Effect of the aqueous extract of African Mistletoe, *Tapinanthus sessilifolius* (P. Beauv) van Tiegh leaf on gastrointestinal muscle activity


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Received 9 October 2001; revised 16 January 2002.

Effects of the aqueous extract of *T. sessilifolius* on the gastrointestinal muscle were investigated on smooth muscle preparations isolated from rabbit jejunum, guinea pig ileum and on gastrointestinal transit in mice. Elemental analysis of the extract was also carried out. The aqueous extract of *T. sessilifolius* evoked a concentration dependent contraction of the rabbit jejunum and guinea pig ileum. The contractions evoked by the extract were not attenuated either by atropine or mepyramine, but they were completely blocked by verapamil. The elemental analysis revealed the presence of Mg, Zn, Fe, Cu, and very high concentration of Ca. The intraperitoneal LD$_{50}$ in mice was found to be 1500 mg/kg. The aqueous extract of *T. sessilifolius* possesses active components that may be mediating the observed biological activity through calcium mobilization.

The African mistletoe, *Tapinanthus sessilifolius* P. Beauv van Tiegh (Loranthaceae) is a semi parasitic plant widely distributed throughout Northern and Southern Nigeria. It is found growing on variety of evergreen trees. Unlike true parasite, which depends on its host for all nutrients, mistletoes take only water and minerals and grow all years round along branches of the host never touching the ground and it remains evergreen. In some parts of Africa including Nigeria, the plant is termed all "healer" plant and the aqueous extract of the dried leaves could serve as a remedy for diabetes, hypertension and other metabolic disorders. The dried leaves of the European mistletoes, *Viscum album* has been studied. There are no reports in the literature about the effects of this widely used plants on the gastrointestinal system. This study was therefore, undertaken to investigate the effects of African mistletoe on the gastrointestinal smooth muscles.

Materials and Methods

Plant material—The leaves of *T. sessilifolius* were harvested in November 1995 in Jos, Plateau State of Nigeria. The plant was harvested from the host, *Psidium guajava*. The plant material was identified and authenticated by Prof. Z.O. Gbile (UNDP) Consultant Taxonomist, Department of Medicinal Plant Research and Traditional Medicine, National Institute for Pharmaceutical Research and Development (NIPRD), Abuja. Voucher specimen No. FH1 105336 was deposited at the Forestry Research Institute, Ibadan and NIPRD, Abuja.

Preparation of the extract—The fresh leaves of *T. sessilifolius* were air dried for 7 days and crushed into powder using a pestle and mortar. 32 g of the powdered leaves were macerated in a litre of distilled water for 24 hr with occasional shaking. This was filtered and the resulting solution freeze-dried. The mean yield was found to be 13 g.

Acute toxicity (LD$_{50}$)—Five groups, each consisting of 5 mice of both sexes were used for the test. Groups 1 - 4 were injected i.p. with varying doses (10, 100, 1000 and 2000 mg/kg) of the extract, while group 5 which served as control signs and symptoms of toxicity over a 24 hr period. Death within this period was recorded. The LD$_{50}$ was estimated using the method of Lorke.

Pharmacological studies

Rabbit jejunum—Rabbits weighing between 1.5-3.0 kg of either sex were used. The rabbits were killed by a blow on the head, exsanguinated and the abdomen opened. Segments of the jejunum 2-3 cm long were removed and dissected free from adhering mesentery. The tissues were mounted in a 20 ml organ bath containing Tyrode’s solution of the following composition (mM): Na$^+$ 149.2, K$^+$ 2.7, Ca$^{2+}$ 3.6, Mg$^{2+}$...
2.2.1, Cl 145.3, H_3PO_4 0.4, HCO_3 11.9 and glucose 5.0. This was aerated with air and maintained at 37° ± 1°C. A load of 0.5 g was applied. A 60 min equilibration period was allowed during which the physiological solution was changed every 15 min. At the end of the equilibration period, the effect of acetylcholine (2.75 × 10^{-9} M) and the extract (0.1-0.8 mg/ml) were evaluated. The effect of atropine (5 × 10^{-9} M) on the responses evoked by acetylcholine (4.4 × 10^{-9} M) and extract (0.1-1.6 mg/ml) were investigated. In a separate study, the effect of the cumulative extract on intestinal strips pretreated with verapamil (2.04 × 10^{-5} M) was investigated. In another experiment, the tissue was allowed to equilibrate for about 30 min. The control baseline tracing was recorded on Ugo Basile Unirecorder before the solution was replaced with a calcium free Tyrode’s solution. The cumulative extract (0.1-0.8 mg/ml bath) was added to the tissue after 2 min interval to restore spontaneous jejunum contractions. The tissue response was recorded on Ugo Basile Unirecorder. In the third experiment, the control baseline was recorded on a recorder before changing to calcium free Tyrode’s solution. The tissue was challenged with verapamil (1 μg/ml). At 2 min intervals, the extract (0.1-3.2 mg/ml) was added into a tissue bath. Determinations were done in quadruplicates. The responses were recorded isometrically on Ugo Basile Unirecorder 7050.

