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Original Article

IN VITRO ANTI-EMETIC EFFECT OF METHANOL ROOT EXTRACT OF *TERMINALIA AVICENNIOIDES* GUILL. & PERR. ON ISOLATED RABBIT ILEUM

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Abstract

Background: *Terminalia avicennioides* is a medicinal plant used traditionally in Nigeria to treat numerous ailments and disorders which include coughs, purgative and emetic. **Objective:** This study scientifically evaluated the probable mechanism of the anti-emetic activity of methanol root extract of *Terminalia avicennioides* Guill. & Perr. on histaminergic and serotonergic receptors of isolated rabbit ileum. **Methodology:** Fresh root bark was extracted using methanol and the antiemetic effect was evaluated by experimenting on a small segment (3 cm) of isolated rabbit ileum, using the data capsule (Ugo Basile) and single chamber isolated tissue apparatus. Varying concentrations of histamine and serotonin (0.05, 0.1, 0.2, and 0.4 mg/ml) were used to obtain contractions as they act on histamine and serotonergic receptors respectively. **Results:** META (0.05 mg/ml, 0.1 mg/ml, 0.2 mg/ml, and 0.4 mg/ml) exhibited a significant concentration-dependent decrease at $p<0.05$ in histamine-induced and serotonin-induced contractions on isolated rabbit ileum from the data capsule reading with percentage inhibition of 43.22, 53.96, 75.35, 85.58 % and 21.62, 49.50, 78.67 and 86.96 % respectively for the different concentrations. **Conclusion:** The study showed that methanol root extract of *Terminalia avicennioides* acts on cholinergic (muscarinic), histaminergic, and serotonergic receptors of isolated rabbit ileum by inhibitory spasmolytic action against smooth muscle contractility, hence its ability to possess anti-emetic effect to relieve vomiting.

Keywords: Anti-emetic; Histamine; Rabbit ileum; Serotonin; *Terminalia avicennioides*.

Introduction

Gastrointestinal disorder is the term used to refer to any condition or disease that occurs within the gastrointestinal tract. It includes conditions such as constipation, irritable bowel syndrome, haemorrhoids, anal fistula, diarrhoea, vomiting (emesis), perianal abscesses, inflammatory bowel syndrome, colitis, colon polyps, and cancer among others.[1]

The essential part of the digestion process involves the controlled progression of ingested food or chyme along the gastrointestinal tract (GIT) which results in the supply of nutrients, water, and electrolytes to various parts of the body. A decrease in gastrointestinal (GI) motility can lead to stasis of chyme in the intestine which increases the quantum of bacterial growth and may cause the breakdown of the barrier leading to bacterial translocation to other organs of the body. An increase in motility, on the other hand, interferes with the digestion and absorption process and can lead to diarrhoea; vomiting, and malabsorption syndrome.[2] Stimulation of sympathetic nerves inhibits peristaltic movements, while parasympathetic

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stimulation increases contractile movement. Most *in vitro* experiments involve the investigation of drug action on the contractions of longitudinal gastrointestinal muscles.

The study of gastrointestinal motility by *in vitro* techniques may help determine the therapeutic potential of newer drugs in gastrointestinal disorders as well as the effect of pathological conditions on gastrointestinal motility.[2] Pharmacological management of these disorders involves the use of commercially available allopathic drugs that produce their antiemetic action by interacting with various receptors in the gastrointestinal tract (GIT) that are closely related and are associated with potential adverse effects such as dry mouth associated with the use of anticholinergics, sedation, anxiety, restlessness among others. These then, heighten the need for natural product researchers to explore natural anti-emetics with fewer adverse effects and better tolerability.[3]

Traditional medicinal practices worldwide have employed herbal remedies for the prevention and treatment of different diseases with negligible side effects. Scientific research and clinical trials have confirmed the efficacy of several plants in the treatment and prevention of several conditions.[4]
Despite the availability of modern medicine in some communities, herbal medicines (medicinal plants) have continued to maintain popularity for historical and cultural reasons, in addition to their efficacy and cheaper cost.[4]

The medicinal effects of Nigerian plants are attributed to the interaction of phytochemicals (such as alkaloids, tannins, phenols, saponins, flavonoids, and essential oils) and bioactive compounds contained in their tissues.[5] These active principles are responsible for their effectiveness against many forms of ailments and enable the plant parts to function as herbs or therapeutic agents, producing biological activity in animals and humans.[6]

