muscle, supporting the traditional claim that the plant is purgative.

1. Introduction

The plant *Tephrosia vogelii* (*T. vogelii*) Hook. f. (Fabaceae) is widely grown in the tropics and subtropics [1], especially in Africa and India [2]. It is used for numerous ethnomedical purposes, including purgative and emetic ones [2,3]. Although its use as insecticide and piscicide has been a subject of scientific investigations for many years [4–10], its purgative activity has received little scientific attention. The methanol extract of *T. vogelii* leaves induced contraction of the guinea pig ileum [11], and increased the spontaneous contractions of the rabbit jejunum [12]. The effects of the extract on the intestinal smooth muscle were mediated, apparently, through muscarinic cholinergic receptors, involving the mobilization of extracellular calcium ions [13]. Agents that increase intestinal motility, including cholinergic agonists such as acetylcholine (ACh), are good laxatives because they decrease absorption of salt and water, secondary to decreased transit time [14,15]. In sufficiently high dosage, many laxatives promote catharsis, which implies purgation and a more fluid evacuation [14].

The aim of the present study was to determine the modulating role of the methanol extract of *T. vogelii* leaves on ACh-induced intestinal motility.

2. Materials and methods

2.1. Plant collection and authentication

Fresh leaves of *T. vogelii* were harvested close to the end of the rainy season (15th October) at Bafai-Gora village near

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Zango-Kataf (10°52′ N, 7°43′ E), Northern Nigeria. The plant was identified and authenticated by a botanist, Mallam Sabo A. Abubakar, in the Savannah Herbarium, National Animal Production Research Institute, Shika-Zaria, Nigeria.

2.2. Preparation of the crude extract

The leaves were air-dried, and ground into powder with a grinding machine ("CHRISTY" Chelmsford–England, Machine Type 8 Lab. Mill) with sieve diameter of 0.5 mm^2 at 8 000 r/min. Methanol extraction (methanol, Analar®, BDH, England) of the dried ground leaves was carried out using a Soxhlet extractor. The extract was evaporated to dryness under pressure using a rotary evaporator. Assay extract was prepared by dissolving the methanol extract in deionized water and filtering the solution obtained with medical cotton wool [11–13].

2.3. Animals

Rabbits (*Oryctolagus cuniculus*) (n = 4) of both sexes, weighing 1 742–1 842 g, were purchased from Department of Animal Science, Ahmadu Bello University, Zaria, Nigeria. They were kept in rabbit cages and housed in an animal pen in the Faculty of Pharmaceutical Sciences, Ahmadu Bello University, Zaria, under husbandry conditions for rabbits until use. They were fed with chicken grower's mash (Pfizer) combined with cabbage or grasses. Tap water was provided *ad libitum*. The rabbits were generally used according to guideline for use and care of animals [16].

2.4. Isolation and preparation of rabbit jejunum

Modified Magnus technique was used [13,17]. Briefly, the rabbits were stunned, and the abdomen opened with a pair of scissors. The intestines were gradually removed and sections of the jejunum were cut. Suitable lengths (2–3 cm) were fixed with a tissue clamp and suspended in 25 mL organ bath (Ugo Basile S.R.L., Varese, Italy) containing Tyrode's solution. The Tyrode's solution comprised the following compounds in g/L of deionized water: NaCl, 8.0; KCl, 0.2; $MgCl_2$, 0.1; $CaCl_2$, 0.2; $NaH_2PO_4 \cdot 2H_2O$, 0.05; $NaHCO_3$, 1.0; glucose, 1.0. The solution was oxygenated with air bubbles using an air pump (Gast®, Benton Harbor, Michigan, USA), and maintained at 37°C using a thermocirculator (Churchill Thermocirculator, Perivale, Middlesex, England). The lower end of the tissue was attached to an oxygenated tube, while the upper end was fixed to an isometric force transducer. A preload of 1 g was chosen. The transducer was connected to a microdynamometer, model 7050 (Ugo Basile, Milan, Italy, www.ugobasile.com), set at a paper speed of 24 mm/min and sensitivity of 2.0 mV, for recording of responses. After a pre-incubation time of 30 min, the experiments were started.

