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Some Gastrointestinal Effects of the Aqueous Root Extract of *Plumbago Zeylanica* (Lead Wort)

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ABSTRACT: The effects of the aqueous root extract of *Plumbago zeylanica* (PLZ, family, Plumbaginaceae) on the gastrointestinal tract (GIT) function was evaluated in normal intestinal transit and gastric ulcer-induced models in rodents. The extract PLZ given orally at 250 and 500 mg kg^{-1} produced a significant ($p<0.05$) decrease in the gastric lacerations induced by both indomethacin and alcohol, similar to that of cimetidine 100 mg kg^{-1}. The extract in selected dose range of 250-2500 mg kg^{-1} were administered intraperitoneally (i.p) to groups of overnight fasted mice (n = 5) an hour before intragastric administration of charcoal meal and the results obtained showed that only high doses of 1250 and 2500 mg kg^{-1} produced significant ($p<0.05$) dose-dependent decrease in intestinal propulsion in rodents. A similar inhibitory effect on the rhythmic contraction of rabbit ileum was obtained *in-vitro* with concentrations of 28.8-344.0 µg ml^{-1} of PLZ. The latter observed effect was similar to that produced by adrenaline (3.9×10^{-7} M) and the adrenergic antagonists- prazosin and propranolol used together with the herbal drug, revealed that PLZ effect is partly mediated through the adrenergic mechanism. The effects of atropine and carbachol on PLZ did not show any cholinergic involvement. Phytochemical analysis revealed the presence of alkaloids, tannins, cardiac glycosides, phlobatanins, anthraquinones, reducing sugars, saponins and flavonoids and the i.p route produced an LD_{50} value of 5296.6 mg kg^{-1}. The study established the scientific basis for the use of PLZ in the treatment of gastric ulcer and diarrhoea, as well as its possible mechanism of action and relative safety when ingested.

Keywords: *Plumbago zeylanica* Lead Wort, antiulcer, antidiarrhoeal, gastrointestinal, aqueous extract.

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INTRODUCTION

Indigenous people traditionally employ a wide range of plants and plant materials to maintain their health. One of the most common health problems is GIT disorders, a manifestation of ill state of the GIT function which includes amongst others, gastritis, peptic ulcer, emesis, constipation and diarrhoea. For example, world-wide occurrence of diarrhoea accounts for more than 5-8 million deaths each year in infants and small children less than 5 yrs (Gabriel *et al*, 2004; Farthing 2002). The World Health Organization (WHO) estimation for the year 1998, were about 7.1 million deaths due to diarrhoea (Park, 2000).

The plant PLZ commonly known as Lead Wort-White flowered and Ceylon Lead Wort (Nyananyo and Olowokudejo, 1986) is one of the plants employed for treating GIT disorders, especially diarrhoea and ulcer. PLZ is distributed as weed throughout the tropical and subtropical countries of the world (Chetty *et al*, 2007). It is a rambling subscandent perennial herb or under shrub with green branches, stems somewhat woody, spreading terate and glabous. Also, the leaves are alternate, ovate or oblong with narrow petiole (Chetty *et al*, 2007). Its flowers are red and bisexual, with axillary and terminal elongated spikes. Roots are coloured light yellow when fresh and reddish brown when dry.

The root has been reported to have numerous therapeutic uses (Chetty, *et al*, 2007), among which

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are antifertility, antidermal, diuretic, antimicrobial, antiulcer and antidiarrhoeal activities.

The present study aimed at investigating the antidiarrhoeal and antiulcer potentials of PLZ as well as its possible mechanism of action.

MATERIALS AND METHODS

Experimental Animals

Swiss albino mice (15-20g) and Wistar rats (100-150g) of either sex were kept and maintained under ideal laboratory conditions of temperature (25±3ºC) and light/dark periodicity (12/12 hr)
The animals were fed with standard rat chows (Neimeth Livestock Feeds Ikeja, Nigeria). Food was withheld twelve to sixteen hours before experiments but there was free access to water.

Plant Materials

Fresh roots of PLZ were collected from Mushin area of Lagos State, Nigeria. The plant was identified by Mr. Adeleke of the Department of Pharmacognosy, Faculty of Pharmacy, University of Lagos, Lagos-Nigeria, where a voucher specimen (voucher No. PCGH410) was deposited. It was also authenticated by Professor J.D. Olowokudejo, of the Department of Botany, University of Lagos.