Terminalia avicennioides Guill & Perr, is a plant of medicinal importance common in Africa and Asia. It is found commonly growing in the savannah region of West Africa. In Nigeria, the plant is found in Guinea and Sahel Savannah.[4] It was reported to be used by traditional medical practitioners to treat a variety of conditions which include inflammation, oxidative stress,, and cytotoxicity in both humans and animals.[7]
In traditional folklore medicine, root bark extract of *Terminalia avicennioides* Guill & Perr. showed interesting antibacterial, anti-ulcer, antihelminthic, and antiemetic activities both *in vivo* and *in vitro*.[4] The leafy parts of the plant have astringent properties and are used to treat dysentery.[8] In humans, decoction from the leaves and bark is used as laxative and diuretic and also has anti-emetic activity.[3] *T. avicennioides* also has domestic uses where the dried bark is used as bee hives in Ghana, while its gummy exudate can also be used to prepare perfumes.[9] Further research into its efficacy and safety is needed to enhance its effective utilisation.[8]
Nausea and vomiting (emesis) are common in the advanced form of diseases. Nausea may be defined as an unpleasant feeling of the need to vomit often accompanied by autonomic symptoms such as pallor, sweat, salivation, tachycardia, and diarrhoea whereas vomiting is the forceful expulsion of gastric contents through the mouth or nose through a complex reflex involving coordinated activities of the diaphragm, gastrointestinal tract, and the abdominal muscles.[15]

Specific conditions like gastritis, poisoning, or non-specific disorders such as brain tumours, elevated intracranial pressure, and overexposure to ionizing radiation could cause vomiting, mediated through the coordinated activity of central and peripheral receptors of serotonin ($5HT_{1A}$, $5HT_3$, $5HT_4$), dopamine (D_2), Histamine (H_1), muscarinic cholinergic (Ach-M), cannabinoids (CB_1), opioids (μ_2), neurokinins and gamma-aminobutyric acid ($GABA\ B_1$). These receptors arise from the vomiting centre, located in the dorsolateral border of the reticular formation of the medulla on the floor of the fourth ventricle of the brain referred to as the chemoreceptor trigger zone (CTZ). Drugs that act on the CTZ are centrally acting drugs. Prolonged and excessive vomiting alters the body's electrolyte balance, leading to dehydration. The multifactorial nature of nausea and vomiting and the associated adverse effects of anti-emetic drugs has led to advances in the development of natural remedies with fewer side adverse effects.[14]

Terminalia avicennioides have been shown scientifically to possess anti-emetic activity[3]. Further studies are needed to prove the exact

mechanism of action by which the plant elicits its anti-emetic effect.[3] There is some evidence that suggests that the efficacy of an anti-emetic drug is directly related to its binding affinity for a specific receptor.[15]

Five dopamine receptors have recently been identified (D_1–D_5). Historically only D_2 receptors were associated with the emetogenic pathway (antagonized by drugs such as haloperidol and metoclopramide) but D_3 receptors are also now felt to be involved in animal studies.[10] Other important receptors involved in the emetic pathway include histamine, acetylcholine (Ach), endorphins, gamma-aminobutyric acid (GABA), and cannabinoids.

The choice of anti-emetic is based on clinical assessment of the emetogenic pathway being triggered, and receptors involved, where information sent to higher centres of the brain is relayed to the vomiting centre and CTZ via neurotransmitters that transmit information to the brain.[16] Neurotransmitters stimulate and activate the vomiting reflex through the afferent pathways [17] and motion sickness which is due to labyrinth stimulation, cytotoxic drugs, hormonal changes during the early weeks of pregnancy,[18] postoperative nausea and vomiting, and toxins such as alcohol that can induce the life-saving physiological response of vomiting to these circulating foreign particles.[15,16,19,20].