2.5. Application of test substances to the isolated rabbit jejunum

Calculated volumes of the constituted extract and ACh (acetylcholine hydrochloride, Sigma, St. Louis, USA) were measured separately and introduced individually into the organ bath containing the tissues, by using 1 mL (insulin) syringes, after which the same concentrations of both were introduced simultaneously. The test substances were allowed to be in contact with the isolated tissues for 25 s, after which the tissues were washed three times by emptying and refilling the organ bath with fresh Tyrode's solution. The paper speed button on the recording microdynamometer was switched off before washing the tissues, and the tissues were allowed to recover before it was switched on. The time interval between each application of test substances was 5 min.

2.6. Statistical analysis

In all cases, the frequency (in contractions per minute, cpm) and amplitude (in mm) of spontaneous activity were taken as an average over 25 s before and 25 s after treatment with test substances. The data were expressed as mean ± SEM. The Student's t-test was used to determine differences between test and control values [18]. Values of $P < 0.05$ were considered statistically significant.

3. Results

Two samples-recorded tracings of the effects of ACh and/or *T. vogelii* leaf extract were shown in Figure 1. A combination of ACh (2.0×10^{-9} g/mL) and the extract (2.0×10^{-4} g/mL) induced an apparently similar increase in amplitude of contraction to that evoked by ACh alone. The responses were reversed after each wash-out. When the concentration of ACh in the bathing medium was reduced to 2.0×10^{-10} g/mL, its combination with the extract (2.0×10^{-4} g/mL) increased the amplitude of contraction of the tissue, apparently similar to that induced by ACh alone at a higher concentration of 4.0×10^{-10} g/mL. Subsequent application of the extract (2.0×10^{-4} g/mL) alone increased the amplitude of contraction of the tissue, which was apparently similar to that produced earlier by ACh (2.0×10^{-10} g/mL) alone. Thus, the application of ACh (4.0×10^{-10} g/mL) alone elicited an increase in contraction, with a predominant increase in the tonicity of the smooth muscle contraction. This tonic contraction became more pronounced, when ACh was applied into the medium at a lower concentration of 2.0×10^{-10} g/mL. A second treatment of the preparation with combined ACh and the extract at 2.0×10^{-10} g/mL and 2.0×10^{-4} g/mL, respectively, gave rise to a more tonic contraction, even after washing. Thereafter, treatment of the smooth muscle with the extract at the usual concentration of 2.0×10^{-4} g/mL singly induced tonic contractions of the smooth muscle.

The effects of ACh and/or *T. vogelii* leaf extract on the frequency and amplitude of contraction of the isolated rabbit jejunums (n = 4) were shown in Table 1. ACh at a high bathing concentration of 2.0×10^{-9} g/mL slightly increased the frequency ($P > 0.05$), but highly increased the amplitude ($P < 0.001$) of contraction of the isolated tissue by 26.1% ± 16.7% and 685.7% ± 61.1%, respectively. At a lower bathing concentration of 2.0×10^{-10} g/mL, ACh increased significantly ($P < 0.001$) both the frequency and amplitude of contractions of the isolated jejunum by 47.6% ± 9.5% and 568.0% ± 60.6%, respectively. When the extract was added alone to the bathing medium of the tissue at the concentration of 2.0×10^{-4} g/mL, the frequency of contraction of the tissue increased insignificantly ($P > 0.05$) by 77.8% ± 66.5%, from 5.4 ± 1.8 cpm to 9.6 ± 2.8 cpm, but the amplitude rose

