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Evaluation of anti-diarrhoeal effect of four medicinal plants on castor oil-induced gastrointestinal motility in mice

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ABSTRACT

Acorus calamus rhizome, Pongamia glabra leaves, Aegle marmelos unripe fruit and Psidium guajava root-bark have been reported to be extensively used in the Indian ayurveda system of medicine for the treatment of diarrhoea and dysentery. However, the traditional claims need to be validated by a suitable experimental model. Therefore, the present study was undertaken to evaluate the effect of methanol extract of the four plants for their anti-diarrhoeal potential by using gastrointestinal tract motility test after charcoal meal administration in castor oil-induced diarrhoeal mice. All the plant extracts showed significant ($p<0.001$) reduction in gastrointestinal motility in charcoal meal test in rats as compared to the control when tested at 7.5 and 15 mg/kg. The result scientifically establishes the anti-diarrhoeal effect of the plant extracts and substantiates the use of this herbal remedy as a non-specific treatment for diarrhoea in folk medicine. However, the study has to be extended further to elucidate the exact mechanism by which it prevents the intestinal movement and the phytochemicals which directly take part in bringing their anti-diarrhoeal effect have to be identified.

Keywords: Herbal drugs; Traditional medicine; Gut motility; Phytochemicals; Diarrhoea; Swiss albino

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INTRODUCTION

Diarrhoeal diseases are one of the leading causes of morbidity and mortality in developing countries and are responsible for the death of millions of people each year [1]. Modern estimate suggests that approximately 2.5 billion cases of diarrhoea occur every year which results in 1.5 million deaths among children under the age five. Diarrhoea still remains the second leading cause of infant mortality [2]. Diarrhoea can be defined as the increased frequency of bowel movements accompanied by a loose consistency of stools [3]. The conditions of diarrhoea are particularly dangerous in infants and young children because of the rapidity with which serious dehydration occur. Secretory diarrhoea is the common form of acute diarrhoea which occurs as a result of increased intestinal secretion or decreased intestinal absorption of fluid and electrolytes or by altered motility of gastro-intestinal tract [4]. Available medications such as loperamide and bismuth subsalicylate may be beneficial; however they may be contraindicated in certain situations [5]. Hence there has been a continuing search of drugs that might inhibit diarrhoea without side effects and as adjunct to oral dehydration therapy. Plants on the other hand, have been the foundation of traditional medicine system throughout the world and continue to nurture mankind with new remedies. WHO has encouraged the use of herbal medicines for the prevention and treatment of diarrhoea since the 1980s [6] due to their affordability as well as abundance.

The rhizomes of *Acorus calamus* (Family: Acoraceae; Tamil name: Vasambu) commonly known as sweet flag are considered to possess anti-spasmodic, carminative and anthelminthic properties and also used for treatment of epilepsy, mental ailments, chronic diarrhoea, dysentery, bronchial catarrh, intermittent fevers and tumours. It is listed as an insecticide, an antifungal agent, an antibacterial agent and a fish toxin [7]. *Pongamia glabra* (Family: Fabaceae; Tamil name: Pungai) commonly known as Indian Beech has been used in the treatment of abscess, bronchitis, diarrhoea, itches, piles, skin diseases, tumours, painful rheumatic joints, ulcers, whooping cough, quench dipsia in diabetes, blood purifier and as an antiseptic to treat wounds and cuts. Some of the reported activities of this

plant include antioxidant, antimicrobial, anti-inflammatory, antiulcer, antihyperglycemic amongst others [8]. *Aegle marmelos* (Family: Rutaceae; Tamil name: Vilvam) is commonly known as Bael. The greatest medicinal value, however, has been attributed to its fruit and the unripe fruit is said to be an excellent remedy for diarrhoea and is especially useful in chronic diarrhoeas [9, 10]. Root of *Psidium guajava* (Family: Myrtaceae; Tamil name: Goyyaa) commonly known as guava is used in West Africa as a decoction to relieve diarrhoea, coughs, stomach ache, dysentery, toothaches, indigestion and constipation; while in Philippines, Fiji and South Africa, the roots are used in the form of decoction and poultice, as an astringent in ulcers, wounds and in treatment of diarrhoea [11]. In ayurveda, the root-bark is successfully employed in chronic infantile diarrhoea in the form of concentrated decoction [12].