Guinea pig ileum—Ileal strips about 2-3 cm long were obtained from guinea pig either sex (300-400 g). The intestinal content was removed by washing with Tyrode’s solution and the mesenteric residues were eliminated. Preparations were set up for recording of isometric responses in a 20 ml double-jacketed organ bath containing Tyrode’s solution at 37° ± 1°C continuously bubbled with air under a 1 g of load. After an initial equilibration period of about 60 min. Concentration-response curves for histamine (4.5 × 10^{-7} × 10^{-6} M) and the extract (0.1-1.6 mg/ml) were obtained. The effects of the extract and histamine on mepyramine (2.5 × 10^{-6} M) pretreated ilea strips were also investigated. Responses were recorded on Ugo Basile Unirecorder 7050.

Small intestinal transit—The effect of T. sessilisfolius on small intestinal transit in anaesthetized mice was tested using the charcoal meal method. Animals were pretreated with an intraperitoneal injection of the extract (100, 200 and 400 mg/kg); control mice were injected with normal saline 10 ml/kg. The charcoal meal, a suspension containing 10% charcoal in 5% acacia gum (0.1 ml/10 g) was administered in transgastrically with the aid of a stomach tube 30 min after the i.p. injection of the extract and normal saline. Mice were sacrificed after 30 min and the intestine was rapidly removed and laid on a white paper for inspection and measurement of distances transverse by the charcoal. The length transverse by the charcoal marker was calculated as a percentage of the intestine length. In some experiments, phentolamine (1 mg/kg), atropine (0.25 mg/kg), yohimbine (1 mg/kg) and verapamil (1 mg/kg) were given subcutaneously 10 min before intraperitoneal injection of the extract.

Elemental analysis—The method of Huldt and Christian were used. Estimation of cation was carried out with flame technique of Hitachi Model 80-80 Polarize Zeeman Atomic Absorption Spectrophotometer. Air acetylene was used as the fuel.

Results obtained in ppm were converted to mg/100 g as indicated below.

\[
\frac{axbxc}{c} = \text{mg/100 g sample}
\]

where a = AAS value in ppm

b = Dilution of wet ash sample

c = Volume in which ash was dissolved

c = Weight of sample

Statistical analysis—The results were expressed as mean ± SE. Significance of differences between control and treated groups were determined using the Student’s t-test.

Results and Discussion

The preliminary data obtained revealed that the aqueous extract of T. sessilisfolius evoked a concentration dependent contraction on the rabbit jejunum (Fig. 1). The extract also caused a concentration dependent contraction of the guinea pig ileum (data not shown).

![Fig. 1](image-url)
Table 1—Effect of phenolamine, atropine and verapamil on the activity of *T. sessilifolius* on small intestinal transit

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (mg/kg)</th>
<th>Intestinal length travelled (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Distilled water</td>
<td>10 ml/kg</td>
<td>57.8 ± 1.6</td>
</tr>
<tr>
<td><em>T. sessilifolius</em></td>
<td>100</td>
<td>75.2 ± 2.0*</td>
</tr>
<tr>
<td></td>
<td>200</td>
<td>89.4 ± 2.6*</td>
</tr>
<tr>
<td></td>
<td>400</td>
<td>95.5 ± 1.7*</td>
</tr>
<tr>
<td>Phenolamine + <em>T.</em></td>
<td>1 + 200</td>
<td>87.8 ± 1.6</td>
</tr>
<tr>
<td><em>sessilifolius</em></td>
<td>0.25 + 200</td>
<td>88.5 ± 2.62</td>
</tr>
<tr>
<td>Verapamil + <em>T.</em></td>
<td>1 + 200</td>
<td>41.2 ± 1.8*</td>
</tr>
</tbody>
</table>

Activity was not restored (Fig. 3b). The element composition of the leaf extract of *T. sessilifolius* in mg per 100 dry weight of powdered extract are: Na 1.00, Ca 364, Mg 20, Mn 2.3, Fe 1.3, Cu 0.91, Zn 14.6, and Cd 0.12. Our elemental analysis showed a very close link between calcium ions and the observed biological activity. The evidence in favour of involvement of calcium channels in mediating the effect of the extract was further strengthened by studies on the gastrointestinal transit, where verapamil was found to inhibit significantly the transit of charcoal meal (Table 1). This suggests therefore, that the contractile effects exhibited by the extract could be mediated through the enhancement of calcium in flux and/or activating the release of intracellular calcium from stores in the sarcoplasmic reticulum. The preliminary phytochemical screening revealed the presence of saponins, flavonoids, tannins and these constituents are known to be bioactive.

**Acknowledgement**

The work is supported by a grant from the National Institute for Pharmaceutical Research and Development, Abuja, Nigeria. The authors gratefully acknowledge the technical assistance of Adamu Mohammed, Hauwa Abdullahi and David Akunika.
secretarial assistance of Charles Balogun is highly appreciated.

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