

DZENDA et al: Contraction of ileum by *T. vogelii*.

CONTRACTION OF ISOLATED GUINEA PIG ILEUM BY *TEPHROSIA VOGELII*
EXTRACT

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SUMMARY

An investigation was carried out on the isolated guinea pig ileum, using the crude methanolic leaf extract of *Tephrosia vogelii* Hook. f., and with the aim of determining its effects on contraction of intestinal smooth muscle. Modified Magnus technique was employed in setting up the tissue. Acetylcholine (ACh), histamine and serotonin (5-hydroxytryptamine, 5-HT) were used as standard agonists with which the effects of the extract of *T. vogelii* were compared. The extract concentration-dependently (3.2×10^{-5} - 4.10×10^{-3} g/ml) contracted the isolated muscle tissue ($n = 7$) in a similar fashion as did ACh (4.0×10^{-9} - 5.12×10^{-7} g/ml), histamine (4.0×10^{-9} - 5.12×10^{-7} g/ml) and 5-HT (3.2×10^{-8} - 4.10×10^{-6} g/ml). The maximal contractile effect (E_{max}) of the extract on the tissue was found to be 48.39 %, 48.61 % and 61.56 % that of ACh, histamine and 5-HT, respectively. The effective concentration of the extract that produced half of its maximal effect (EC_{50}) was calculated to be 2.37×10^{-4} g/ml, whereas the values for ACh, histamine and 5-HT were 2.10×10^{-8} , 3.76×10^{-8} and 1.88×10^{-7} g/ml, respectively. In conclusion, the methanolic leaf extract of *T. vogelii* exerted stimulatory effects on the intestinal smooth muscle which may be likened to those of standard agonists like ACh, histamine and 5-HT. The results provided some scientific basis for the use of *T. vogelii* leaves as a purgative in traditional medicine.

KEY WORDS: Acetylcholine, Histamine, Ileum, Purgative, *Tephrosia vogelii*.

INTRODUCTION

Tephrosia vogelii Hook. f. (*Fabaceae*) is widely grown in the tropics, especially in Africa and India, for numerous ethnomedical and veterinary uses (Lambert *et al.*, 1993); for instance as purgative and emetic (Burkill, 1995). Whereas its use as piscicide (Bossard, 1993; Ibrahim *et al.*, 2000) and insecticide (Chiu, 1989; Kaposhi, 1992; Ogendo *et al.*, 2004; Koono and Dorn, 2005) has been extensively studied, its action as a purgative has not been widely investigated (Dzenda *et al.*, 2006). Since most purgatives exert their action by increasing the contractile movements of the intestines, thereby decreasing gastro-intestinal tract (GIT) transit time (Brunton, 1996), the aim of the present study was to investigate the effects of *T. vogelii* extract on the contraction of intestinal smooth muscles. It was conceivable that the results of the present study may contribute to the establishment of a scientific basis for the use of *T. vogelii* as purgative.

MATERIALS AND METHODS

Preparation of plant extract

Fresh leaves of *Tephrosia vogelii* were collected at Bafai-Gora, 12 km south of Zonkwa (9°47' N, 8°18' E), Kaduna State, Nigeria. The plant was identified and authenticated by Mal. S. Abubakar, Savannah Herbarium, National Animal Production Research Institute, Shika, Zaria; with voucher specimen number: T. Dzenda 1. The leaves were air-dried, and ground into powder using a grinding machine ("CHRISTY" Chelmsford - England, Machine type 8 lab. mill) with sieve diameter of 0.5 mm² at 8,000 rpm. Crude methanolic (methanol, Analar[®], BDH, England) extraction of the leaves was carried out using a Soxhlet extractor. The extract was evaporated to dryness under pressure using a rotary evaporator, which yielded 18.53% w/w. Assay extract was prepared by dissolving the extract in de-ionized water and then filtering with medical cotton wool. Extract stock solution of 100 mg/ml was prepared, from which serial dilutions were made to obtain relevant concentrations.

