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Presence of Laxative and Antidiarrheal Activities in *Periploca aphylla:* A Saudi Medicinal Plant

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Abstract: *Periploca aphylla* (Family: Asclepiadaceae), is native to Saudi Arabia and is used as purgative. The aim of this study was to investigate the gut modulatory effect of the aqueous (Pa.Aq) and n-hexane (Pa.Hex) fractions of *P. aphylla* and to investigate their mechanism of actions. The aqueous (Pa.Aq) and n-hexane fractions (Pa.Hex) of the *P. aphylla* were studied using the *in-vivo* and *in-vitro* experiments. The laxative and antidiarrheal activities were conducted in mice while isolated rabbit jejunum and guinea-pig ileum preparations were used to investigate their mode of action. In the *in-vivo* experiments, Pa.Aq showed atropine-sensitive laxative effect in mice at the doses of 30 and 100 mg kg^{-1}, while Pa.Hex showed opposite effect providing 40 and 80% protection from diarrhea at the same doses. In the *in-vitro* experiments, Pa.Aq showed atropine-sensitive spasmogenic effect in isolated rabbit jejunum and guinea-pig ileum, while Pa.Hex showed spasmolytic effect by inhibiting the spontaneous and high K^+-induced contractions in isolated rabbit jejunum, similar to verapamil; a standard calcium channel blocker (CCB). The CCB activity was confirmed when Pa.Hex dose-dependently (0.03-0.1 mg mL^{-1}) shifted the Ca^{++} concentration-response curves to the right with suppression of the maximum response, similar to verapamil. These data indicate that the laxative effect mediated through cholinergic pathways is attributed to the presence of water soluble (polar) constituent(s), while the antidiarrheal effect exhibited by non-polar constituent(s) through Ca^{++} antagonist effect is perhaps meant by nature to offset the excessive gut stimulation effect which could have been otherwise harmful.

Key words: *Periploca aphylla*, laxative, cholinergic, antidiarrheal, calcium channel blocking activity

INTRODUCTION

The genus Periploca, belonging to family Asclepiadaceae, comprises of about 12 species (Rehman *et al*., 2003). It is represented in Saudi Arabia by four species: *P. aphylla* Dcne (Zahran and Younes, 1990), *P. somaliensis* Browicz (Abdel-Sattar *et al*., 2009), *P. visciformis* Vatke (Al-Farhan *et al*., 2005) and *P. Brevicoronata* (Goyder and Boulos, 1991). *P. aphylla*, locally known as Suwwas, is a leafless shrub widely distributed in South Hijaz and Najd regions of Saudi Arabia (Chaudhary and Al-Jowaid, 1999). It is used by Saudi indigenous medicine practitioners as stomachic, laxative and for treatment of swellings and cerebral fever (Kazimierz, 1966; Baquar, 1989), while its latex is applied as a poultice in tumors and swellings (Al-Yahya *et al*., 1990). Phytochemical studies on different species belonging to genus Periploca resulted in the isolation and structure identification of different classes of secondary metabolites such as pregnane glycosides from *P. sepium* and *P. calophylla* (Itokawa *et al*., 1987; 1988a-c; Xu *et al*., 1990; Deepak *et al*., 2012) and cardinolides from *P. sepium* and *P. forrestii* (Xu *et al*., 1990, Li *et al*., 2010, 2012). In addition, triterpenes, lignanes (Rehman *et al*., 2003) and flavonoids (Yang-Min *et al*., 2010) were also reported from genus Periploca.

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Biological studies of extracts and isolated compounds from different Periploca species showed to possess cytotoxic immunosuppressive (Feng *et al*., 2008), antibacterial (Rehman *et al*., 2003), antioxidant (Iqbal *et al*., 2012), hepatoprotective and antiprotozoal (Al-Musayeib *et al*., 2012) activities. However, to the best of our knowledge, no study exists in the literature to validate its medicinal use in gut disorders such as constipation. Knowing that the laxative effect is usually present in the aqueous fractions and that the laxative effect usually co-exists in nature with spasmolytic component present in the non-polar fractions, this study was conducted on the aqueous and n-hexane fractions using both *in-vivo* and *in-vitro* studies to validate the medicinal use of plant in gut disorders.

