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Antidiarrhoeal activity of ethanolic extract of roots of *morinda pubescens* J.E. Smith (Rubiaceae)

*** Kavitha Chandran CI, Indira G**

Department of Pharmacognosy, Govt. Medical College, Thiruvananthapuram, Kerala State, India

Abstract
The plant *Morinda pubescens* (Rubiaceae) is a small, ever green plant which is native to southern India and it has been reported to possess a number of medicinal properties. The purpose of present study was to evaluate the antidiarrhoeal activity of the ethanolic extract of the roots of *Morinda pubescens* which is used traditionally as folk medicine, by using castor oil induced diarrhoea model. The parameters of this study were number of diarrhoeal episodes and mean weight of stool of mice. The percentage protection in extract treated animals showing diarrhoea was compared with castor oil treated and loperamide treated animals. The results revealed that the ethanolic extracts significantly reduced diarrhoea in mice with reduction in weight of stools.

Keywords: *Morinda pubescens*, Antidiarrhoeal, Castor oil, Loperamide, Mice

Introduction
Diarrhoeal diseases are one of the leading causes of morbidity and mortality in developing countries and responsible for the death of millions of people each year. There are large numbers of epidemiological and experimental evidence pertaining to worldwide acute diarrhoeal disease, which is one of the principal causes of death in infants [1]. Despite immense technological advancement in modern medicine, many people in the developing countries still rely on the healing practices and medicinal plants for their daily health care needs. Therore, the World Health Organization encouraged studies for the treatment and prevention of diarrhoeal diseases depending on traditional medicinal practices [2]. A range of medicinal plants with antidiarrhoeal properties has been widely used by the traditional healers; however the effectiveness of many of these antidiarrhoeal traditional medicines has not been scientifically evaluated [3].

Morinda pubescens J.E. Smith commonly known as Aal or Indian Mulbery is a species of flowering plant in the family Rubiaceae, native to Southern Asia**.** Traditionally the leaf juice was given orally to children before food for easy digestion. The charred leaves made into a decoction with mustard were a favourite domestic remedy for infantile diarrhoea [4]. The expressed juice of leaves was externally applied to gout to relieve pain. The leaves are administered internally as a tonic and febrifuge. They were useful in gastropathy, dyspepsia, ulcerative stomatitis, wounds, gout, inflammation, hernia, sarcocele and fever [5]. The roots were styptic, constipating, anti-inflammatory, alexeteric and tonic, and were useful in haemorrhages, dysentery, inflammations, boils and general debility [6]**.** *Morinda pubescens* its medicinal potential has yet to be studied scientifically, and, therefore, this present study was initiated with the aim of investigating the medicinal and therapeutic properties of *M. pubescens* by evaluating its effects on wound healing in injured mice using two different concentrations of ethanolic extract of *M. pubesens*.

Materials and methods
Plant material
The plant specimens (roots) for the proposed study were collected from localities of Kariavattom, Thiruvananthapuram. The collected plants were carefully examined and authenticated in Department of Botany, University of Kerala, Kariavattom, Thiruvananthapuram. A voucher specimen (Voucher No.KUBH-5867) has been deposited for future reference.

Animals
The protocol for conducting the wound healing experiments in mice was approved by the Instituitional Animal Ethics Committee, Govt. Medical college, Thiruvananthapuram (Regn No: 01/01/2015/MCT). Swiss albino mice of both sexes weighing 20-25 g were used for the study. The animals were kept in the standard condition. The room temperature was maintained 22±2 ^{0}C with food and water *ad libitum*. The animals were transferred to the laboratory at least 1h before start of experiment.

Preparation of *M. pubescens* root extract
The collected roots were washed with running tap water to remove adhering materials and cut into small pieces. Then, the roots were dried at a temperature not exceeding 50 °C. These dried materials were cut into small pieces and then pulverized mechanically into coarse powder. The coarse powder obtained by passed through the sieve No. 18. Then this coarse powder was extracted with absolute alcohol by soxhlet apparatus for 3 h. ethanolic extract was obtained in rotary evaporator. The semisolid mass of ethanolic extract was stored in a dessicator.