Preparation of Extract

The fresh roots of PLZ were washed with distilled water, drained and dried in the oven at a temperature of 40ºC for 24-36h after which they were chopped into smaller pieces. The chopped bits (200g) were boiled for 60 min. in 2.5 L distilled water. After cooling and macerating for 12h at room temperature, the mixture was filtered using a white muslin cloth and the filtrate evaporated to dryness in an oven set at 55ºC, to obtain a solid residue (14.4g) referred to as the extract from which appropriate concentrations were made in distilled water for experiments.

Sources of Drugs

Prazosin, propranolol, adrenaline, atropine, carbachol were obtained from Sigma
While loperamide (Jansen-Cilag, Belgium) was from a local Pharmacy shop at Idi-Araba, Surulere, Lagos-Nigeria.

Acute Toxicity Test

The acute toxicity (LD_{50}) of the extract was estimated in 30 Swiss albino mice by the i.p route as described by Miller and Tainter (1944). Five groups of animals (n=5), were separately administered doses of 2,500-8,500 mg kg^{-1} and the number of deaths within 24 h in each group was recorded, while one group was given distilled water (10 ml kg^{-1}) via the same route and observed similarly. The animals were observed for a further 14 days for signs of delayed toxicity. LD_{50} was estimated from the graph of percentage mortality (converted to probit) against log-dose of the extract. The extract when given orally in doses of 10,000-25,000mg kg^{-1} did not produce any mortality.

Phytochemical Tests

The freshly prepared extract was subjected to simple chemical tests as described by Trease and Evans (1983).

Studies on Small Intestinal Transit

The effect of the extract on small intestinal transit in mice was tested using the charcoal meal method (Akah, 1989; Di Carlo *et al*, 1994). Animals were pre-treated with i.p doses of the extract (250 and 500 mg kg^{-1}) and controls received 10ml kg^{-1} distilled water. One hour after, charcoal meal (5% charcoal in 10% aqueous solution of tragacanth; 0.3 ml /mouse) was given orally and all the animals sacrificed 30 min thereafter to measure the length of distance traversed by the marker. Other agonists and antagonists similarly administered were loperamide (5.0 mg kg^{-1}), carbachol (0.1 mg kg^{-1}), prazosin, yohimbine, propranolol (1.0 mg kg^{-1}) and atropine (0.25 mg kg^{-1}). All antagonists were given subcutaneously (s.c) 15 min before PLZ. Results were expressed as ±S.E.M. and the significance of difference between means was tested by the students' t-test. Results were regarded as significant when $p<0.05$.

Ethanol-Induced Gastric Ulcer

Acute gastric ulcerations were induced by ethanol (Robert *et al*, 1979). Absolute ethanol (1.0ml/animal) was administered intragastrically to control rats and rats pre-treated 60 min before oral dosing with 250 and 500 mg kg^{-1} PLZ. One hour after ethanol administration, animals were killed by chloroform euthanasia and the stomach of the animals excised and cut along the greater curvature. Ulcer index was determined using a graduated transparency.

Indomethacin-Induced gastric ulcer

Acute gastric ulcerations were induced by 30mg kg^{-1} indomethacin administered by oral cannulation 1h after pre-treatment with 250 and 500 mg kg^{-1} PLZ and distilled water (Ueki *et al*, 1958). Five hours after

indomethacin therapy, animals were killed and sacrificed as above.

Studies on Isolated Rabbit Ileum

Segments of rabbit ileum (2 cm long) removed from freshly killed animals were suspended in a 50 ml organ bath containing aerated tyrode solution filled up to 40 ml mark and maintained at 37ºC. The tension on the tissue was 1.0 g and the tissue was equilibrated for 60 min during which the bathing solution was replaced every 10 min. At the end of the equilibration period, responses were established non-cumulatively for the extract, acting for 45 sec. The responses were recorded using a 3 min time cycle.

A graded dose response effect was obtained with the aqueous extract (28.8-344.0 µg ml^{-1}) and compared with the response produced by adrenaline (3.9×10^{-7} M). Adrenergic antagonists and atropine were also used together with the extract in order to establish its probable effect on cholinoceptors and adrenoceptors.

RESULTS

Phytochemical Analysis

PLZ was found to contain diverse bioactive agents as shown (Table 1)

Table 1:
Phytochemical Analysis of PLZ root extract.