MATERIAL AND METHODS

Plant material: The aerial parts of *P. aphylla* were collected in March 2008, from Oyoun village located near Al-Baha city in the south western part of the Kingdom of Saudi Arabia. The plant was identified by Professor Mohammed Yousef, Pharmacognosy Department, College of Pharmacy, King Saud University, Saudi Arabia. A voucher specimen was deposited at the department.

Extract preparation: The air-dried powdered aerial parts (200 g) were exhaustively extracted by ethanol (70%) using Soxhlet apparatus for 8 h. The dried alcoholic extract was then dispensed in 250 mL water and then successively partitioned with several portions of n-hexane (5×350 mL), chloroform (5×350 mL), ethyl acetate (5×350 mL) and n-butanol (5×350 mL). The obtained extracts were separately dehydrated by passing over anhydrous sodium sulfate, evaporated to dryness under reduced pressure, kept in desiccator over anhydrous calcium chloride and saved for further investigation.

The aqueous and n-hexane fraction of the titled plant was screened for its laxative and antidiarrheal effect, while the other extracts were saved for further phytochemical and biological studies.

Drugs: The following reference chemicals were obtained from the sources specified: acetylcholine chloride (Ach), loperamide hydrochloride, carbamoylcholine (carbachol), verapamil hydrochloride, potassium chloride (Sigma Chemical Company, St. Louis, MO, USA) and castor oil (Karachi Chemical Industries, Karachi, Pakistan). Chemicals used for making physiological salt solutions including potassium chloride, calcium chloride, glucose, magnesium chloride, magnesium sulfate, potassium dihydrogen phosphate, sodium bicarbonate, sodium dihydrogen phosphate and sodium chloride were obtained from Merck (Darmstadt, Germany). All chemicals used were of the highest purity grade. Stock solutions of all the chemicals were made in distilled water and the dilutions were made fresh in normal saline on the day of experiment.

Animals: BALB/c mice (weighing 20-25 g), local breed rabbits (weighing 1-1.5 kg) and guinea pig (400-500 g) of either sex, were housed at the animal house of the Aga Khan University under a controlled environment (23-25°C). The animals were kept in their respective cages with sawdust (changed at every 48 h) and were fasted for 24 h before starting the experiment. In routine, they were given tap water ad libitum and a standard diet consisting of (g kg^{-1}): flour 380, fiber 380, molasses 12, NaCl 5.8, nutrivet L 2.5, potassium metabisulfate 1.2, vegetable oil 38, fish meal 170 and powdered milk 150. The experiments were performed with the rulings of the Institute of Laboratory Animal Resources, Commission on Life Sciences, National Research Council (NRC, 1996).

In-vivo experiments

Laxative activity test: Mice fasted for 6 h before the experiment were placed individually in cages lined with clean filter paper. The animals were divided into seven groups (n = 6); the first group acting as the negative control and administered saline (10 mL kg^{-1}, p.o.), while the next group received CCh (1 mg kg^{-1}, i.p.) which served as the positive control. The third and fourth groups received orally, 30 and 100 mg kg^{-1} of Pa.Aq, respectively. To determine the mechanism underlying its laxative effect, separate sets of mice (group # 5, 6 and 7) were pretreated with atropine (10 mg kg^{-1}, i.p.) one hour before administration of the extract or CCh. After 18 hours, the feces production (total number of feces and total number of wet feces per group) in all animals was counted and the percentage increase in wet feces relative to that of total fecal output was recorded which was considered as the laxative effect (Khan *et al*., 2012).

Antidiarrheal activity: The method previously used in our lab was followed with some modifications. Mice (20-25 g) of either sex were fasted for 24 h before the experiment. The animals were housed in individual cages and divided in four equal groups, for each n = 5. The first group received saline along with normal saline (10 mL kg^{-1}, p.o.), acted negative control. The second and third groups received Pa.Hex 30 and 100 mg kg^{-1}, respectively. Fourth group received loperamide (10 mg kg^{-1}), as positive control. Afterwards, the cages were inspected for the presence and absence of typical diarrheal droppings; the absence was noted as a positive result, indicating protection from diarrhea.