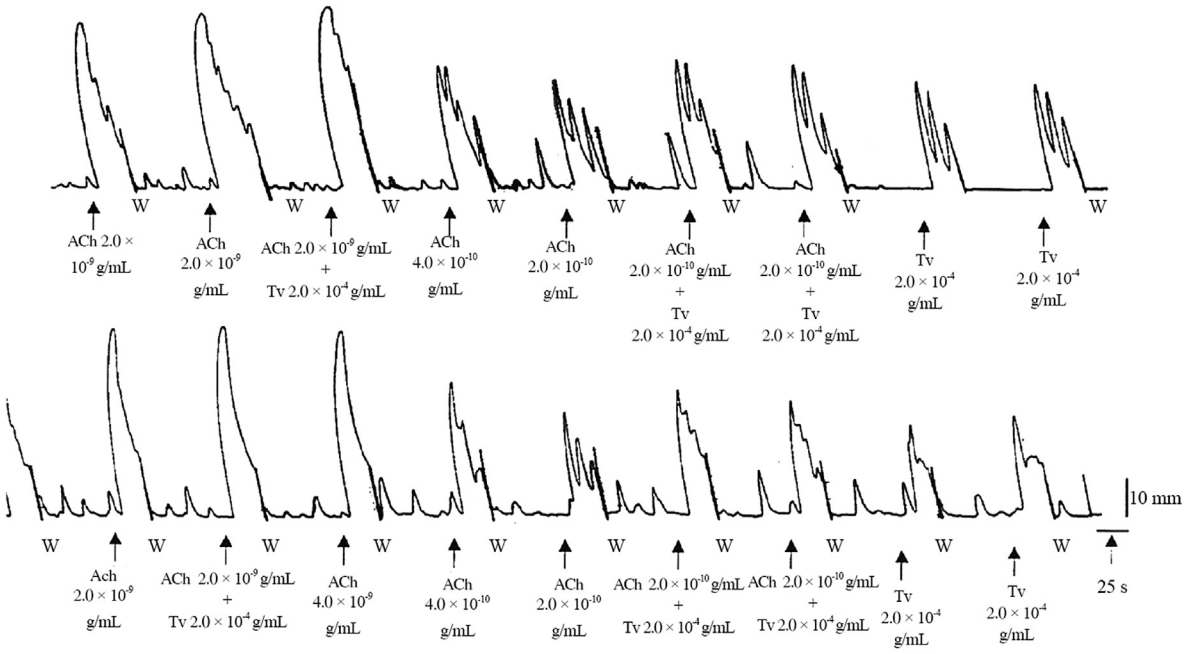

Figure 1. Effects of ACh and *T. vogelii* leaf extract on *in vitro* contraction of rabbit jejunum segments.
Tv: *T. vogelii* leaf extract; W: Wash-out.

Table 1
Effects of interaction between ACh and *T. vogelii* leaf extract on the frequency and amplitude of contraction of isolated rabbit ($n = 4$) jejunums.

Test substance (g/mL)	Frequency			Amplitude		
	Control (cpm)	Test (cpm)	% Change	Control (mm)	Test (mm)	% Change
ACh (2.0×10^{-9})	6.9 ± 1.4	8.7 ± 0.9	26.1 ± 16.7^{NS}	4.0 ± 1.2	31.3 ± 1.8	685.7 ± 61.1***
ACh (2.0×10^{-10})	6.3 ± 0.6	9.3 ± 0.3	47.6 ± 9.5***	3.3 ± 1.0	21.7 ± 1.9	568.0 ± 60.6***
T. vogelii leaf extract (2.0×10^{-4})	5.4 ± 1.8	9.6 ± 2.8	77.8 ± 66.5^{NS}	2.9 ± 0.7	15.9 ± 0.8	455.2 ± 38.1***
ACh (2.0×10^{-9}) + Tv (2.0×10^{-4})	8.1 ± 1.3	6.9 ± 1.7	−14.8 ± 8.6^{NS}	3.3 ± 0.9	44.9 ± 3.1	1263.8 ± 69.0***
ACh (2.0×10^{-10}) + Tv (2.0×10^{-4})	2.7 ± 0.3	8.7 ± 0.9	222.2 ± 25.9***	8.1 ± 2.5	25.8 ± 1.7	217.0 ± 40.9***

Values: Mean ± SEM; ^{NS}: $P > 0.05$; ***: $P < 0.001$.

significantly ($P < 0.001$) by 455.2% ± 38.1%, from 2.9 ± 0.7 mm to 15.9 ± 0.8 mm.