Test		*Inference*
Alkaloids	Dragendorff	+++
	Meyer	+++
	Wagner	+++
Saponins	Frothing	++
	Emulsion	++
Tannins	Bromine water	+++
Cardiac Glycosides	Legal	+++
	Kedde	++
	Libermann's test	++
	Salkowski	++
Flavonoids	Ferric chloride	+++
	Lead acetate	++
	Sodium hydroxide	+++
Anthraquinones	Bomtrager	+
Phlobatanins		++
Reducing sugar	Fehling	++
	Resorcinol	++

Acute Toxicity Study

The oral route did not produce any mortality at the dose range investigated, however, i.p doses of 3750, 5000, 6000 and 8,500 mg kg^{-1} caused deaths in the treated mice and the LD_{50} was interpolated as 5296.6 mg kg^{-1} (Figure 1). The behavioural changes observed in the animals after 2h of PLZ (oral and i.p) were mouse writhing, reduced activity and anorexia.

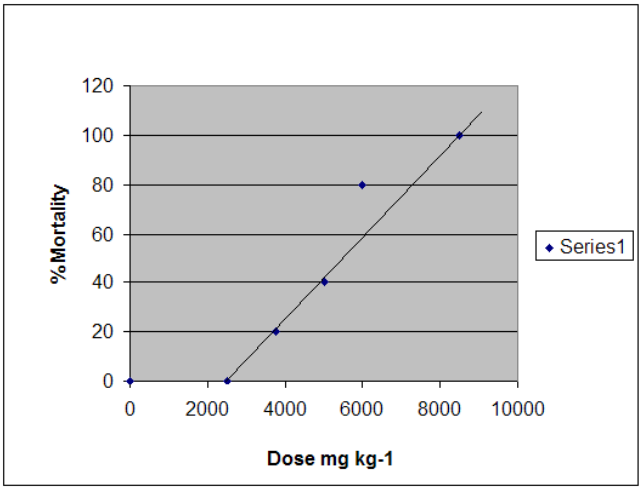

Figure 1:
Acute Toxicity of i.p. doses of PLZ in mice (LD_{50} = 5296.6 mg kg^{-1}).

Table 2:
Effects of PLZ and other agents on intestinal transit in mice

Treatment (i.p)	*Dose (mg kg^{-1})*	*Length traversed (cm)*	*% Inhibition*
Control	-	34.90±0.66	-
PLZ	1250	16.20±0.42 *	53.58
PLZ	2500	5.20±0.21 *	85.10
Loperamide	5.0	0	100.00
Carbachol	0.1	43.70±0.46	
PLZ+Carbachol	0.1&2500	41.36±0.18	
Prazosin+PLZ	0.1&2500	8.44±0.71	75.80
Propranolol+PLZ	0.1&2500	2.20±0.04	93.69
Yohimbine+PLZ	0.1&2500	2.30±0.46	93.40

* $p<0.05$

Intestinal Transit

The charcoal meal in the controls travelled 34.90 cm of the total intestinal length (Table 2). Low doses of the extract 250 and 500 mg kg^{-1} did not produce any effect on the propulsive movement of the gut whereas,

larger doses of 1250 and 2500 mg kg^{-1} reduced intestinal transit in a dose-related manner and produced significant ($p<0.05$) 53.58% and 85.10% inhibition respectively (Table 2). The adrenergic antagonists did not produce any observable inhibition of PLZ effect.

Absolute Alcohol-Induced gastric Ulcer

Absolute alcohol produced gastric ulcerations in all the animals treated. Pre-treatment with aqueous extract of PLZ (250 and 500 mg kg^{-1}) orally, induced a significant and dose-dependent inhibition of gastric ulcers(Table 3). Effect of cimetidine, a histamine H_2-receptor antagonist was found to be greater than the herbal drug.

Indomethacin-Induced gastric Ulcer

Indomethacin produced gastric ulcer in all the treated animals. PLZ produced a significant and dose dependent inhibition of gastric ulceration in all the treated rats. However, misoprostol offered a better protection (Table 4).