Preparation of rabbit jejunum

The research was conducted in accordance with

the internationally accepted principles for laboratory animal use and care as contained in the European Community guidelines (EEC Directive of 1986; 86/609/EEC). Modified Magnus' technique (Vogel and Vogel, 1997) was used. Seven local rabbits of both sexes, and weighing 742-1,042 g were stunned and eviscerated. The intestines were gradually removed and the jejunum was cut into sections of 2-3 cm. Each section was fixed with a tissue clamp and suspended in 25 ml organ bath (SRI), containing Tyrode's solution. The solution was oxygenated with air bubbles using an air pump (Gast[®], Benton Harbor, Michigan-USA), and maintained at 37°C using a thermocirculator. A preload of 1 g was chosen. The transducer was connected to a microdynamometer 7050 (Ugo Basile, Milan, Italy), at a paper speed of 24 mm/min and sensitivity of 2, for recording of responses. A pre-incubation time of 30 min was allowed.

Application of test substances to the isolated guinea pig ileum

Calculated volumes of the constituted extract and drug solutions were introduced into the organ bath containing the tissues, using 1 ml (insulin) syringes. The extract and drugs were allowed to be in contact with the isolated tissues for 25 seconds, after which the tissues were washed three times by emptying and refilling the organ bath with fresh Tyrode's solution. The time interval between application of each drug was 5 min. Increasing concentrations of acetylcholine (ACh, acetylcholine hydrochloride, Sigma, USA), histamine (histamine hydrochloride, Sigma, USA), 5-hydroxytryptamine (5-HT, 5-hydroxytryptamine creatine sulphate, May and Baker, England) and the extract of *T. vogelii* were introduced into the bathing medium until maximum effect was obtained for each agonist.

Statistical analysis

The data were expressed as mean \pm standard error of the mean (Mean \pm SEM), and subjected to Student's *t*-test. Values of $P < 0.05$ were considered significant. Calculation of the mean

effective concentrations (EC_{50}) was done using linear regression and according to the method of least squares (Snedecor and Cochran, 1980).

RESULTS

The results of the study are presented in Table I and in Figures 1 and 2. The isolated guinea pig ileum produced no apparent spontaneous contraction, and no rhythmic contractions were observed in the ileum before addition of test substances. Application of extract to the bathing medium at the concentration of 3.2×10^{-5} g/ml did

not elicit any significant contraction of the guinea pig ileum (Fig. 1d). A gradual increase in the concentration of the extract to 1.28×10^{-4} g/ml induced an increase in the amplitude of contraction, which lasted about 25 s. Higher concentration of the extract (2.56×10^{-4} g/ml) further increased amplitude of the contraction. At 5.12×10^{-4} g/ml, amplitude of the contraction progressively increased, but the duration of the tonic contraction was shorter than those obtained at lower concentrations.

TABLE I: E_{max} , concentration producing E_{max} , EC_{50} and intrinsic activity values of acetylcholine (ACh), histamine, 5-hydroxytryptamine (5-HT) and the methanolic leaf extract of *Tephrosia vogelii* obtained on the isolated guinea pig ileum (n=7).

Test Substance	E_{max} (mm) (Mean \pm SEM)	E_{max} (%) (Mean \pm SEM)	Concentration Producing E_{max} (g/ml)	EC_{50} (g/ml)	Intrinsic Activity
ACh	30.71 ± 4.77^a	100.00 ± 15.53^a	1.280×10^{-7a}	2.10×10^{-8a}	1.0000 ^a
Histamine	30.57 ± 5.50^a	99.54 ± 17.91^a	2.560×10^{-7b}	3.76×10^{-8b}	0.9954 ^a
5-HT	24.14 ± 5.41^b	78.61 ± 17.62^b	2.048×10^{-6c}	1.88×10^{-7c}	0.7861 ^b
Extract	14.86 ± 3.52^c	48.39 ± 11.46^c	1.024×10^{-3d}	2.37×10^{-4d}	0.4839 ^c

^{a, b, c, d} = Values in the same column with different superscript alphabets are significantly different ($P < 0.05$); n = Number of animals; SEM = Standard error of the mean; E_{max} = Effective maximum tissue response; EC_{50} = Effective concentration producing half the maximal response; Intrinsic Activity = Proportionality constant.