Materials and methods

Equipment and Materials

Materials used for this study include; *hp* computer, Forced transducer (7003-F), Data Capsule Ugo Basile (17304), USB cable, single chamber isolated organ bath of 20 ml capacity, syringes (1 ml, 5 ml, 10 ml), suture thread and needle, Dissection pan and kit, aeration machine, power cable and tubing, regulator clip, glass reservoir, tyrode's physiological salt solution, tank supplier, white transfer rubber tank of 10 liters for preparation of tyrode's physiological salt solution, electronic analytical weighing balance (max.180 g), measuring cylinders (25 ml,100 ml) electric hot plate, beakers (200 ml,1000 ml), desiccator and refrigerator.

Chemicals and Reagents

The reagents include; Atropine, sodium chloride (NaCl), potassium chloride (KCl), sodium hydrogen phosphate (NaH_2PO_4), calcium chloride ($CaCl_2$), magnesium chloride hexahydrate ($MgCl.6H_2O$), and D-glucose (Sigma Aldrich, US), histamine (M&B, Nigeria Plc.), serotonin (5-HT)(Kernel Ltd., UK).

Experimental Animals and Housing Conditions

Two rabbits (male and female) were obtained from Gombe local market' kasuwan kaji' and were kept at room temperature in the Animal House, Department of Pharmacology, Faculty of Pharmaceutical Sciences, Gombe State University. The rabbits were allowed free access to water and poultry feed processed with soya beans powder. They were fasted for food but allowed free access to water for 24 hours before the commencement of the experiment. The entire study was carried out in a pharmacology laboratory, Department of Pharmacology and Therapeutics, Faculty of Pharmaceutical Sciences, Gombe State University, Gombe, Nigeria according to the Gombe State University ethical guidelines for the handling and use of laboratory animals.

Plant Collection Identification and Extraction Procedure

Branches of *Terminalia avicennioides* along with its roots (with documented voucher number 90023) were collected from Samaru Village in Zaria, Nigeria, identified by Mal. Namadi Sunusi, of the herbarium unit of the Department of Biological Sciences, Ahmadu Bello University, Zaria, Kaduna State were washed, peeled, sliced into smaller pieces, and air dried under shade for 10 days. The air-dried root bark was size reduced to powder using a mechanical grinder and weighed using the analytical weighing balance. Powder weighing 1 kg was extracted using cold maceration method for 48 hours with 70 % v/v methanol in water, with occasional shaking and filtration. The filtrate was concentrated under a controlled temperature (45- 50 °C) over a water bath followed by air drying on a wide surface. The methanol root extract obtained was weighed and encoded META (methanol extract of *T.avicennioides*), the percentage yield was calculated and it was stored in an air-tight container and placed in a desiccator until required for use.

Experimental Protocols

A healthy rabbit was euthanized in a chloroform container (2 cm) and the stomach was cut open and

the ileum was isolated, washed in normal saline solution, and cut open (about 2 cm in length) according to the method described by Magnus[22]. It was then mounted to the Iworxs data capsule (17304) using force-transducer model 7003-F which was then suspended in the organ bath clamped on the stainless steel rod stand then connected to the computer with DIN-DIN cable filled with tyrode physiological solution to the mark. It was then connected to the power supply. The circulator was turned on with the chamber at 37°C.

The Effect of Methanol Root Bark Extract of *T. avicennioides* (META) on Histamine-induced Contraction

The tissue was stabilised using a submaximal concentration of histamine 0.1 mg/ml and tissue was considered stabilised after three (3) stock concentrations gave a constant response of smooth muscle contractions for forty minutes. The administration was started from the lowest concentration of histamine 10^{-6} mg/ml to the highest concentration of 10^{-2} mg/ml. The cycle below was repeated for each concentration till ceiling effect was obtained.

Step I: 0 minutes, start normal record
Step II: 30 seconds, add histamine and record the stimulatory effect
Step III: 30 seconds, wash three times and wait for one minute
Step IV: 30 seconds normal record
Step V: 30 seconds, add the META
Step VI: 30 seconds, add histamine and observe the effect

The following cycle was repeated in the presence of extract (META) administered at 0.05 mg/ml, 0.1 mg/ml, 0.2 mg/ml, and 0.4 mg/ml. The effect was measured in grams (g) as the force of contraction (mean contraction ± standard error of mean). The percentage % inhibition of response produced by the extract was calculated as:

Percentage Inhibition % = [(A-B)/A ×100]

Where A and B are contractions in grams of control and treated group respectively and (n=4).