Table 4: Effect of PLZ and cimetidine on alcohol-induced ulcer

Treatment	*Dose (mg kg^{-1})*	*Ulcer index (±SEM)*	*% Inhibition*
Control	-	68.36±1.39	-
PLZ	250	46.90±0.26 ^{a,,b}	31.40
PLZ	500	32.54±0.21$^{a, b}$	52.40
Cimetidine	100	8.52±0.43	87.50

$^{a, b}$ *$p<0.05$ with control and cimetidine respectively.*

Table 5: Effects of PLZ and misoprostol on indomethacin-induced gastric ulcer

Treatment	*Dose mg kg^{-1}*	*Ulcer index (±SEM)*	*% Inhibition*
Control	-	53.48±2.05	-
PLZ	250	27.13±1.64 $^{a, b}$	49.27
PLZ	500	7.68±0.22 ^{a}	85.64
Misoprostol	0.2	4.13±0.37	92.28

$^{a, b}$ $p<0.05$ control and misoprostol respectively

Isolated Rabbit Ileum

The extract (28.8-344.0 µg ml^{-1}) produced a concentration-dependent and sustained relaxation of the intestine (Table 7). Adrenaline (3.9×10^{-7} M) – induced relaxation of the preparation was also recorded for comparison. While the results obtained did not reveal any interaction of PLZ with the cholinergic receptors, adrenergic antagonists, both prazosin and propranolol were able to attenuate the smooth muscle relaxation produced by the herbal drug (Table 5).

Table 6: Effect of PLZ and other agents on rabbit ileum

Treatment	*Concentration (µg ml^{-1})*	*Height of contraction (mm) (±SEM 5 Experiments)*
PLZ	28.8	3.40±0.2
PLZ	57.4	2.30±0.5
PLZ	230.0	0.30±0.2
PLZ	344.0	0
Adrenaline	3.9×10^{-7} M	0
Prazosin +Adrenaline	1.5×10^{-7} M & 3.9×10^{-7} M	2.0±0.0
Propranolol +Adrenaline	2.0×10^{-7} M & 3.9×10^{-7} M	1.2±0.2
Praz.+ Prop+Adren.		1.2±0.3
Praz+PLZ	1.5×10^{-7} M & 230.0 µg ml^{-1}	2.6±0.4
Prop+PLZ	2.0×10^{-7} M & 230.0 ’’	2.6±0.2
Praz+ Prop+PLZ		2.6±0.2

DISCUSSION

The plant *P.zeylanica* has been used by traditional herbal practitioners in the treatment of ulcer and other GIT symptoms. The present study aimed at evaluating the effect of the aqueous root decoction of the plant on GIT motility, gastric-induced ulcerations and the rhythmic contractions of the rabbit ileum.

In the normal intestinal transit, low doses produced no recognizable effect with the mean values of 34.30±1.64 and 32.70±0.53 for 250 mg kg^{-1} and 500 mg kg^{-1} respectively. Control recorded a close range of 34.90±1.49. However, high doses of 1250 mg kg^{-1} and 2500 mg kg^{-1} produced a dose-dependent inhibition of the intestinal transit (Table 2). The results of the *in-vitro* study showed a remarkable dose-dependent relaxation on the GIT (rabbit ileum, Table 5, Figures 2&3). The observed effect of PLZ could substantiate its folklore use as an antidiarrhoeal agent. In this present report, the extract induced relaxation of the intestine was attenuated to an extent by alpha and beta adrenoceptor antagonists, prazosin and propranolol (1 mg kg^{-1}).