Though these plants have been traditionally acclaimed for the treatment of diarrhoea and its associated disorders, literature search did not reveal any scientific authentication on the anti-diarrhoeal property of the methanol extract of the various aforementioned parts. Hence this research attempts at a possible investigation on the anti-diarrhoeal properties of the plant extracts by performing gastro-intestinal motility test using charcoal as a marker.

MATERIALS AND METHODS

Plant collection

Plant materials were collected locally in and around Vellore District, Tamilnadu, India. All the plants in the study were identified by a Botany Professor, Voorhees College, Vellore. Plant parts used were *Acorus calamus* rhizome, *Pongamia glabra* leaves, *Aegle marmelos* unripe fruit and *Psidium guajava* root-bark. The plant materials were thoroughly washed with distilled water and shade-dried at room temperature (25±2ºC) for two weeks.

Extraction

Dried plant materials (500 g) were powdered using a manual grinder and was taken in a flat-bottomed flask and was first defatted with n-hexane twice in the cold. Then the material was soaked in methanol and kept under constant shaking. After 48 hours, the extract was filtered. Nearly 80% of the solvent was removed by distillation on water bath at atmospheric pressure and the last trace was removed under reduced pressure.

Experimental animals

Adult male Swiss albino mice weighing 25 – 30 g were obtained from the animal facility at the Department of Pharmacology, Christian Medical College, Vellore. The animals were housed within the facility and maintained on standard rodent feed and water *ad libitum.* On transfer to the work area, the animals were allowed 14 days for acclimatization.

Small intestine motility test

Gastrointestinal motility test was performed according to the standard method with minor modifications [13]. Mice were fasted for 12 hours. Charcoal meal test was conducted on 10 male mice in each group placed in separate cages. A suspension containing 10% active charcoal in 1.5% arabic gum was used as a marker. Each animal in the group was orally administered with methanol plant extracts at two different concentrations: 7.5 mg/kg and 15 mg/kg. The control mice received only saline. After 30 minutes, both the control and the experimental mice received orally 0.1 ml of charcoal suspension followed by the oral administration of castor oil. 20 minutes after the charcoal meal, the animals were sacrificed by cervical dislocation and the intestine from the pylorus up to ileo-caecal junction was excised and aligned parallel to a ruler. The distance traversed by the charcoal marker was measured and expressed as percentage of the total intestinal length.

Statistical analysis

Data were expressed as mean ± SD of ten replicates and statistical analysis was carried out employing Students t-test.

RESULTS AND DISCUSSION

When compared to the control group, the plant extracts decreased propulsion of the charcoal meal through the gastrointestinal tract in a dose-related manner (Table 1). The plant extracts at higher concentrations produced a significant (p<0.001) decrease in the distance travelled by the marker suspension, when the small intestinal transit was accelerated with castor oil. However, among the four extracts, *A. marmelos* unripe fruit extract at 7.5 mg/kg and 15 mg/kg dosage produced the highest reduction (57% and 29.6% respectively) of gastrointestinal motility which was significant (p<0.001) compared to the control producing a marked decrease in the propulsion of charcoal meal through gastrointestinal tract. And though all the extracts reduced the intestinal motility, *P. glabra* leaves extract showed the greatest propulsion of the charcoal meal at 7.5 mg/kg of the extract.

Table 1: Effect of the methanol plant extracts on gastro-intestinal motility

Treatment	Dose (mg/kg) (30 min before castor oil administration)	Movement of charcoal meal (%)
Control	0.1 ml	80.9±1.8
Acorus calamus	7.5	60.0±1.9***
	15	32.4±1.2***
Pongamia glabra	7.5	67.6±1.2***
	15	42.1±1.0***
Aegle marmelos	7.5	57.0±1.9***
	15	29.6±1.6***
Psidium guajava	7.5	61.4±1.5***
	15	35.2±1.0***

*Values are represented as mean±SD (n=10) and ***p<0.001 were considered statistically significant*