***In-vitro* experiments:** The spasmogenic/spasmolytic activities were studied on isolated rabbit jejunum and guinea pig ileum preparations as described previously (Syed Taqvi *et al*., 2006; Janbaz *et al*., 2013; Khan *et al*., 2012). Approximately 2 cm long segments of jejunum or ileum were suspended in tissue baths containing Tyrode's solution maintained at 37°C and aerated with carbogen (95% O_2 and 5% CO_2). Intestinal responses were recorded isotonically using Bioscience transducers attached to Powerlab Data Acquisition System (AD Instruments, Sydney, Australia) linked to a computer installed with Labchart software (version 6). The tissues were allowed to equilibrate for 30 min prior to addition of any chemical substance. The tissues were stabilized following repeated exposure to 0.3 μM acetylcholine (3-5 times) after washing with the Tyrode's solution until the sub-maximal responses of uniform amplitude were obtained. The observed modulation of spontaneous rhythmic contractions were used to test spasmogenic or spasmolytic activity in isolated rabbit jejunum preparation, whereas, induction of contraction with test or control drugs above that of the basal tone was used to measure spasmogenic activity in guinea-pig ileum.

Calcium channel blocking activity: To assess whether the spasmolytic activity of the test substances was mediated through calcium channel blockade, high concentration of potassium as, K^+ (80 mM), was used to depolarize the jejunal preparations (Farre *et al*., 1991). K^+ (80 mM) was added to the tissue bath which produced a sustained contraction. Test material and standards were then added in a cumulative fashion to obtain concentration-dependent inhibitory responses. The relaxation of intestinal preparations, pre-contracted with high K^+, was expressed as percent of the control pre-contraction. To confirm the calcium antagonist activity of test substances, the tissue was allowed to stabilize in normal Tyrode's solution which was then replaced with Ca^{++}-free Tyrode's solution containing EDTA (0.1 mM) for 30 min in order to remove Ca^{++} from the tissues. This solution was further replaced with K^+-rich and Ca^{++}-free Tyrode's solution, having the following composition: KCl 50, NaCl 91.04, $MgCl_2$ 1.05, $NaHCO_3$ 11.90, NaH_2PO_4 0.42, glucose 5.55 and EDTA 0.1 mM, with pH of 7.4. Following an incubation period of 30 min, control concentration-response curves of Ca^{++} were obtained. When the control concentration response curves of Ca^{++} were found super-impossible (usually after two cycles), the tissues were pretreated with the test material for 60 min to test the possible calcium channel blocking effect. The concentration response curves of Ca^{++} were reconstructed in the presence of different concentrations of the test material.

Statistical analysis: The data expressed are Mean±standard error of mean (SEM, n = number of experiments) and the median effective concentrations (EC_{50} values) with 95% confidence intervals (CI). The concentration-response curves (CRCs) were analyzed by non-linear regression while Chi-square-test for antidiarrheal assay. All the graphs, calculations and statistical analysis were performed using GraphPad Prism 4 for windows (GraphPad Software, San Diego, California, USA).

RESULTS AND DISCUSSION

Keeping in view the medicinal use of *P. aphylla* in gastrointestinal disorders, its aqueous (Pa.Aq) and n-hexane (Pa.Hex) fractions was investigated to rationalize its medicinal use. In the in-vivo study for its laxative activity, Pa.Aq treatment produced 65.16±1.22% and 87.5±4.74% (mean±SEM, n=6) wet feces in mice at 30 and 100 mg kg^{-1}, respectively. The positive control, CCh (1 mg kg^{-1}) produced 87.66±3.27 % wet feces, while the saline treated group did not form any wet feces as expected. To see if the laxative effect has any cholinergic component, animals were pretreated with atropine (10 mg kg^{-1}) which reduced the effect to 22.83±6.96 % and 40.66±13.71 %, at respective doses of 30 and 100 mg kg^{-1}; further details are shown in Table 1. Increased production of wet feces is indicative of laxative activity, similar to the effect of carbachol, a standard cholinergic agonist and

Table 1: Effect of atropine on the laxative activity of the aqueous fraction of *periploca aphylla* (Pa.Aq)

