



## POTENTIAL EVALUATION OF ANTI-DIARRHEAL ACTIVITY OF LEAVES OF *AGANOSMA DICHOTOMA* IN MICE

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### ABSTRACT

The plant *Aganosma dichotoma* is widely used as traditional medicine in different aborigines which is supported by this investigation. It belongs to Apocynaceae family. The aim of the study was to evaluate the methanolic extract of leaf of *Aganosma dichotoma*'s different fractionates as anti-diarrheal agents. The methanolic extract of leaves of *Aganosma dichotoma* (400 mg/kg) exhibited very statistically significant anti-diarrheal activity with a 68.27 % reduction of diarrhea compared to the standard of loperamide 69.23 %. The anti-diarrheal activity shows *Aganosma dichotoma* statistically significant at the higher dose compared to dose of 200 mg/kg. Therefore we can assume that, the anti-diarrheal activity of *Aganosma dichotoma* was dose dependent.

**Keywords:** *Aganosma dichotoma*; Anti-diarrheal activity screening, Castor oil-induced diarrhea, Leaves, Loperamide drug.

### INTRODUCTION

Diarrhea is the passage of 3 or more loose or liquid stools per day, or more frequently than is normal for the individual. It is usually a symptom of gastrointestinal infection, which can be caused by a variety of bacterial, viral and parasitic organisms<sup>1</sup>. About 1.7 to 5 billion cases of diarrhea occur per year<sup>2,3</sup>. It is most common in developing countries where most of the young people's were affected by diarrhea on average three times a year<sup>2</sup>. Worldwide, as of 2012, it is the second most common cause of deaths in children less than five (0.76 million or 11 %) <sup>2,4</sup>. Frequent episodes of diarrhea are also a common cause of malnutrition and the most common cause in those less than five years of age<sup>2</sup>. Prevention of infectious diarrhea is by improved sanitation, clean drinking water and hand washing. Breast feeding for at least six month is also recommended as is vaccination against rotavirus. Oral rehydration solution (ORS), which is clean water with modest amounts of salts and sugar, along with zinc tablets are the treatments of choice. This treatment has been estimated to have saved 50 million children in the past 25 years<sup>2</sup>. A wide range of plants with anti-diarrheal properties is widely used for the treatment of diarrhea. Some of new plant also included in the current list and day by day the list is going to enlarging<sup>5</sup>. In the current study, the anti-diarrheal activity was studied of the methanolic extract of *Aganosma dichotoma* Macro and Microscopic characteristics of leaves of *Aganosma dichotoma* along with the numerical values, fluorescence characteristic<sup>6</sup>. The plant *Aganosma dichotoma* contains the Kampferol, phenolic acid also<sup>7</sup>. One investigation shows that, *Aganosma dichotoma* shows sedative and hypnotics effect in moderately<sup>8</sup>. The Plant leaf extract also shows a moderate an anti-microbial and with a good thrombolytic activity where an investigation of antimicrobial and others free radical scavenging, brine shrimp lethality, antimicrobial and thrombolytic study was conducted<sup>9</sup>.

### MATERIALS AND METHODS

#### Collection of plant sample

The plants of *Aganosma dichotoma* were collected in September, 2012 from Sylhet, Bangladesh. A voucher specimen was deposited in the Bangladesh National Herbarium, Mirpur, Dhaka and is tagged with the accession number of 39644.

#### Preparation of Plant Extract

2 kg of leaves were collected for separation. The leaves were initially dried in normal air and then in mechanical drier at 60-70°C. The dried leaves were grounded to make coarse powder with a mechanical grinding machine and powder was kept in a clean closed glass container. During grinding the leaves sample, the grinder was thoroughly cleaned to avoid the contamination and cross contamination with any remnant of previously grinded material or with the foreign materials. 200 g of the powder sample were soaked in 650 ml methanol for the purpose of extraction. The regular shaking were perform for 15 days on a shaker machine and also as well as in manual basis. After 15-20 days, the plant extract were filtered with the filter paper with the help of Buchner funnel. Finally, the filtrate was concentrated by evaporating the solvent at a temperature of 40°C by water bath. After all these procedure, a paste like deep radish green concentrate was obtained<sup>10</sup>.

#### Drugs and chemicals

Loperamide were used for the standard drug to conduct the study. Loperamide were collected from Bangladeshi largest company "Square Pharmaceuticals Limited".

#### Experimental animal

Swiss-albino mice of either sex, aged 4-5 weeks, obtained from the Animal Resource Branch of the International Centre for Diarrheal Diseases and Research, Bangladesh (ICDDR) were used for the experiment. Each of these mice was weighed from 25-40 g. They were housed in standard polypropylene cages and kept under controlled room temperature (24 ± 2°C; relative humidity 60-70 %) in a 12 h

light-dark cycle and fed ICDDR; B formulated rodent food and water (*ad-libitum*). As these animals are very sensitive to environmental changes, they are kept before the test for at least 3-4 days in the environment where the experiment will take place. Institutional animal ethical committee (IAEC) was approved (HET-CU2012/1) experimental protocol requirements which supervised by Department of Pharmaceutical Sciences, North South University.

