INTRODUCTION

In developing countries, a majority of people living in rural areas almost exclusively use traditional medicine in treating all sorts of diseases including diarrhea. Diarrhea is a major health problem especially for children under the age of 5 and up to 17% of children admitted in the paediatrics ward die of diarrhea. Worldwide distribution of diarrhea accounts for more than 5-8 million deaths each year in infants and children below 5 years old especially in developing countries. According to W.H.O. estimates for 1998, about 7.1 million deaths were caused by diarrhea. The incidence of diarrheal diseases still remains high despite the efforts of many governments and international organizations to curb it. It is therefore important to identify and evaluate available natural drugs as alternatives to currently used anti-diarrheal drugs, which are not always free from adverse effects [1,2]. A range of medicinal plants with anti-diarrheal properties is widely used by traditional healers. However, the effectiveness of many of these anti-diarrheal traditional medicines has not been scientifically evaluated.

In the normal adult, 7-8 liters of water and electrolytes are secreted daily into the gastrointestinal tract. This, together with dietary fluid, is absorbed by epithelial cells in the small and large bowel. Water follows the osmotic gradients which result from shifts of electrolytes across the intestinal epithelium, and sodium and chloride transport mechanisms are central to the causation and management of diarrhea, especially that caused by bacteria and viruses. The energy for the process is provided by the activity of Na+/K+ ATPase.

Absorption of sodium into the epithelium is effected by:

Sodium-glucose-coupled entry. Glucose stimulates the absorption of sodium and the resulting water flow also sweeps additional sodium and chloride along with it (Solvent drag). This important mechanism remains active in diarrhea of various etiologies and improvement of sodium and water absorption by glucose (and amino acids) is the basis of oral rehydration regiments (see below). Absorption of sodium and water in the colon is stimulated by short-chain fatty acids (see below, cereal-based ORT).

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Sodium-ion-coupled entry, $\text{Na}^+$ and $\text{Cl}^-$ enter the epithelial cell, either as a pair or, as seems more likely, there is a double exchange, $\text{Na}^+$ (extracellular) with $\text{H}^+$ (intracellular) and $\text{Cl}^-$ (extracellular) with $2\text{OH}^-$ or $2\text{HCO}_3^-$ (intracellular) [3-5]. The *Dioscorea oppositifolia* is a perennial twining vine belong to the family Dioscoreaceae. The leaves are arranged oppositely with heart shape. It is distributed worldwide, the perennial tuber of *Dioscorea oppositifolia* is well known, due to the triterpenoid compound [6]. The tuber contains about 20% starch, 75% water, 0.1% vitamin $\text{B}_1$ and 10 to 15mg vitamin $\text{C}$. It also contains mucilage, amylase, aminoacids and glutamine [7]. The tubers are sometimes used as an herbal tonic. It stimulates the stomach and spleen and has on effect on lungs kidneys. The tubers have been eaten for the treatment of poor appetite, chronic diarrhea, asthma and dry cough [8]. Hence the present work is carried out to evaluate the effect of methanol extract of *Dioscorea oppositifolia* an adult wistar rats with anti diarrhoeal activity against castor oil-induced-diarrhoea model in rats.

**MATERIAL AND METHODS**

**Plant Material**

The tubers of *Dioscorea oppositifolia* were collected from Talakona forest, Chittoor District of Andhra Pradesh, India, in the month of November, 2009. The plant was authenticated by Prof. P. Jayaraman, Director of National Institute of Herbal Science, West Tambaram, Chennai. The voucher specimen (PARC/2009/430) of the plant was deposited at the college for further reference.

**Preparation of extraction**

The tubers of *Dioscorea oppositifolia* was dried in shade and pulverized in grinder-mixer to obtain a coarse powder. It was then passed through 40 mesh sieve. Weight quantity to continuous hot extraction with methanol in soxhlet apparatus for 48 hours. The extract was evaporated under reduced pressure using rotary evaporator until all the solvent has been removed to given an extract methanol extract of *Dioscorea oppositifolia* 12.85% w/w.