Intestinal diseases are one of the main causes of death in infants, and it is therefore important to identify and evaluate commonly available natural drugs as alternative to currently used anti-diarrhoeal drug [14]. Often diarrhoea is considered as a consequence of altered motility and fluid accumulation in intestinal tract [15]. In this study, methanol extracts of four medicinal plants were evaluated for their anti-diarrhoeal effect in castor oil-induced intestinal motility. Diarrhoea can be induced by castor oil through the production of active metabolite ricinoleic acid. Ricinoleic acid increases peristaltic activity and produces permeability changes in the intestinal mucosal membrane to electrolytes and water. More precisely, castor oil elevates the biosynthesis of prostaglandin which results in irritation and inflammation of the intestinal mucosa to stimulate the motility and secretion [16]. Gastro-intestinal motility describes the contraction of the muscles that mix and propel contents in the gastrointestinal tract. Charcoal meal test in mice is a method used to study the effect of drugs on the motility of intestine [17].

Among the four tested methanol extracts, *A. marmelos* unripe fruit extract exhibited a good retention of charcoal suspension in the intestine. *A. marmelos* has been reported to be effective in chronic cases of diarrhoea due to the presence of large quantities of mucilage acting as demulcent. Decoction of *A. marmelos* unripe fruit has been shown to affect the production and action of certain enterotoxins such as cholera toxin and *E. coli* heat labile toxin; and thereby affecting bacterial colonization to the gut epithelium [18] which may be due to the presence of coumarins and tannins in fruit that act in synergism. Having accounted in controlling infectious diarrhoea, *A. marmelos* ethanolic extract had been proved to have potency to reduce the transit time in the small intestine comparable to atropine administration [19]. Thus this study confirms the folk claim of *A. marmelos* unripe fruit to control diarrhoea.

A. calamus rhizome extract also exhibited a greater retention time of the intestinal content which may be due to the presence of β-asarone which was found as a major component in the crude methanol fractions [7]. The anti-motility and anti-diarrhoeal effects of *P. guajava* root can be attributed because of its astringent nature and may be due to quercetin which showed a similar effect with the leaf extract of *P. guajava* by relaxing smooth muscles and inhibiting bowel contraction, probably by inhibiting intracellular calcium release from the sarcoplasmic reticulum [20]. However, the active principle in root-bark has not been mentioned so far in previous works.

Moreover, the *P. glabra* leaves extract had an increased transit time with the other tested extracts but considerably lesser than the control which may be attributed to the presence of steroids, saponins and glycosides in relatively large amount when compared to alkaloids, flavonoids, tannins and triterpenes [8]. The anti-diarrhoeal activity of the extract may also be due to the presence of tannins which denature proteins in the intestinal mucosa by forming protein tannates and subsequently reduce secretion [21].

A high rate of intestinal absorption might lead to a decrease in intestinal accumulation and together with reduced intestinal motility may result in increased transit time. Another mechanism had been previously proposed to explain the diarrhoeal effect of castor oil which includes inhibition of intestinal Na^+K^+ ATPase activity, thus reducing normal fluid absorption. It is possible that the extracts were able to inhibit electrolyte permeability due to castor oil and prostaglandins release. Suppression of the intestinal fluid accumulation by the extract might also suggest the inhibition of gastrointestinal motility [22]. Since the pharmacological activity of any plant is contributed by the phytochemicals present in the plant extracts [23], here the phyto-compounds must have been responsible for the anti-diarrhoeal property. Though further investigation is required to identify the compounds in these extracts, the positive result can be correlated to the phyto-constituents which acted synergistically in reducing secretion and prolonging the intestinal content to facilitate intestinal absorption.

CONCLUSION

To conclude, the four medicinal plants tested for gastro-intestinal motility proved to be a better therapeutic agent for diarrhoea. It can be assumed that the anti-diarrhoeal action of the extracts was mediated by an anti-secretory mechanism and also by reducing gastro-intestinal motility contributed by the phytochemical agents possessed by the plant extracts. Although, further investigation is required to identify the compounds in the extract and the exact mechanism which contributes to increase in the intestinal content retention time, this study confirms the traditional usage of these plants as anti-diarrhoeal agents.

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