The minimum amplitude of contraction of the ileum was obtained at the concentration of 3.2×10^{-5} g/ml, while the maximum contraction was obtained at the concentration of 2.048×10^{-3} g/ml. *Tephrosia vogelii* extract concentration of 4.096×10^{-3} g/ml induced a lower amplitude and shorter duration of contraction compared to that obtained at the lower concentration (2.048×10^{-3} g/ml) (Fig. 1d). The crude methanolic leaf extract of *Tephrosia vogelii* elicited the effective maximum tissue response (E_{max}) of 14.86 ± 3.52 mm on the isolated guinea pig ileum (n = 7) at a final bath concentration of 1.024×10^{-3} g/ml (-log. 2.99) (Fig. 2).

The extract concentration that produced half of this maximal response (EC_{50}) was calculated to be 2.37×10^{-4} g/ml whereas those of ACh, histamine and 5-HT were 2.10×10^{-8} , 3.76×10^{-8} and 1.88×10^{-7} g/ml, respectively (Table I). Although the EC_{50} value of the extract was in the order of 10^{-4} , those of the standard test substances were in the order of 10^{-7} (5-HT) and 10^{-8} (ACh; histamine). The maximal contractile action of the extract on the isolated guinea pig ileum was calculated to be 48.39% that of ACh, 48.61% that of histamine and 61.56% that of 5-HT.