Acute Oral Toxicity Study
Acute toxicity studies of ethanolic extract of *Morinda pubescens* in Swiss albino mice will be carried out as per OECD guidelines 425 limit test [7]**.**

Six female albino mice are required
Procedure: Animals should be fasted prior to dosing, with food but not water should be withheld for 3-4 hours. Following the period of fasting, the animals should be weighed and the test substance administered. The fasted body weight of each animal is determined and the dose calculated according to the body weight. After the substance has been administered, food may be

withheld for further 1-2 hours in mice [8].

Limit test

The limit test is primarily used in situations where the experimenter has information indicating that the test material is likely to be nontoxic, i.e., having toxicity below regulatory limit doses.

Limit dose at 2000mg/kg

Dosed one animal at the test dose. If the animal dies, conduct the main test to determine the LD50. If the animal survives, dose four additional animals sequentially so that a total of five animals are tested. If three animals die, the limit test is terminated and the main test is performed. The LD50 is greater than 2000mg/Kg if three or more animals survive. If an animal unexpectedly dies late in the study, and there are other survivors, it is appropriate to stop dosing and observe all animals to see if other animals will also die during a similar observation period. Late deaths should be counted the same as other deaths.

The LD50 is less than the test dose (2000mg/kg) when three or more animals die.

The LD50 is greater than the test dose (2000mg/Kg) when three or more animals survive.

Observations

Animals are observed individually at least once during the first 30 minutes after dosing, periodically during the first 24 hours (with special attention given during the first 4 hours), and daily thereafter, for a total of 14 days. The duration of observation should not be fixed rigidly. It should be determined by the toxic reactions and time of onset and length of recovery period, and may thus be extended when considered necessary. The times at which signs of toxicity appear and disappear are important, especially if there is a tendency for toxic signs to be delayed.

Antidiarrhoeal activity by *In vivo* Method

Swiss albino mice of both sexes weighing 20-25 g were used for the study. They were fasted overnight and brought to the lab on the day of the experiment. They were weighed and housed individually in cages with no access to drinking water. Loperamide at a dose of 2 mg/kg p.o. was administered to the standard group animals [8]. Test group animals received ethanol extract of *Morinda pubescens* 200 and 300 mg/kg p.o. The control group animals were dosed with distilled water (1ml/100g p.o). One hour after dosage; administered castor oil orally to all animals in a dose of 1 ml/100g. After four hours, stools were collected on non-wetting paper [9]. Antidiarrhoeal activity was expressed as % protection, in comparison with the control. Percentage protection was calculated by using the following formula

Percentage Protection = (Sc-St/Sc) x 100

Where,

Sc is mean stool weight of control

St is mean stool weight of test

Statistical analysis

Statistical analysis was done using one way ANNOVA followed by Dunnet's test. P values lesser than 0.05 were considered as significant.

Table 1: Effect of Morinda pubescens on Castor oil induced diarrhoea in mice

Groups	Treatment	Total no of defecation in first 4 hrs	Total weight of feaces in first 4 hrs.
Control	Castor oil (0.5 ml, p.o.) + Distilled water (0.5 ml p.o.)	21.67±0.988**	0.0825±0.0015**
Loperamide (standard)	Castor oil (0.5 ml, p.o.) +Loperamide (2 mg/kg p.o.)	4.83±0.3073**	0.2983±0.009**
Ethanolic extract (Test I)	Castor oil (0.5 ml, p.o.) + Ethanolic extract (200 mg/kg p.o.)	8.167±0.307**	0.036±0.008**
Ethanolic extract (Test II)	Castor oil (0.5 ml, p.o.) + Ethanolic extract (300 mg/kg p.o.)	4.833±0.401**	0.030±0.0006**