Group No.	Treatment	Dose (mg kg^{-1})	Defecation/group	Number of wet feces/group	% of wet feces
1	Saline (p.o. mL kg^{-1})	10	3±0.38	0	0
2	Carbachol (p.o.)	1	11±0.52**	10±0.51***	87.66±3.27
3	Pa.Aq (p.o.)	30	9±0.82**	5.83±0.54*	65.16±1.22
4		100	11.16±1.30**	9.83±0.54**	87.50±4.74
5	Carbachol+Atropine (i.p.)	1+10	3.66±0.56**	0.5±0.22***	20±7.91
6	Pa.Aq (p.o.)+Atropine (i.p.)	30+10	4.33±0.71**	0.83±0.166***	22.83±6.96
7		100+10	4.16±0.70**	1.5±0.34***	40.66±13.71

Values shown are mean±S.E.M, n = 6. *p<0.05, **p<0.01 and ***p<0.001 show a comparison of group No. 2, 3 and 4 vs. group No. 1 (One-way ANOVA followed by Dunnett's test), group No. 5 vs. group No. 2, group No. 6 vs. group No. and group No. 7 vs. group No. 4 (unpaired t- test)

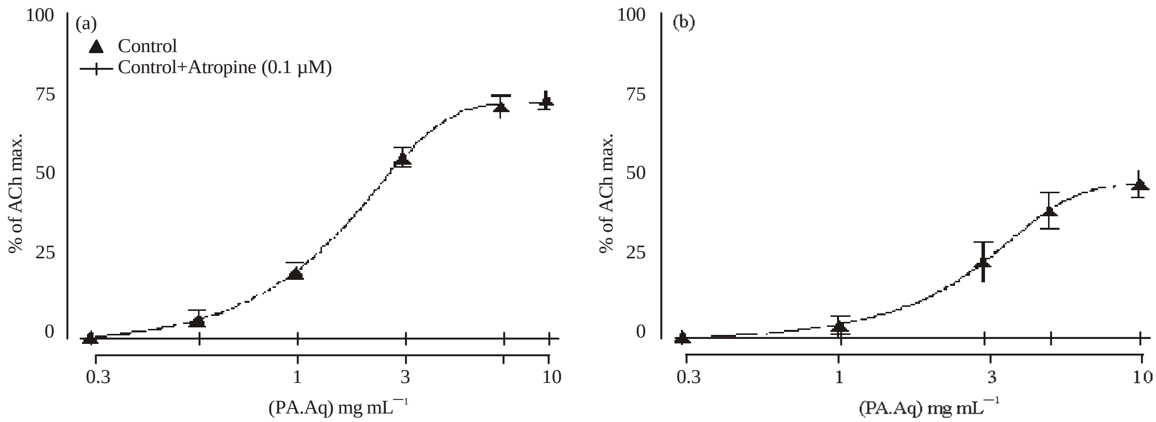

Fig. 1(a-b): Effect of the aqueous fraction of *Periploca aphylla* (Pa.Aq) on spontaneous contraction of rabbit jejunum (a) and guinea pig ileum (b) preparations. The values shown are mean±SEM from 4 to 5 determinations

accelerator of intestinal contents (Brown and Taylor, 2006). When studied in the in-vitro studies, the aqueous extract increased motility both in rabbit jejunum and guinea-pig ileum and this stimulatory effect was abolished in the presence of atropine (Fig. 1), a muscarinic receptor blocker (Gilani *et al*., 1997), confirming the presence of some Ach-like component(s) in gut stimulant action. Ach is a neurotransmitter of the parasympathetic nervous system and is known to cause gut stimulation through the activation of M_3 muscarinic receptors subtype (Brown and Taylor, 2006); hence, the presence of Ach-like constituents explains its medicinal use in constipation and as digestive aid. This study is in line with common observation that the laxative activity of plants is usually mediated through cholinergic pathways which concentrates in the aqueous fractions (Ghayur and Gilani, 2004; Gilani *et al*., 2005; Mehmood *et al*., 2011; Khan *et al*., 2012).

It is also observed that the laxative activity in natural products usually co-exists with anti-diarrheal or antispasmodic activity, usually separated in the non-polar fractions (Gilani *et al*., 2005; Khan *et al*., 2012); thus the n-hexane fraction was studied, to see if it also possesses antidiarrheal and antispasmodic activities.

In the castor oil-induced diarrhea in mice, Pa.Hex showed 20 and 60% protection at respective doses of 30 and 100 mg kg^{-1} vs. castor oil untreated group, whereas, loperamide, a standard antidiarrheal agent (Reynolds *et al*., 1984), showed complete protection (Table 2).