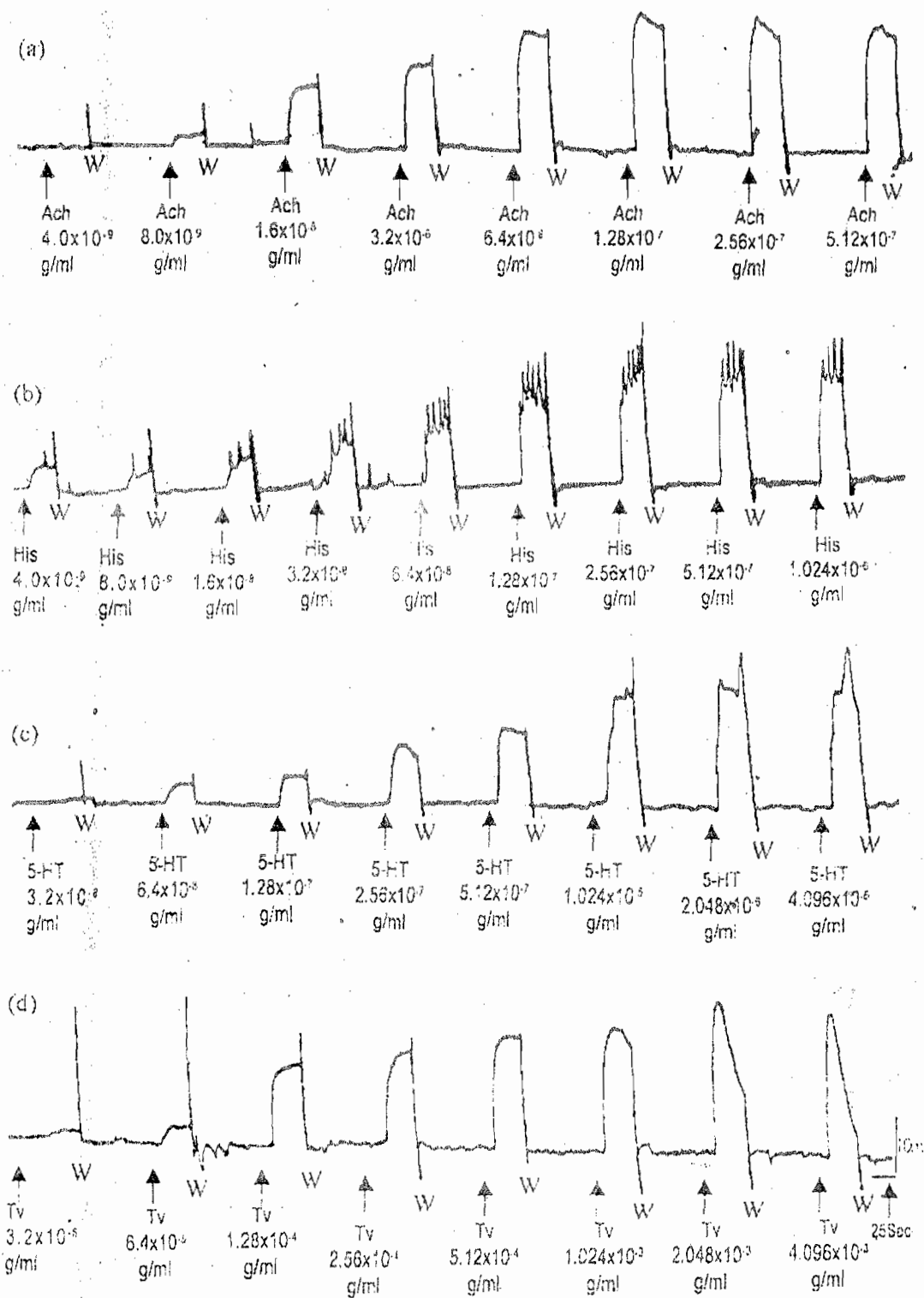


FIGURE 1: Concentration-dependent effects of acetylcholine (Ach) (a), histamine (His) (b), 5-hydroxytryptamine (5-HT) (c), and *Tephrosia vogelii* leaf extract (Tv) (d) on the isolated guinea pig ileum.