Effect of Methanol Root Bark Extract of *T. avicennioides* (META) on Serotonin (5 HT) induced Contraction

The tissue was stabilised using a submaximal concentration of 0.2 ml serotonin (0.1 mg/ml) and the tissue was considered stabilised after three (3) concentrations produced the same response of smooth muscle contractions. Drug administration was from the lowest concentration of 10^{-6} to the highest concentration of 10^{-2} mg/ml. The step above was repeated in the presence of extract (META) administered at 0.05 mg/ml, 0.1 mg/ml, 0.2 mg/ml, and 0.4 mg/ml respectively and the effect was measured in gram force of contraction as (mean ± standard error of mean (SEM). The percentage % inhibition of response was calculated as:

Percentage Inhibition (%) = [(A-B)/A ×100]Where A and B are contractions in grams (g) of the control and treated group and (n=4).

Statistical Analysis

The values obtained in this study were expressed as a mean ± SEM, The data were analysed statistically using SPSS version 20. One-way analysis of variance (ANOVA), followed by Dunnett's post hoc test for multiple comparisons was used to determine the level of significance between means with a p-value less than or equal to 0.05 considered as significant.

Results

Effect of Methanol Root Bark Extract of *T. avicennioides* (META) on Histamine-induced Contraction on Isolated Rabbit Ileum

The administration of 0.2 ml histamine (0.1 μg/ml) produced 3.432 g ± 0.405 contractions in the positive control group. Multiple comparisons showed that the extract (META) produced a concentration-dependent decrease of 1.949 g, 1.580 g, 0.846 g, and 0.495 g in concentration respectively when extract concentrations of 0.05, 0.1, 0.2, and 0.4 mg/ml were used respectively for the study. Thus the META decreased histamine-induced contraction significantly at p values less than 0.05, 0.005, and 0.001 respectively.

Table 1. Effect of META on Histamine-induced contraction on isolated rabbit ileum

Group (mg/ml)	Contraction (g)± SEM	% inhibition
Hist (0.2)	3.432 ± 0.405	-
Hist + META(0.05)	1.949 ± 0.161*	43.22
Hist + META (0.1)	1.580 ± 0.517**	53.96
Hist + META (0.2)	0.846 ± 0.164***	75.35
Hist + META (0.4)	0.495 ± 0.222***	85.58

Values presented as mean ± SEM, n=4. Value significantly different compared to the histamine (positive control group) at *p< 0.05, ** p< 0.005, ***p < 0.001 (one-way ANOVA, Dunnett's test). Key: Methanol extract of *Terminalia avicennioides (*META), Histamine (Hist).

Effect of Methanol Root Bark Extract of *T. avicennioides* (META) on Serotonin (5-HT) induced Contraction on Isolated Rabbit Ileum

Administration of 0.2 ml serotonin (0.1 µg/ml) produced 2.531 g ± 0.842 contractions in the positive control group. Multiple comparisons show that administration of META on serotonin-induced contractions produced a concentration-dependent decrease of in contraction 1.984 g, 1.278 g, 0.540 g, and 0.330 g in contraction respectively of the rabbit ileum at extract concentrations of 0.05, 0.1, 0.2 and 0.4 mg/ml were used respectively. Hence, the extract significantly ($p \leq 0.05$) decreased serotonin-induced contractions at 0.2 and 0.4 mg/ml respectively.

Table 2. Effect of META on serotonin-induced contraction on isolated rabbit ileum

Group (mg/ml)	Contraction (g)± SEM	% inhibition of contraction
5-HT (0.2)	2.531 ± 0.842	-
5-HT+ META (0.05)	1.984 ± 0.576	21.62
5-HT+ META (0.1)	1.278 ± 0.302	49.5
5-HT+ META (0.2)	0.540 ± 0.269*	78.67
5-HT +META (0.4)	0.330 ± 0.153*	86.96

Values presented as mean contractions ± SEM, n=4. Values significantly decreased (for 0.2 and 0.4 mg/ml of META) compared to the serotonin (positive control group) at* p< 0.05 (one-way ANOVA, Dunnett's test)

Key: Methanol extract of *Terminalia avicennioides* (META), Serotonin (5-HT).