Values are presented as mean ± SEM, (n=6); ***p*< 0.001, Dunnets t- test as compared to control

Table 2: Percentage inhibitions of defecation of loperamide and different concentration of ethanolic extracts

Groups	Treatment	Percentage inhibition of Defecation
Control	Castor oil (0.5 ml, p.o.)+ Distilled water (0.5 ml p.o.)	-
Loperamide (standard)	Castor oil (0.5 ml, p.o.)+Loperamide (2 mg/kg p.o.)	63.74±1.49%**
Ethanolic extract (Test I)	Castor oil (0.5 ml, p.o.)+ Ethanolic extract (200 mg/kg p.o.)	57.24±1.12%**
Ethanolic extract (Test II)	Castor oil (0.5 ml, p.o.)+ Ethanolic extract (300 mg/kg p.o.)	63.14±1.15%**

n=6, the results were expressed as Mean ± SEM to show difference in groups. The differences are considered significant when, **P<0.001

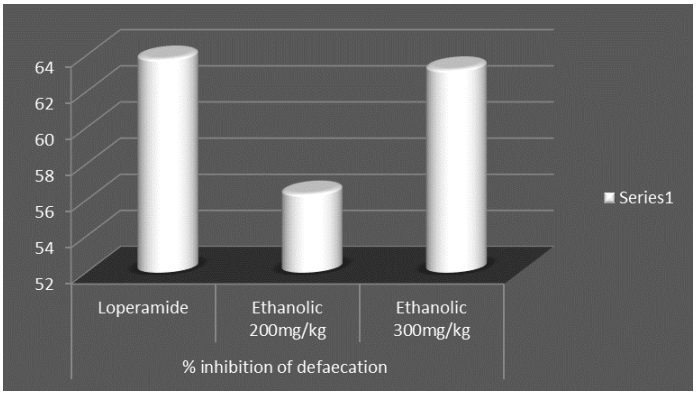

Fig 1: Percentage inhibition of defecation of standard with different concentration of ethanolic extract

Results

Acute toxicity

The acute oral toxicity study showed that oral administration of ethanolic extracts of *M. pubescens* roots to the mice up to 2000 mg/kg dose neither showed mortality nor any visible clinical signs of general weakness in the animals. So indicated that the toxic dose was above 2000 mg/kg.

Antidiarrhoeal effects

In this study we investigated and compared a possible antidiarrhoeal activity of crude ethanolic extract obtained from roots of *M. pubescens* in mice. Oral administration of loperamide 2mg/kg and ethanolic extracts (200 and 300 mg/kg) showed that there was significant dose- related inhibition of defecation frequency when compared to the negative control. The ethanolic extract of *M. pubescens* administered at the dose of 200 and 300 mg/kg showed 57.24% and 63.14% reduction in diarrhoeal episodes respectively. This shows significant reduction in diarrhoeal episodes with maximum effect at 300 mg/kg dose level. Whereas the standard group, Loperamide a standard, antidiarrhoeal drug treated animal at 2 mg/kg showed significant reduction in diarrhoeal episodes (63.74%). The study reveals that the ethanolic extracts exhibited significant antidiarrhoeal activity. The remarkable antidiarrhoeal effect of *M. pubescens* root extracts against castor oil induced diarrhoea model proves to its efficacy in an extensive range of diarrhoeal conditions [10].

Discussion

Diarrhoea results from an imbalance between the absorptive and secretory mechanisms in the intestinal tract, which is accompanied by an excess loss of fluid in the faeces [11]. Castor oil causes diarrhoea due to its active metabolite, ricinolic acid, which stimulates the peristaltic activity in the small intestine, leading to changes in the electrolyte permeability of the intestinal mucosa. In this study, the ethanolic extract of *M. pubescens* significantly reduced the faecal output produced by castor oil. The remarkable antidiarrhoeal effect of *M. pubescens* root extracts against castor oil induced diarrhoea model proves to its efficacy in an extensive range of diarrhoeal conditions [12].

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