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RESEARCH ARTICLE

Evaluation of the Antidiarrheal Activity of Ethanolic Extract of *Diospyros virginiana* in Rats

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ABSTRACT

The ethanolic extract of the leaves and bark of *Diospyros virginiana* was screened for antidiarrheal effects. The extract was evaluated for castor oil- induced diarrhea and intestinal transit in rats. *Diospyros virginiana* significantly dose-dependently reduced frequency of stooling in castor oil-induced diarrhea and intestinal motility in rats. Ethanolic leaves extracts reduces diarrheal burden than ethanolic bark extract. These findings suggest that the ethanolic extract of the leaves and bark of *Diospyros virginiana* may contain some biologically active ingredients that are active for the treatment of diarrhea in herbal traditional medicine.

KEYWORDS

Antidiarrheal activity, Castor oil, Char coal meal, Ethanolic extracts

INTRODUCTION

Diarrhea is characterized by an increased frequency of bowel movements, wet stools and abdominal pain[1]. It is a leading cause of malnutrition and deaths among children in the developing countries of the world today. According to the World Health Report, diarrhea is the cause of 3.3% of all the deaths worldwide. The worldwide distribution of diarrhea[2] accounts for more than 5-8 million deaths each year in children who were aged less than 5 years. The use of traditional medicine to combat the consequences of diarrhea has been emphasized by the WHO in its diarrhea control programme[3]. Many synthetic chemicals are available for the treatment of diarrhea, but they have some side effects.

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The natural drugs are used as anti-diarrheal drugs, which are not always free from adverse effects.

Plants have been a valuable source of natural products for maintaining human health for many years. More recently, there has been a greater search for natural therapies. The use of herbal drugs in the treatment of diarrhea is a common practice in many African countries[4]. About 80% of individuals from developed countries receive traditional medicines including compounds derived from medicinal plants. Such medicinal plants can be exploited since it has been shown that they are important sources of new chemical substances with potential therapeutic effects[5]. In developing countries like India, a majority of people who live in the rural areas almost exclusively use traditional medicines in treating all sorts of diseases, including diarrhea[6].

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Diospyros virginiana is a persimmon species commonly called the American Persimmon, Common Persimmon, Eastern Persimmon, "'Simmon", "Possumwood", or "Sugar-plum". This is a well-known indigenous tree, growing in woods and fields. Persimmons have been used to lubricate the lungs and strengthen the spleen and pancreas[7]. They improve energy and contain enzymes that help damaged cells and foreign microbes be broken down. Persimmons have a special affinity for the large intestines and heart. Persimmons have been used to treat bronchitis, catarrh, cough, diarrhea, dysentery, goiter, hangover, hemorrhoids and hiccoughs. The bark has been used in intermittent and both it and the unripe fruit have been beneficial in various forms of disease of the bowels, chronic dysentery, and uterine hemorrhage; used in infusion, syrup, or vinous tincture[8].

Seeds and fruits are generally low in crude protein, crude fat, and calcium but high in nitrogen-free extract and tannin[9.] The inner bark and unripe fruit are sometimes used in treatment of fevers, diarrhea, and hemorrhage. Indelible ink is made from fruit. Persimmon is valued as an ornamental because of its hardiness, adaptability to a wide range of soils and climates, its lustrous leaves, its abundant crop of fruits, and its immunity from disease and insects.

Hence, the present study was undertaken to evaluate the possible anti-diarrheal activity of the leaves and bark extract *D.virginiana* of which is used commonly in Indian traditional medicine, by using various validated models and to find out if the folk medicinal use has a scientifically justified basis.

MATERIALS AND METHOD

Plant Collection and Preparation of the Extract

D.virginiana belongs to the family *Ebenaceae* was collected from Coonoor, Nilgiris District, Tamil Nadu, India and identified by the special key given Cambell flora. The leaf and bark of *D.virginiana* were washed with sterile distilled water. After, the leaves and bark were shade dried and powdered by using pestle and mortar. 25g of powder was filled in the thimble and extracted successively with ethanol using a Soxhlet extractor for 48 h. The extracts were concentrated using rotary flash evaporator and preserved at 5°C in airtight bottle until further use. The ethanolic extracts of the plant was diluted with distilled water and was administered orally to mice.

Animals

Swiss albino rats weighed about 100-130 g of either sex were divided into five groups of six animals each. The animals were deprived of food for 24 hours before the commencement of the experiment, but water was allowed adlibitum.

Experimental Design

Swiss albino rats were divided into five groups of 6 animals each.