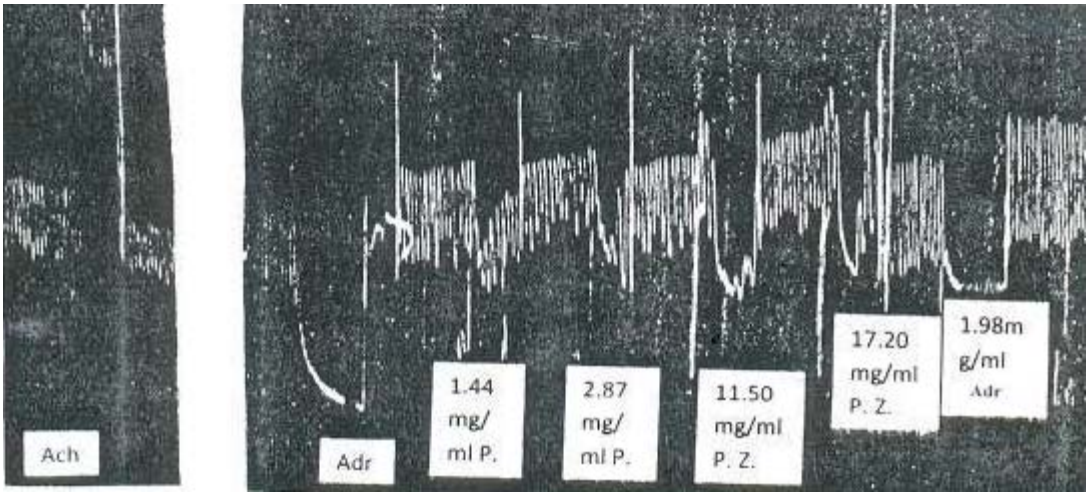

Fig. 2:
Concentration dependent responses of PLZ on isolated rabbit ileum. Ach (5.5µM) and Adr (0.39µM) induced responses are shown

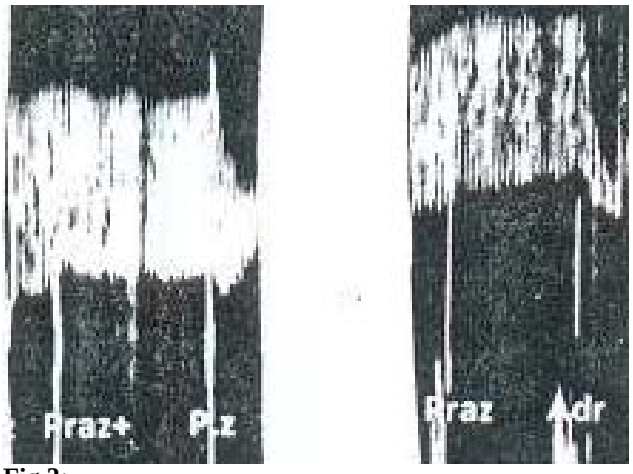

Fig.3:
Effect of prazocin on the relaxant effect of PLZ (230ug/ml) and adrenaline (3.9 x 10^{-7})

Interaction of PLZ with both alpha and beta adrenoceptors as observed in this study might not be the sole mechanism of GIT relaxation of the herbal drug, since the latter contained diverse bioactive agents including flavonoids, which exert inhibitory effect on the normal intestinal transit (Bennet, 1992; Binder 1992). Furthermore, there are many mechanisms that control intestinal functions, such as intrinsic and extrinsic nerves, autacoids and hormones, all these might be contributory in the git effect of PLZ.

Oral doses of 250 and 500 mg kg^{-1} reduced the lacerations induced by both alcohol and indomethacin in a dose-dependent manner (Tables 3&4). However, PLZ was found to be more efficacious in inhibiting indomethacin-induced ulcer than in the alcoholic model and this finding suggests that the drug plant possibly acts through a similar mechanism as the prostaglandin in protecting against gastric ulceration. Several reports have shown that flavonoids inhibit gastric acid secretion and also protect against experimental ulcer (Parmar and Ghosh, 1981; Motilva *et a*l, 1992). Flavonoids have also been reported to inhibit the activity of histidine decarboxylase (Hayes and Foreman, 1987, Kouturek *et al*, 1986 and Di Carlo *et al*, 1994) and thus reduce the formation of histamine in the gastric mucosa. Apart from the latter effect, they are also known to be free scavenger (Baumann *et al*, 1980 and Cavallini *et al*, 1978). Furthermore, tannins have been reported to form a fine pellicle of coagulated protein over the lining of the alimentary tract, thus protecting inflamed mucous membranes (Oliver Bep 1960).

Yuan-Chuen and Tung-Liang 2005 reported the inhibitory effect of PLZ on *H. pylori*, a bacterium that has been implicated in gastric ulceration (Rang *et al*, 2003).

From the fore-going, it is projected that PLZ acts through different mechanisms to protect against ulcerogens.

The oral extract of the plant in the present investigation produced no mortality up to a dose of 25,000 mg kg^{-1} but the i.p dosing was found toxic at a

dose range of 3750-8,500 mg kg^{-1} , giving an LD_{50} of 5296.6 mg kg^{-1} .

In conclusion, the present study has substantiated folkloric use of PLZ in both diarrhoea and gastric ulcerations. The former action is speculated to be mediated through adrenergic mechanism, while its antiulcer effect has been projected to be cytoprotective. The relative safety of PLZ when ingested, especially through the oral route has also been established.

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