**Acute Oral Toxicity Study**

The procedure was followed by using OECD 423 (Acute Toxic Class Method). The acute toxic class method is a step wise procedure with three animals of a single sex per step. Depending on the mortality or moribund status of the animals and the average two to three steps may be necessary to allow judgment on the acute toxicity of the test substance. This procedure results in the use number of animals while allowing for acceptable data based scientific conclusion. The method used to defined doses (5, 50, 500, 2000 mg/kg body weight) the results allow a substance to be ranked and classified according to the Globally Harmonized System (GHS) for the classification of chemicals which cause acute toxicity.

**Experimental Procedure**

Albino wistar rats weighing 150-250 gm were used for the study. The starting dose level of methanol extract of *Dioscorea oppositifolia* was 2000 mg/kg body weight p.o as most of the crude extracts posses LD$_{50}$ value more than 200 mg/kg p.o. Dose volume was administered 0.2ml per 100gm body weight to overnight fasted rats with were ad libidum. Food was withheld for a further 3-4 hours after administration of methanol extract *Dioscorea oppositifolia* L. and observed for signs for toxicity. The body weight of the rats before and after administration were noted that changes in skin and fur, eyes, mucous membranes, respiratory, circulatory, autonomic and central nervous system and motor activity and behavior pattern were observed and also sign of tremors, convulsions, salivation, diarrhea, lethargy, sleep and coma were noted. The onset of toxicity and signs of toxicity also noted.

The acute toxicity of methanol extract of *Dioscorea oppositifolia* leaves was determined as per the OECD guideline no. 423 (Acute Toxic Class Method). It was observed that the test extract was not lethal to the rats even at 2000mg/kg dose. Hence, 1/10$^{th}$ (200mg/kg) and 1/5$^{th}$ (400mg/kg) of this dose were selected for further study [9].

**Animals used**

Healthy adult wistar rats (150-250gm) were obtained from the animal house in Sree VidyaniKethan College of Pharmacy, Tirupati, Andhra Pradesh. The animals were maintained in a well ventilated room with 12:12 hour light or dark cycle in polypropylene cages. The animals were fed with standard pellet fed (Hindustan Lever Limited, Bangalore) and water was given *ad libitum*. Ethical committee clearance was obtained from institutional animal ethics committee (IAE) of CPCSEA (Ref. No./IAEC/XIII/05/SVCP/2009-2010).

**Castor oil-induced diarrhoea**

Rats were divided into four groups of six animals each, diarrhoea was induced by administering 1ml of castor oil orally to rats. Group I served as control (2 ml/kg, i.p. saline), group II received atropine (3 mg/kg, i.p.) served as standard and group III and IV received the methanol extract of *Dioscorea oppositifolia* (400 and 200 mg/kg, p.o), 1hrs before castor oil administration. This numbers of both wet and dry diarrhoea droppings were counted every hour for a period 4 hrs mean of the positive control group consisted of animals given an intra peritoneal injection of saline (2 ml/kg, ip) [10].
Small intestinal transit
Rats were fasted for 18 hrs and divided into four groups of six animals each. Group I received 2ml of castor oil orally with saline 2 ml/kg intra peritoneal, group II received atropine (3 mg/kg, i.p.), group III and IV received MEDO 400 and 200mg.kg p.o respectively, 1 hrs before the administration of castor oil. One ml of marker (10% charcoal suspension in 5% gum acacia was administered orally 1 hrs after castor oil treatment. The rats were sacrificed after 1 hrs and the distance traveled by charcoal meal from the pylorus was measured and expressed as percentage of the total length of the intestine from the pylorus to caecum [11].

Statistical analysis
The data was expressed as mean ± S.E.M. (standard error of the mean) student’s t-test was used for the ANOVA followed by the result regarded as significant at p<0.001.

RESULTS
ACUTE TOXICITY STUDY
The body weight of the rats before and after administration were noted that there is no changes in skin and fur, eyes, mucous membranes, respiratory, circulatory, autonomic and central nervous system and motor activity and behavior pattern were observed and also no sign of tremors, convulsions, salivation, diarrhoea, lethargy, sleep and coma were noted. The onset of toxicity and signs of toxicity also not there. In further study there was no toxicity/ death were observed at these levels.