Group I: Normal control

Group II: Castor oil control

Group III: Castor oil + Loperamide (4 mg/5ml)

Group IV: Castor oil + *Diospyros virginiana* bark (500mg/kg)

Group V: Castor oil + *Diospyros virginiana* leaves (500mg/kg)

Effect of Extracts on Castor oil Induced Diarrhea - Fecal Score Method

First grouped served as the control and received distilled water. All other four groups received castor oil at a dose of 0.1ml per animal orally. The second group served as castor oil control. Thirty minutes after castor oil administration, the third group received loperamide. The fourth group received ethanolic extract of *Diospyros virginiana* bark at the level of 500mg/kg and the fifth group received ethanolic extract of *Diospyros virginiana* leaves at 500mg/kg. Following administration, the animals were placed separately in cages with filter paper, which was changed every hour. The total number of faces and diarrheal faces excreted was recorded for a period of 76 h. The total

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score of diarrheal faces of control group was considered that of 100%. The results were expressed of inhibition.

Effect of Extract on Intestinal Transit Time

This method was done using charcoal as a diet marker. First group served as the control and received water. All the other four groups received castor oil at a dose 0.1ml per animal orally. The second group served as castor oil control. Thirty minutes after castor oil administration, the third group received loperamide. The fourth group received ethanolic extract of *Diospyros virginiana* bark at the level of 500mg/kg and the fifth group received ethanolic extract of *Diospyros virginiana* leaves at 500mg/kg. Each animal was given 1ml of charcoal meal orally (3% deactivated charcoal in 10% aqueous feed) after 30 minutes of castor oil administration. All animals were scarified after 30minutes of charcoal meal administration and the distance covered by the charcoal meal in the intestine, from the pylorus to the caecum was measured and expressed as a percentage of distance moved. All the values were expressed as mean ± standard deviation.

RESULTS AND DISCUSSION

Group-IV and Group-V showed a considerable percentage of protection against diarrhea in experimental animals. In the fecal score assay, at 500 mg/kg concentration (Group - V) extract yielded 93.12 % and Group - IV extract yielded 61.20 % of inhibition in defecation and group III ie., Loperamide (4mg/ 5ml) treated group showed only 31.19 % protection (Table-1).

Study of gastrointestinal transit is one of the parameters included in the antidiarrheal study. Charcoal meal was given to the animal to track the nature of gastrointestinal mobility. Castor oil + *Diospyros virginiana* leaves at 500 mg/kg concentration (Group - V) reduced the gastrointestinal (GI) mobility up to the level of 79.95 % and Castor oil + *Diospyros virginiana* bark (Group - IV) at the same concentration reduced 62.7 % gastrointestinal (GI) mobility whereas Loperamide treated animal group (Group -III) showed only 45.10 % reduction of gastrointestinal (GI) mobility (Table-2). Ethanolic leaves extracts reduces diarrheal burden than ethanolic bark extract.

Table 1: Effect of *Diospyros virginiana* extracts on castor oil induced diarrhea in mice

Groups	Protection (%)	Number of faeces in 4 hrs	Inhibition of defaecation %)
Group-I	100	5.4±0.32	-
Group-II	0	18.6±0.76	-
Group-III	33	13.8±0.44	31.19
Group-IV	83.4	7.2±0.21	61.20
Group-V	100	5.8±0.44	93.12

*Values are given as mean ± standard deviation

Table 2: Effect of *Diospyros virginiana* extracts on intestinal transit

Groups	Mean intestinal length (cm)	Mean distance travelled by charcoal (cm)	GI transit inhibition (%)
Group-I	88.56±0.3847	79.3±0.8366	89.54
Group-II	92.18±0.1788	39±0.790	42.30
Group-III	90.24±0.7829	40.7±0.4472	45.10
Group-IV	48.8 ± 2.1	30.6 ± 1.8	62.70
Group-V	88.3±0.3741	70.6±0.4183	79.95

Castor oil causes diarrhea due to its active metabolite, ricinolic acid[10], which stimulates peristaltic activity in the small intestine, leading to changes in the electrolyte permeability of the intestinal mucosa. Its action also stimulates the release of endogenous prostaglandin[11]. In this study, aqueous extract exhibited a significant

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anti-diarrhoeal activity. Its effect did not depend on the dose. The results were similar to that of the standard drug Loperamide (4mg/5ml) with regard to the severity of diarrhea. Aqueous extract significantly reduced gastrointestinal transit as observed by the decrease in gastrointestinal motility of charcoal meal. The extract also led to a marked reduction in the weight and the volume of the gastrointestinal contents.