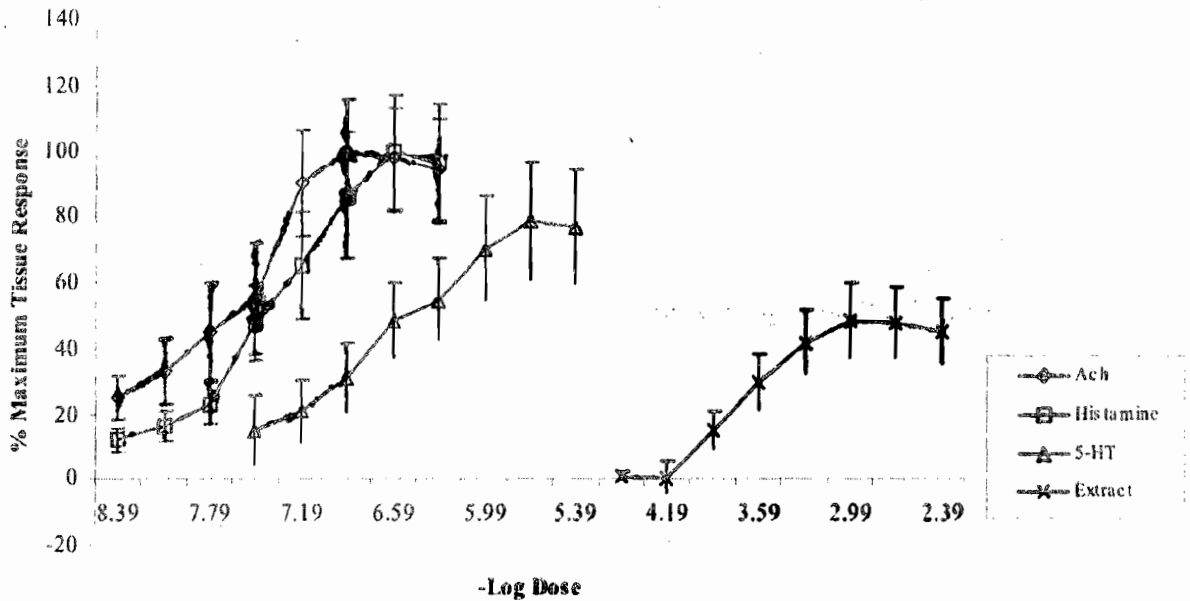


FIGURE 2: Comparative contractile effects of acetylcholine (ach), histamine, 5-hydroxytryptamine (5-HT), and *Tephrosia vogelii* leaf extract on isolated guinea pig ileum.

DISCUSSION

The results of the present study showed that the methanolic extract of *Tephrosia vogelii* leaves concentration-dependently contracted the isolated guinea pig ileum in a similar fashion as did ACh, histamine and 5-HT (Fig. 1). The results agreed with that of a related study carried out on the isolated rabbit jejunum (Dzenda *et al.*, 2006). ACh induced the highest contraction of the isolated guinea pig ileum, followed closely by histamine, then 5-HT, and lastly the extract (Fig. 2). The results indicated that ACh and histamine were most efficacious, followed by 5-HT, and the extract. Furthermore, ACh had the lowest EC₅₀ value, followed closely by histamine, then 5-HT, and the extract. The results showed that ACh and histamine were most potent, followed by 5-HT, and then the extract. They confirmed the action of the test neurotransmitters (Caputo, 1978; Horowitz *et al.*, 1996; Uguru *et al.*, 1997), and further demonstrated that of the extract on the tissue. These findings agreed with those of Dzenda *et al.* (2004, 2005) that *T. vogelii* leaf extract potentiated the action of ACh and acted on muscarinic receptors *in vitro*.

The relatively low efficacy and potency of the extract obtained in the present study could be due to its crude state. Investigation of the extract showed that *Tephrosia vogelii* leaves contained flavonoids, which include deguelin, tephrosin (Sharma and Khanna, 1975; Marston *et al.*, 1984; Lambert *et al.*, 1993), elliptone (Sharma and Khanna, 1975), isoquercetrin, quercetin, rutin (Marston *et al.*, 1984), rotenolone (Lambert *et al.*, 1993) and rotenone (Barnes and Freyre, 1967; Sharma and Khanna, 1975; Lambert *et al.*, 1993). The efficacy of the extract could be due to the presence of one or more of the biologically active principles (Galeffi, 1980). However, it is not uncommon for crude extracts from plants to possess two opposite effects because of the nature of their numerous active principles (Anwar *et al.*, 1999). Quercetin, for example, has been reported to inhibit spontaneous phasic contractions of the guinea pig ileum concentration-dependently (Galvez *et al.*, 1996).

The results of the present study demonstrated that the extract of *T. vogelii* leaves induced a graded contraction of the smooth muscle of the GIT, the severity of which depended on the

concentration of the extract. This was evidenced by the progression and increases in the amplitude of smooth muscle contraction with increase in the concentration of the extract in the tissue bathing medium. The results were consistent with the use of the plant as a purgative (Burkill, 1995), thus confirming that the extract may be a potent stimulant of GIT smooth muscle. Furthermore, the results demonstrated that the active principles contained in the plant extract were capable of increasing the force of contraction of the smooth muscle as evidenced by the increase in amplitude of contraction. Thus, the action of the extract increased GIT flux, which may be responsible for the purgative activity of *Tephrosia vogelii* leaf extracts.

CONCLUSION

The crude methanolic extract of *Tephrosia vogelii* leaves exerted stimulatory effects on the intestinal smooth muscle, which may be likened to those of standard agonists like ACH, histamine and 5-HT. The results provided some scientific basis for the use of *T. vogelii* leaves as a purgative.

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