Castor oil-induced diarrhea
The results of the present study showed that the methanolic tuber extract of Dioscorea oppositifolia L. produced a statistically significant reduction in the severity and frequency of diarrhoea produced by castor oil in a dose dependent manner. MEDO significantly inhibited defecation compared to control group (2 ml/kg i.p)

Small intestinal transit
The MEDO (200-400 mg/kg p.o) significantly inhibited castor oil induced intestinal fluid accumulation and the volume of intestinal content up to compared to control additionally the MEDO had significantly reduced the castor oil induced intestinal transit (p<0.001) compared to control group.
The acute toxicity tests for the MEDO were performed and it was found that was safe in 2000 mg/kg body weight.

Fig. 1. Physiological Mechanism of diarrhea
Fig. 2. Effect of methanolic extract of Dioscorea oppositifolia L. tubers on castor oil induced diarrhoea in rats

![Bar chart showing mean defecation in 4 hours for different groups.]

Fig. 3. Effect of methanolic extract of Dioscorea oppositifolia L. tubers on castor oil induced small intestinal transit in rats

![Bar chart showing % of intestinal transit for different groups.]

Table 1. Acute Oral Toxicity Studies of MEDO

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Groups</th>
<th>Dose/kg b.w</th>
<th>Weight of animals</th>
<th>Signs of Toxicity</th>
<th>Onset of Toxicity</th>
<th>Duration of Study</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>MEDO</td>
<td>2000 mg</td>
<td>160 g 160 g</td>
<td>No signs of Toxicity</td>
<td>Nil</td>
<td>14 days</td>
</tr>
<tr>
<td>2.</td>
<td>MEDO</td>
<td>2000 mg</td>
<td>180 g 180 g</td>
<td>No signs of Toxicity</td>
<td>Nil</td>
<td>14 days</td>
</tr>
<tr>
<td>3.</td>
<td>MEDO</td>
<td>2000 mg</td>
<td>200 g 200 g</td>
<td>No signs of Toxicity</td>
<td>Nil</td>
<td>14 days</td>
</tr>
<tr>
<td>4.</td>
<td>MEDO</td>
<td>2000 mg</td>
<td>160 g 160 g</td>
<td>No signs of Toxicity</td>
<td>Nil</td>
<td>14 days</td>
</tr>
<tr>
<td>5.</td>
<td>MEDO</td>
<td>2000 mg</td>
<td>180 g 180 g</td>
<td>No signs of Toxicity</td>
<td>Nil</td>
<td>14 days</td>
</tr>
<tr>
<td>6.</td>
<td>MEDO</td>
<td>2000 mg</td>
<td>200 g 200 g</td>
<td>No signs of Toxicity</td>
<td>Nil</td>
<td>14 days</td>
</tr>
</tbody>
</table>
the diarrhoeal effect of castor oil. It has not been possible to define its correct mechanism of action. MEDO may act against to above any one of the mechanism [15-18].

The MEDO significantly reduced the castor oil induced intestinal transit as compared with control group. In this study, atropine decreased intestinal transit possibly due to its anti-cholinergic effect [19]. In castor oil induced diarrhoea, the liberation of ricinoleic acid results in irritation and inflammation of the intestinal mucosa, leading to release of prostaglandins, which results in stimulation of secretion [20] by prevents the reabsorption of NaCl and water. Probably MEDO increased the reabsorption of NaCl and water by decreasing intestinal motility as observed by the decrease in intestinal transit by charcoal meal.

In conclusion, the present study has shown that Dioscorea oppositifolia L. is a potential therapeutic option in the effective management of diarrhoea, thus justifying its widespread use by the local population for these purposes. Concerted efforts are being made to fully investigate the mechanisms involved in the pharmacological activities of Dioscorea oppositifolia L. and phytochemical studies are also in progress to isolate and characterize the active constituents of Dioscorea oppositifolia L. The isolated compound may serve as useful prototypes of anti-diarrhoeal drugs of natural origin possessing the desired pharmacological activities while lacking certain untoward effects.

