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Purgative activity of the aqueous leaf extract of *Peninanthus longifolius* Miers

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Abstract

Objective: To investigate the claimed purgative property of aqueous leaf extract of *Peninanthus longifolius*. Materials and methods: Safety profile was evaluated using acute toxicity test (mice). Phytochemical constituents were determined using standard procedures. Effect of extract on the intestinal muscle was studied in isolated guinea pig ileum in the presence and absence of agonists and antagonists. Activity of the extract on intestinal motility was assessed by charcoal meal test (mice) and faecal output determination (mice). Doses of 125, 250 and 500 mg/kg of the extract were used orally. Results: LD_{50} of the extract of *P. longifolius* was 3.0 ± 0.85 g/kg. Phytochemical constituents include carbohydrates, proteins, tannins, saponins, alkaloids, steroidal aglycones and glycosides. The extract produced contraction in the guinea pig ileum in a dose-related manner. The contraction was antagonized only by atropine but not by hexamethonium or mepyramine. It also increased intestinal motility and faecal output. Conclusion: Aqueous leaf extract of *P. longifolius* produced significant ($p < 0.05$) degree of purgation predominantly through stimulation of cholinergic pathway. This may be attributed to one or more bioactive constituents found in the leaf.

Key words: *Perinanthus longifolius* , Purgative activity, Cholinergic activity.

1. Introduction

Medicinally, purgations are employed in the management of conditions that require evacuation of the bowel. There are many local purgatives which are used in various parts of the world. *Peninanthus longifolius*, Miers (Menispermaceae) is a woody perennial shrub of up to 1.8 m high. The morphology of the plant has been described [1].

It occurs in clusters in the tropical rain forest region of Nigeria. The shrub is used by the natives of Eastern States of Nigeria for treatment of constipation and for "washing dirty stomach". The leaf is also used as an emetic and an expectorant. Depending on patient's body weight and age, natives use one-quarter of the lamina for induction of purgation. The leaf is ground while it is still fresh. The ground form is mixed with little salt and is taken with yam. The ground form may be dispensed in either warm water or alcohol and administered orally.

The induction of purgation commences in less than an hour after ingestion of the drug. The purgation

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is usually stopped by licking some quantity of fresh palm oil. The local name is "*Ochikparakpara*". The present study has been undertaken to verify the claimed purgative property of *P. longifolius* in folkloric medicine.

2. Materials and methods

2.1. Collection of plant materials

The plant was collected in August (1999), from Ezza South Local Government Area of Enugu State, Nigeria. Botanical identification was made by Mr. A. Ozioko of the Department of Botany, University of Nigeria, Nsukka, and voucher specimen have been deposited in the University Herbarium

2.2 Extraction

Locally, the leaves are extracted fresh for induction of purgation. Hence, the leaves were ground into a coarse mass in a mortar. About 200 g of the coarse mass was macerated in 400 ml of distilled water and extracted for 24 hr and filtered with Buchner funnel. Solvent (water) removal afforded a solid extract of 9.6%. Another 200 g of the ground leaves was extracted by macerating in 400 ml of 99% alcohol for 24 h and again filtered. Preliminary experiments indicated that aqueous extract was more potent than the alcoholic extract. Therefore the aqueous extract was used for further experiments.

2.3 Phytochemical tests

Phytochemical screening of the aqueous extract was carried out using standard procedures [2].

2.4 Acute toxicity tests

The acute toxicity (LD_{50}) of the extract was estimated in Swiss albino mice (20 - 25 g) by the oral route [3].

2.5 Effect on isolated guinea pig ileum

Segments of ileum (2 cm long) isolated from freshly killed guinea pigs were suspended in a 20 ml organ bath containing aerated Tyrode's solution at 37°C. The composition of the Tyrode's solution was (mM/L): NaCl 137, $CaCl_2$ 1.0, $NaHCO_3$ 12, NaH_2PO_4 0.2, KCl 0.7, $MgCl_2$ 1.0 and glucose 5.5. The tissues were set up under a tension of 0.5 g and responses were recorded on a kymograph connected to a frontal writing lever attached to a smoked drum. The description of the set up was as described earlier [4].

At the end of 60 min equilibration period, the responses of the tissues to the extract and acetylcholine were established in the presence and absence of atropine, mepyramine and hexamethonium. The effect of the extract on the contraction produced by acetylcholine was also evaluated. Each agonist response was measured on separate tissue and four separate determinations were made for each agonist.

2.6 Effects on small intestinal motility.

Twenty albino mice of either sex (20 - 25 g) were randomly divided into five groups of four animals per group. The animals were starved for 24 h prior to the beginning of the experiments but had free access to water.

One group received 10% aqueous solution of tragacanth (20 ml/kg), the second, third and fourth groups received 125, 250 and 500 mg/kg of the extract respectively while the last group received 40 mg/kg of carbachol. All the administration were by the oral route using a catheter. Five minutes after drug administration, 0.5 ml of a 5% charcoal suspension in 10% aqueous solution of tragacanth powder was administered to each animal orally. The animals were sacrificed 30 min later and the abdomen was opened. The percentage distance traveled by the charcoal plug in the small intestine (from the pylorus to the caecum) in the treatment groups were determined and compared with normal group[5].

Table 1: Dose-response effect of acetylcholine and extract on isolated guinea pig ileum.