The inhibition of gastrointestinal mobility and the reduction in fecal output by substances are the basis of the pharmacological evolution of a potential antidiarrheal agent. It is well known that ricin oleic acid, an active component of castor oil induces intestinal peristalsis, leading to diarrhea. Castor oil induced diarrhea by release in prostaglandin which causes an increase in the net secretion of water[12].

Earlier studies showed that ant dysenteric and antidiarrheal properties of medicinal plants were due to steroids, terpenoids, flavonoids, phenolic compounds, tannins, lignin, carbohydrates and proteins. Hence, tannins, reducing sugars and sterols may be responsible for the mechanism of action of antidiarrheal activity. Antidiarrheal activity of this extract may also be due to the presence of denatured proteins, which form protein tenants. Protein tenants make the intestinal mucosa more resistant and hence, reduce secretion[13]. This can be due to the fact that the extract increased the reabsorption of water by decreasing intestinal motility as observed in the decrease of intestinal transit by charcoal meal. Loperamide, apart from regulating the gastrointestinal tract, is also reported to slow down transit in the small intestine, reduce colon flow rate, and consequently any effect on colonic motility[14]. Atropine significantly reduced intestinal transit time. This is possible due to its anticholinergic effect. However, it did not inhibit castor oil induced enter pooling thereby; suggesting that mediators other than acetylcholine are involved in castor oil induced enter pooling. Furthermore, a decrease in intestinal transit time with atropine could also be due to reduction in gastric emptying.

CONCLUSION

The results of this investigation revealed that the ethanolic extract of leaves and bark of *Diospyros virginiana* contained pharmacologically active substances with anti-diarrheal properties. Further research has to be carried out to fractionate and purify the extract, in order to find out the molecule which is responsible for the antidiarrheal activity which was observed.

REFERENCES

1. World Health Organization, Country Health System Fact Sheet. 2006.
2. Beentje, H., & Sara S. (2001). Plant systematic and phytogeography for the understanding of african biodiversity, *Systematics and Geography of plants*, 71, 284-286.
3. Victora, C. G., Bryce, J., Fontaine, O., & Monasch, R. (2000). Reducing deaths from diarrhoea through oral rehydration therapy. *Bulletin of the World Health Organization*, 78(10), 1246-1255.
4. Inayathulla Shariff, W. R., Asit, K., Sikarwar, A., & Mukesh, S. (2010). Evaluation of the anti-diarrhoeal activity of the Crataeva nurrvala root bark in experimental animals. *International Journal of Pharmacy and Pharmaceutical Sciences*, 2, 158-61.
5. Karnick, C. R. (1994). Pharmacopoeial standards of herbal plants. Vol. I (No. Ed. 1). Sri Satguru Publications. 94-95.
6. Duke, J. A. (1985). Handbook of medicinal herbs. CRC press. Inc., Boca Ratan, Fia. 33-34.
7. Háznagy-Radnai, E., Czigle, S., & Máthé, I. (2007). TLC and GC analysis of the essential oils of Stachys species. JPC-*Journal of Planar Chromatography-Modern TLC*, 20(3), 189-196.
8. Anonymous. (1889). "The persimmon" Gard and Forest, 1889, 2(95), 612.

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9. Barram, I. (1971). A memoir on the distillation of persimmon, *Trans Philos Soc*, 1, 231-234.
10. Watson, W. C., & Gordon, R. S. (1962). Studies on the digestion, absorption and metabolism of castor oil. *Biochemical pharmacology*, 11(3), 229-236.
11. The wealth of India, "A Dictionary of Indian Raw materials and Industrial products", III publications and Information Directorate, New Delhi, 1992, 582-90.
12. Longanga O. A., Vercruysse, A., & Foriers, A. (2000). Contribution to the ethno botanical, phytochemical and pharmacological studies of traditionally used medicinal plants in the treatment of dysentery and diarrhea in the Lamella area", Democratic Republic of Congo (DRC), *J. Ethanopharmacol*, 71(3), 411-23.
13. Chitme, H. R., Chandra, R., & Kaushik, S. (2004). Studies on anti-diarrhoeal activity of Calotropis gigantea R. Br. in experimental animals. *J Pharm Pharmaceut Sci*, 7(1), 70-75.
14. Beentje, H., & Sara, S. (2001). Plant systematic and Phytogeography for the understanding of African Biodiversity, *Systematics and Geography of plants*, 71, 284-286.

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