**DISCUSSION AND CONCLUSION**

Diarrhoea results from an imbalance between the absorptive and secretory mechanisms in the intestinal tract, accompanied by hurry, resulting in an excess loss of fluid in the faeces. At doses of 200 and 400 mg/kg, the methanol extract of Dioscorea oppositifolia L. showed significant anti-diarrhoeal activity against castor oil-induced diarrhoea as compared with the control group. It significantly (P<0.01) reduced the frequency of diarrhoea and consistency of defecations. The MEDO also showed a dose related decrease in castor oil-induced diarrhoea.

Several mechanisms have been supposed to be involved in the diarrhoeal effect of castor oil. These include Castor oil is decreases fluid absorption, increases secretion in the small intestine and colon, and affects smooth muscle contractility in the intestine. Castor oil produces diarrhoeal effect due to its active component of ricinoleic acid, inhibition of intestinal Na⁺,K⁺-ATPase activity to reduce normal fluid absorption, activation of adenyl cyclase, stimulation of prostaglandin formation, platelet-activating factor and recently nitric oxide was contribute to the diarrhoeal effect of castor oil [12-14]. Despite the fact that number of mechanisms has been involved for the diarrhoeal effect of castor oil, it has not been possible to define its correct mechanism of action. MEDO may act against to

**Table 2. Effect of methanolic extract of Dioscorea oppositifolia L. tubers on castor oil induced diarrhoea in rats**

<table>
<thead>
<tr>
<th>Groups</th>
<th>Treatment / Dose (mg/kg)</th>
<th>Mean defecation in 4 hrs.</th>
<th>% inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Disease control (saline2ml/kg i.p.)</td>
<td>24.00 ± 0.36</td>
<td>-</td>
</tr>
<tr>
<td>II</td>
<td>Standard atropine (3mg/kg i.p.)</td>
<td>8.83 ± 0.40***</td>
<td>63.21</td>
</tr>
<tr>
<td>III</td>
<td>MEDO (400mg/kg p.o.)</td>
<td>10.50 ± 0.34**</td>
<td>56.25</td>
</tr>
<tr>
<td>IV</td>
<td>MEDO (200mg/kg p.o.)</td>
<td>13.83 ± 0.31***</td>
<td>42.38</td>
</tr>
</tbody>
</table>

Values of expressed as mean ± SEM, ANOVA followed by student t-test in each group rats ***P<0.001, as compared to castor oil induced group.

**Table 3. Effect of methanolic extract of Dioscorea oppositifolia L. tubers on castor oil induced small intestinal transit in rats**

<table>
<thead>
<tr>
<th>Groups</th>
<th>Treatment / Dose (mg/kg)</th>
<th>Total length of intestine</th>
<th>Distance traveled by marker</th>
<th>% of intestinal transit</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Disease control (saline 2ml/kg i.p.)</td>
<td>105 ± 2.82</td>
<td>75.98 ± 1.35</td>
<td>72.36 ± 0.36</td>
</tr>
<tr>
<td>II</td>
<td>Standard atropine (3mg/kg i.p.)</td>
<td>99.51 ± 4.82</td>
<td>20.57 ± 0.97***</td>
<td>20.67±0.31 ***</td>
</tr>
<tr>
<td>III</td>
<td>MEDO (400 mg/kg p.o.)</td>
<td>106.33 ± 3.04</td>
<td>27.57 ± 1.34***</td>
<td>25.928±0.45 ***</td>
</tr>
<tr>
<td>IV</td>
<td>MEDO (200 mg/kg p.o.)</td>
<td>101.58 ± 2.86</td>
<td>37.61 ± 1.27***</td>
<td>37.025±0.39 ***</td>
</tr>
</tbody>
</table>

Values of expressed as mean ± SEM, ANOVA followed by student t-test in each group rats ***P<0.001, as compared to intestinal transit in rats.
REFERENCES


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