Acetylcholine (μg)	Response (cm)	Extract (mg)	Response (cm)
3	2.7 ± 0.2	22	1.1 ± 0.2
6	4.0 ± 0.3	44	2.4 ± 0.1
12	4.7 ± 0.2	88	3.1 ± 0.3

Values (responces) are expressed as mean ± SEM of four replicates

2.7 Effect on purgation

Twenty albino mice of either sex (20 - 30 g) were randomly divided into five groups of four animals per group. They were starved for 24 h before the commencement of the experiment but had free access to water. The first group received 20 ml/kg of distilled water, the second, third and fourth groups were given 125, 250 and 500 mg/kg of the aqueous extract respectively and the fifth group received 28 mg/kg of Bisacodyl. All the administration were by oral route. They were then allowed free access to the standard diet (Pfizer PLC, Lagos).

Following the administration of the substances the animals were housed singly in cages lined with sheets of white absorbent paper. Water supply was withdrawn and the mice were observed for 24 h during which the numbers of wet droppings were noted [6].

2.8 Statistical analysis

Results were expressed as mean ± standard error of mean. The significance of difference between means of control and treated groups was determined by Student's *t* - test and results were regarded as significant with $p < 0.05$.

3. Result

The oral LD_{50} of the extract in mice was calculated to be 3.0 ± 0.85 g/kg. Phytochemical screening yielded positive reactions for carbohydrates, proteins, flavonoids, tannins, saponin, alkaloids, steroidal aglycones and glycosides (cardiac, anthracene and cyanogenetic). The extract produced dose-dependent contraction in the isolated guinea pig ileum (Table 1). The aqueous extract also potentiated the contraction produced by acetylcholine (not shown).

However, the contraction induced by the extract was inhibited only by atropine but not mepyramine or hexamethonium. The alcoholic extract did not produce any contraction in the guinea pig ileum even in gram concentration. The result of the charcoal meal test is shown in Table 2. The administration of the extract significantly ($p < 0.05$) potentiated in a dose-related manner the charcoal meal transit. The increased intestinal transit produced by 500 mg/kg of the extract was slightly lower than that produced by carbachol (40 mg/kg).

The extract dose-dependently produced purgation (as measured by average number of faeces for 24h) in mice. The induction of purgation was significant at a dose of 250 mg/kg and above (Table 3). It was found that 500 mg/kg of the aqueous extract produced higher degree of purgation than Bisacodyl (28 mg/kg).

4. Discussion

Aqueous extract of *P. longifolius* appear to be relatively safe. This is based on the result of the acute toxicity (LD_{50} of 3.0 ± 0.85 g/kg). The result obtained in the guinea pig ileum and intestinal transit test revealed that the extract hastens intestinal

Table 2: Average percent distance traveled by charcoal mucilage

Drug/dose	Average percentage distance
125 mg/kg Extract	54.9 ± 7.7*
250 mg/kg Extract	63.4 ± 5.2*
500 mg/kg Extract	80.0 ± 9.2*
40 mg/kg Carbachol	92.0 ± 12.2*
20 ml/kg Tragacanth solution	45.8 ± 6.3

*p<0.05 *vs* normal (Tragacnth solution) group; n=4

Table 3: Number of faeces produced by mice when given various doses of drugs

Drug/dose	Number of faeces
125 mg/kg Extract	12 ± 2.5
250 mg/kg Extract	16 ± 2.5*
500 mg/kg Extract	22 ± 2.0*
28 mg/kg Bisacodyl	18 ± 6.9 *
20 ml/kg Distilled water	10 ± 2.1

* p < 0.05 *vs* normal water group; n=4; Values are in mean ± SEM

motility. Induction of ileal contraction has already been proposed as been indicative of ability to stimulate gastrointestinal motility and hence increase in the frequency of expelling faecal loads [7].

Its potentiation of acetylcholine-induced ileal contraction suggests cholinomimetic activity. Furthermore, the blocking of its contractile activity by atropine and not by hexamethonium (which excludes ganglion activity) or mepyramine (which rules out histaminergic action) further suggest that its contractile effect and hence the purgative activity is predominantly mediated through parasympathetic axis. This correlates with earlier finding that intestinal tract is dually innervated, autonomically with the cholinergic innervation causing stimulatory effect [8]; the excitatory neurons of the Auerbach's plexus are chiefly cholinergic[9, 10]. In other words, the extract is likely to produce increased intestinal motility by stimulating the autonomic cholinergic system which in turn stimulates the Auerbach's cholinergic system (which is excitatory).

The exact chemical principle responsible for the observed purgative activity is not known but may be attributed to one of the several bioactive components which we demonstrated their presence in the leaf. Saponins were found to be absent in the alcoholic extract. The fact that this alcoholic extract induced no ileal contractile response even in gram concentration suggest that saponin may be responsible for the observed purgative activity. Although tannins and glycosides have been implicated in purgation [11], it appears that the types present in the plant may not have any contractile effect. It is also possible that the purgative activity is mediated by the interaction of more than one secondary metabolites.

The purgative activity of aqueous extract of *P. longifolius* as manifested by significant induction of contractile response in guinea pig ileum and increased intestinal motility clearly confirm the folkloric purgative activity of the extract and provide pharmacological rationale for its use in traditional medicine.

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