A potential antidiarrheal agent may exhibit its antidiarrheal effect by inhibiting either gut motility and/or electrolyte out flux (Croci *et al*., 1997). The protective effect of Pa.Hex against the castor oil-induced diarrhea in mice, similar to loperamide, suggests that it has either an inhibitory effect on contraction or on electrolyte out flux.

Table 2: Antidiarrheal activity of Perploca aphylla n-Hexane in mice, on castor oil (10 mL kg^{-1})-induced diarrhea

Treatment (p.o.), dose (mg kg^{-1})	No. of mice out of five with diarrhea	% Protection
Saline (10 mL kg^{-1})+Castor oil	5-May	0
Pa.n-Haxane+Castor oil		
30+10	5-Mar	40
100+10	1*/5	80
Loperamide+Castor oil	0**/10	100

*$p<0.05$ and **$p<0.01$vs. Saline+Castor oil treated group (χ^2-test)

To elucidate the possible mechanism(s), Pa.Hex was further studied in the in-vitro experiments.

When tested on spontaneously contracting rabbit jejunal preparation, n-hexane fraction caused dose-dependent inhibition with EC_{50} value of 1.85 mg mL^{-1} (1.26-2.53 mg mL^{-1}; n = 4), thus showing spasmolytic activity. To further characterize the inhibitory effect shown by Pa.Hex in rabbit jejunal smooth muscles, a high concentration of K^+ (80 mM) was used to produce sustained contractions. The Pa.Hex was then added in a cumulative fashion, where it caused a concentration-dependent relaxation of the high K^+-induced contractions with an EC_{50} value of 0.28 mg mL^{-1} (0.24-0.33), as shown in Fig. 2a, thus showing more potency against high K^+-induced contractions, similar to verapamil, a standard Ca^{++} antagonist (Fleckenstein, 1977) which also caused a concentration-related inhibitory effects against high K^+-induced contractions with an EC_{50} value of 0.07 μM (0.04-0.12, n = 4), showing greater potency when compared with that on spontaneous contractions with EC_{50} 0.5 μM (0.40-0.63, n = 4) (Fig. 2b). The greater potency against high K^+-induced contraction, is a typical characteristic of Ca^{++} antagonists (Godfraind *et al*., 1986), This hypothesis was further strengthened when pretreatment of the tissues with Pa.n-Hex (0.03-1 mg mL^{-1}) caused a rightward shift in the Ca^{++}CRCs (Fig. 3a), similar