When ACh at the high concentration of 2.0×10^{-9} g/mL was added to the bathing medium with the extract (2.0×10^{-4} g/mL) at the same time, the frequency of contraction of the isolated smooth muscle tissue decreased non-significantly ($P > 0.05$), while the amplitude of contraction of the tissue rose ($P < 0.0001$) by 1263.8% ± 69.0%, from 3.3 ± 0.9 mm to 44.9 ± 3.1 mm, representing 84.3% increase over ACh (2.0×10^{-9} g/mL) effect alone. A combined application of ACh at the lower concentration of 2.0×10^{-10} g/mL with the extract (2.0×10^{-4} g/mL) significantly ($P < 0.001$) raised the frequency of contraction of the isolated smooth muscle by 222.2% ± 25.9%, from 2.7 ± 0.3 cpm to 8.7 ± 0.9 cpm, representing a 366.7%-increase over ACh (2.0×10^{-10} g/mL) effect alone; while the amplitude of contraction rose by 217.0% ± 40.9%, but represented a 61.8%-decrease over the effect of ACh alone.

4. Discussion

The results show that ACh at a high concentration of 2.0×10^{-9} g/mL and the extract (2.0×10^{-4} g/mL) individually increased significantly the amplitude without any significant effect on the frequency of contraction of the isolated rabbit jejunum. However, a lower concentration of ACh (2.0×10^{-10} g/mL) significantly raised both the frequency and amplitude of contraction of the smooth muscle tissue. Furthermore, combinations of extract and ACh (2.0×10^{-9} g/mL) of high concentration had significant influence only on the amplitude, while that with ACh (2.0×10^{-10} g/mL) of a low concentration increased significantly both frequency and amplitude of contraction of the tissue. The results support the earlier findings that methanol extracts of *T. vogelii* leaves may act on the same muscarinic receptor sites as ACh to induce contraction of intestinal smooth muscle [13]. The results suggest that occupation of more receptor sites by ACh at a high concentration (2.0×10^{-9} g/mL) seemingly did not allow further increase in frequency of contraction by the extract [19–21], while the increase in amplitude of contraction was enhanced.

The results also show that a high concentration of the extract (2.0×10^{-4} g/mL) increased the amplitude of contraction of the tissue similar to those induced by ACh at relatively low concentrations (2.0×10^{-10} g/mL); apparently, because the extract was used in its crude form. Fractional separation/partitioning of the extract and subsequent isolation/identification of the active ingredients with testing of the contractile activity may be required [22–24]. This is because the efficacy of the *T. vogelii* extract may be due to the presence of one or more biologically active principles [1,2]. Indeed, pharmacological

assays have shown that the activity of crude plant extracts is not always due to the main components, but the minor ones, or even to the synergism of all the active principles [22,25,26]. Thus, the activity may be enhanced or diminished after isolation of individual active principles.

The increase in frequency of contraction of the tissue induced by a combination of ACh (2.0×10^{-10} g/mL) and the extract (2.0×10^{-4} g/mL) was more than double that induced by either ACh or the extract when applied singly. The result indicates that the combined action of the extract and ACh on the frequency of contraction of the tissue is synergistic [27]. Furthermore, the combined effect of ACh (2.0×10^{-9} g/mL) and the extract (2.0×10^{-4} g/mL) on the increase in amplitude of contraction of the tissue was more than, but less than double, that of ACh when applied singly. The results suggest that the extract of *T. vogelii* leaves potentiated the contractile action of ACh on the amplitude of contraction of the isolated rabbit jejunum, when submaximal concentrations of both were interacted [28]. The findings of the present study support the use of extracts of *T. vogelii* leaves as purgative in ethnomedical and -veterinary practices.

The extract of *T. vogelii* leaves potentiated the contractile action of ACh on the amplitude of contraction of the isolated rabbit jejunum; the combined action of the extract and ACh on the frequency of contraction of the tissue was synergistic. The findings provide further scientific basis for the ethnomedical and -veterinary use of *T. vogelii* leaf extract as a purgative.

Conflict of interest statement

We declare that we have no conflict of interest.

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