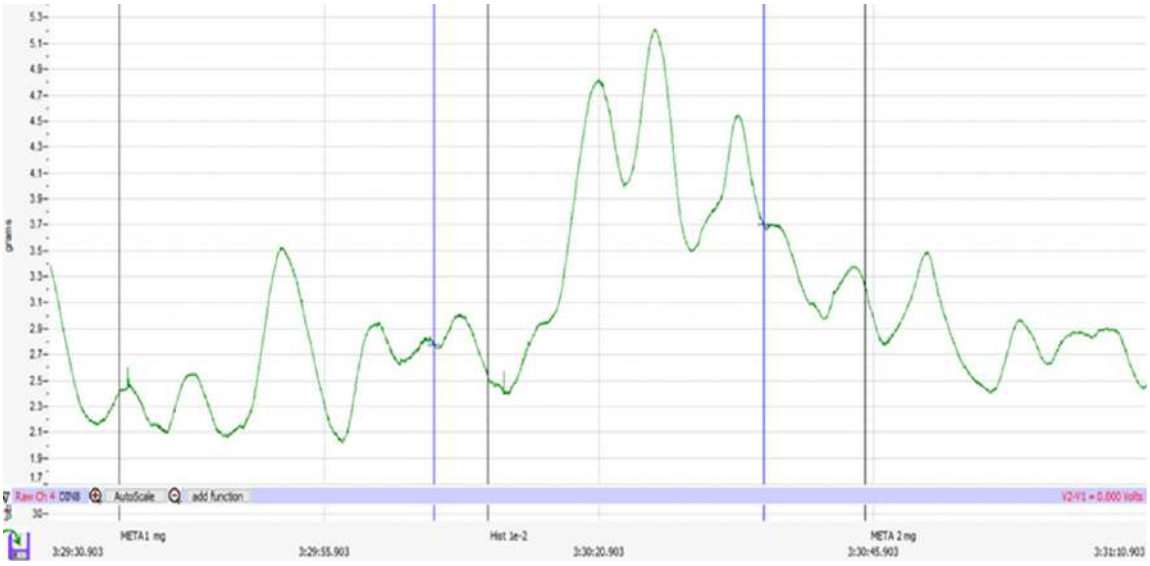

Fig 1: Effect of META on Histamine-induced contraction on isolated rabbit ileum

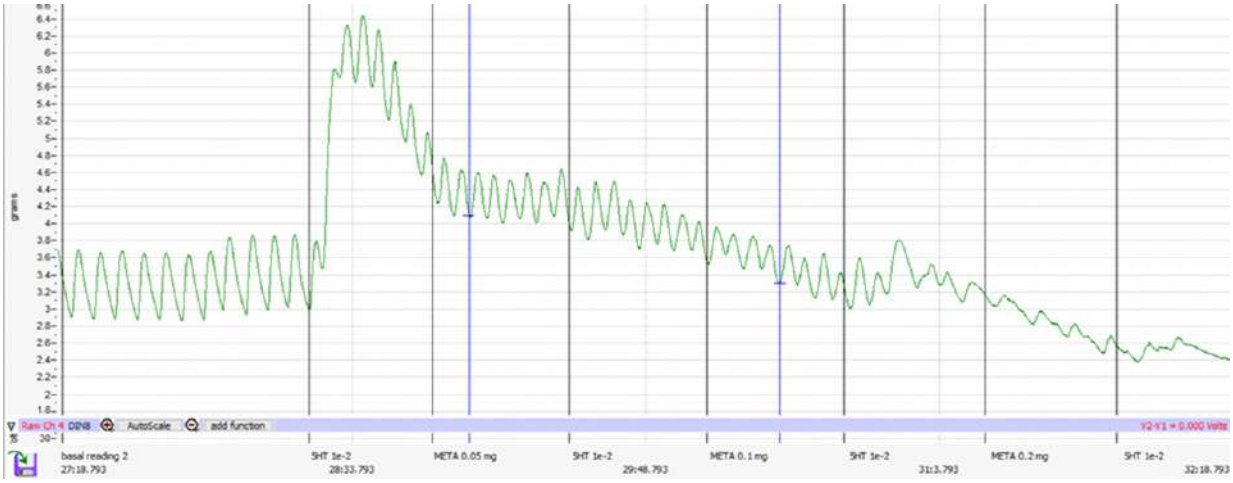

Fig. 2: Effect of META on Serotonin-induced contraction on isolated rabbit ileum.