**Anti diarrheal activity screening  
Castor oil-induced diarrhea in mice**

Thirty experimental animals were randomly selected and were divided into four groups denoted as group-I (Control) and mice of this cage received 1 ml 2 % v/v aqueous Tween 80 orally, group-II (Standard drug treated) and mice of this cage were treated with the reference drug Loperamide at the dose of 50 mg/kg body weight orally, group-III and group-IV (MELI treated) and mice of this cage were treated with methanolic extract of *Aganosma dichotoma* leaves at the dose of 200 and 400 mg/kg body weight by oral route. Before administering the leaves of *Aganosma dichotoma* were

suspended in 2 % v/v aqueous Tween 80. Each animal was placed in an individual cage and the floor lining was changed at every hour. Diarrhea was induced by oral administration of castor oil to each mouse after the above treatment. During an observation period of 5 hours; the number of diarrheic feces excreted by the animals was recorded<sup>10</sup>.

**Statistical analysis**

All data were expressed as mean and Standard error. T-test was used to evaluate the different sample. The statistical significance was accepted at the range of P < 0.001, P < 0.01 and P < 0.05.

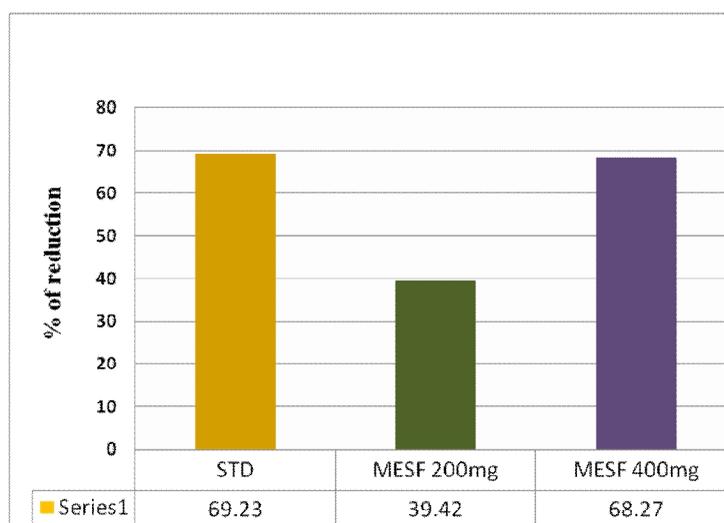
**RESULTS**

**Castor oil induced diarrhea**

The methanolic extract of leaves of *Aganosma dichotoma* was subjected to castor oil induced anti-diarrheal test and the following data are collected. Both dose of 200 mg/kg and 400 mg/kg were administered with Control group as well as with Standard group (Table 1).

**Table 1: Castor oil induced diarrheal model**

Code No.	Number of diarrheal feces (Mean)	% Reduction of diarrhea	t-Test value	Standard error	P-value	Level of significance
STD	2.67	69.23	4.02	0.333	0.0158	Statistically significant
MELI 200 mg	5.25	39.42	1.99	1.031	0.1038	Not statistically significant
MELI 400 mg	2.75	68.27	4.41	0.479	0.0069	Statistically significant



**Figure 1: Anti-diarrheal effect of methanolic extract of leaves of *A. dichotoma* on castor oil (1 mL/mice) induced diarrhea in mice**

**DISCUSSION**

In the condition of diarrhea, a lot of water loss from the body and as a results, the water and electrolyte imbalance develop because of very frequent and excessive loss of water by feces. In some diarrhea the secretory component predominates, while other diarrhea is characterized by hyper motility. Castor oil causes diarrhea due to its active metabolite, ricinoleic acid. This stimulates peristaltic activity in the small intestine<sup>10</sup>. Ricinoleic acid is active metabolites of Castor oil and its make diarrhea by stimulating peristaltic activity in the small intestine and lead to diarrhea thus make water and electrolyte imbalance in the body. The methanolic extract of

leaves of *Aganosma dichotoma* shows a significant level of anti-diarrheal activity in dose of 400 mg/kg. In the dose of 200 mg/kg shows less activity compared to the standard. The methanolic extract of leaves of *Aganosma dichotoma* shows its anti-diarrheal activity in the larger dose and thus we can say its dose dependent (Figure 1).

**CONCLUSION**

In the present investigation, the results demonstrate that the leaves of *A. dichotoma* have significant anti-diarrheal activity as dose dependent manner. The anti-diarrheal exhibited via inhibition of hyper secretion, gastrointestinal motility and

enhance gastric transit time. The leaves of *A. dichotoma* could be used in the diarrhea. Further investigation will lead to isolation of active lead compounds for several biological activity as well as anti-diarrheal lead compounds.

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