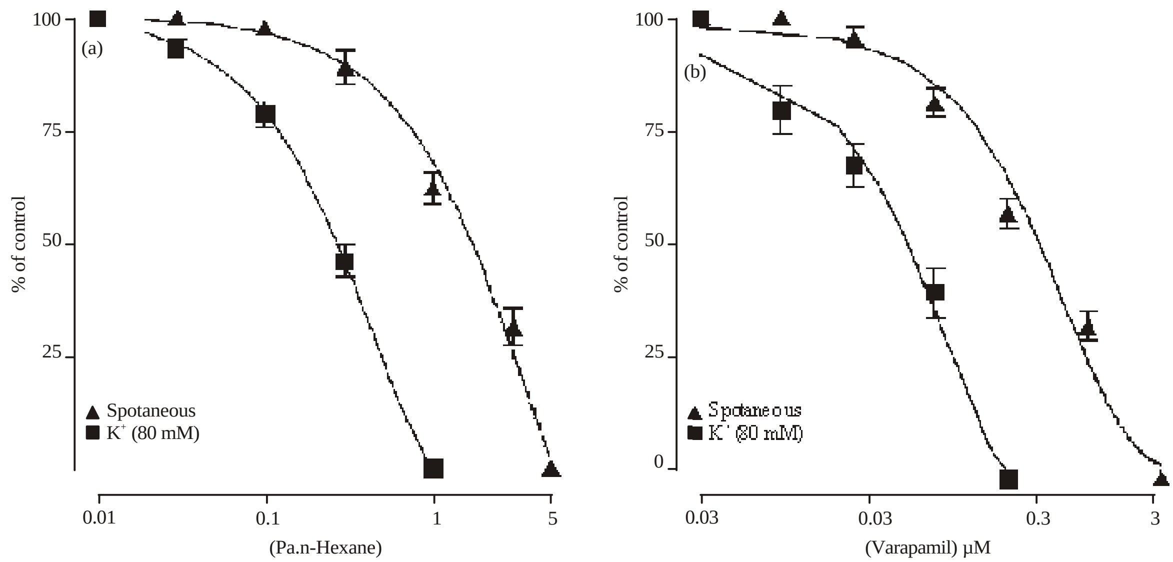

Fig. 2(a-b): Concentration-response curves showing effect of the n-hexane fraction of *Periploca aphylla* (Pa.n-Hexane) (a) and verapamil (b) on spontaneous and high K^+-induced contraction of rabbit jejunum preparations. The values shown are mean±SEM. from 4 to 5 determinations

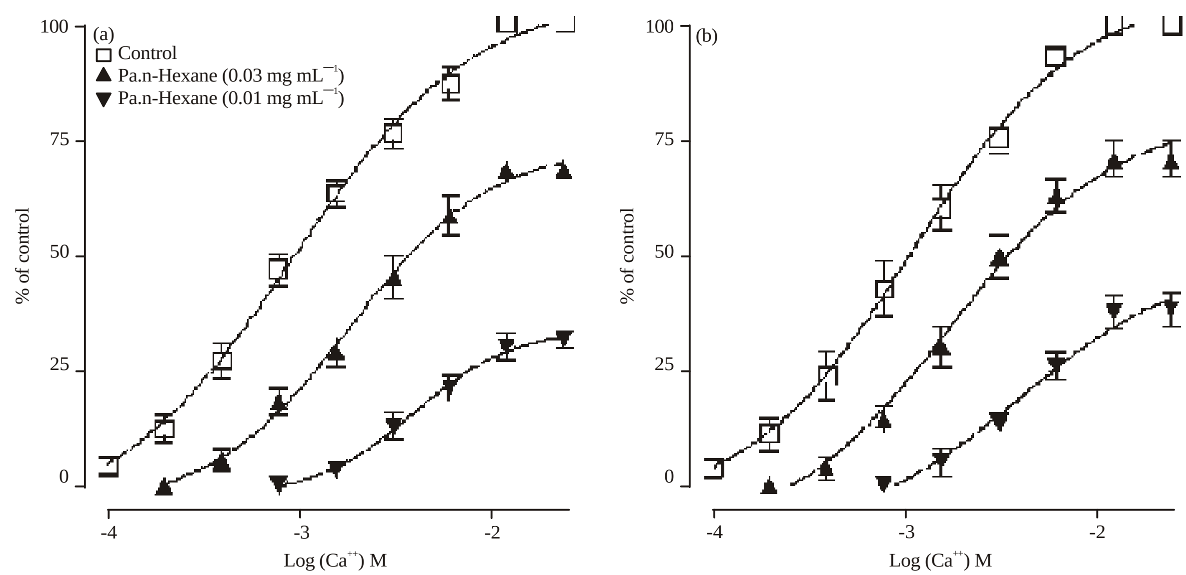

Fig. 3(a-b): Dose-dependent Ca^{++}-concentration response curves (CRCs) of the n-hexane fraction of *Periploca aphylla* (Pa.n-Hexane) (a) and verapamil (b) in isolated rabbit jejunum preparations. Values shown are mean±SEM. from 4 to 5 determinations

to that of verapamil (Fig. 3b). The observed CCB effects of Pa.Hex might be due to the presence flavonoids (Yang-Min *et al*., 2010), as compounds of this class have been reported to possess CCB-like actions (Di Carlo *et al*., 1993; Revuelta *et al*., 1997). However, contribution of other constituents accounting for reported effects cannot be ruled out.

CONCLUSION

These data indicate that the aqueous and n-hexane fractions of *P. aphylla* exhibited gut stimulant and inhibitory activities respectively. The gut stimulatory effect is mediated through cholinergic stimulation while blockade of Ca^{++} influx is involved in the gut inhibitory activities and this study explains the medicinal use of plant in constipation, while presence of inhibitory constituents may be meant by nature to offset the excessive gut stimulation usually seen with chemical drugs used for constipation.

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