Discussion

In medicinal plant studies today, phytochemicals are regarded as potential research compounds because their potential health benefits are not fully scientifically established.[23] Intestinal disorders are generally associated with motility dysfunctions and various plant species showed promising antispasmodic activity via different mechanisms.[24] Other plants aside from *Terminalia avicennioides* reported in the treatment of vomiting[25] include; *Aconitum palmatum* root, *Alhagi pseudalhagi, Cannabis sativa, Ageratum conyzoides, Zingiber officinale,* and others.[25,26]

The processes involved in nausea and vomiting result from continuous interactions between the gastrointestinal tract, including its enteric nervous system, the CNS, and the autonomic nervous system. [27,28] In this study following administration of histamine, contractile effects elicited by histamine on the isolated rabbit ileum is said to be mediated through histamine H_1 receptors.[29] Other studies showed that administration of histamine contracts both the ileal longitudinal and circular smooth muscles through activation of H_1 receptors. [30] Administration of methanol root extract of *Terminalia avicenniodes* significantly decreased histamine-induced contractions in a concentration-dependent manner (*p< 0.05, ** p< 0.005, ***p < 0.001). It has been shown that histamine, thus plays a significant role in signaling for emetic action in the CTZ while some drug molecules like mepyramine, burimamide, and metamide inhibit the histamine-induced emesis.[12]

Thus, the spasmolytic effect of META which significantly reduced the amplitude of contraction is possibly due to the blockade of H_1 receptors predominantly present in the smooth muscle of the gastrointestinal tract.

The contraction of gastrointestinal smooth muscle produced by serotonin according to Mashhadi F.F. *et al.,*[31] is said to be mediated via postsynaptic hyperpolarisation of the enteric neurons, stimulation of serotonergic (5-HT_2, 5-HT_3) receptors alongside the stimulating action on ganglion cells located in the enteric nervous system and antagonism of 5-HT_{1A}receptors.[32] Activation of the 5-HT_4receptors in the enteric nervous system causes increased acetylcholine release, thereby mediating the motility-enhancing or 'prokinetic' effect of selective serotonin agonists such as cisapride. [30] The significant (p-value < 0.05) decrease in serotonin-induced contraction in a concentration-dependent manner shows that META's spasmolytic effect could be via the blockade of serotonin receptors (especially the 5-HT_3, 5-HT_4). It should however be noted that 5-hydroxytryptamine (5-HT) inhibits gastric acid and pepsin secretion, but increases mucosal production, thus having ulcer-protective properties.

This experiment seeks to validate the study conducted by Mohd *et al*., [3] who reported that the META decreases apomorphine-induced pecking in chicks suggesting that it blocks dopaminergic receptors in the chemoreceptor trigger zone (CTZ)

and also may act by interfering with 5-HT_3 receptors present in the peripheral ending of afferent vagal nerves. By comparing it with this study, it showed that this extract targets most of the receptors in the gastrointestinal tract involved in nausea and vomiting which are 5-HT receptors, Dopamine D_2 receptors, and muscarinic acetylcholine (M_3) receptors, which may have a similar mechanisms of action with the conventional anti-emetic drugs targeting these receptors as well.

Phytochemical screening of the methanol root bark extract of *T. avicenniodes* carried out by Mann *et al*.,[31] revealed the presence of tannins, alkaloids, flavonoids, phenols steroids, and glycosides and were reported to possess anti-emetic activity. Similar studies showed that the alkaloid present in META is responsible for its anti-emetic activity.[3] Therefore, the anti-emetic effect of this extract mediated via histamine (H_1) and serotonin receptors (5-HT_2, 5-HT_3, and 5-HT_4) could be due to the presence of alkaloids, glycosides, flavonoids, phenols, and steroids.[33]

This study has scientifically justified the traditional use of *T. avicennioides* as an anti-emetic and antispasmodic.

Conclusion

The study shows that methanol root extract of *T. avicennioides* (META) scientifically possesses anti-emetic activity likely mediated through the blockade of histamine (H_1) and serotonin (5HT_2, 5-HT_3, 5-HT_4) receptor subtypes, which can be used as promising herbal medication, especially for the treatment of emesis.

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