Annals of R.S.C.B., ISSN:1583-6258, Vol. 24, Issue 1, 2020, Pages. 1574 - 1583
Received 15 April 2020; Accepted 23 June 2020.

Evaluation of Anti-Diarrhoeal Activity of Ficus Dalhousiae MiqLeaf Methanolic Extract

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Abstract

India has a dense forest with plenty of medicinal plants which have been used as folklore medicines by the local people for many years. They used different plant parts of Ficus species to treat diarrhoea. Depending on the traditional use of some plants of Ficus genus as antidiarrhoeal, this plant was selected to evaluate antidiarrhoeal activity in animal models. Wistar albino rats weighing 180 to 200 grams were used in this study. There were five groups in each experimental model with six animals in each group. The antidiarrhoeal activity was evaluated by different experimental models namely castor oil-induced diarrhoea and magnesium sulphate induced diarrhoea. The methanolic extract of Ficus dalhousiae leaves showed significant antidiarrhoeal activity against castor oil-induced diarrhoea and magnesium sulphate induced diarrhoea in rats. The methanolic extracts at 100, 200 and 400 mg/kg significantly inhibited diarrhoea. There was a significant dose-dependent anti diarrhoeal effect in both the animal models as compared to the standard drug ($P<0.01$). Based on the results in experimental models, the methanolic extract of Ficus dalhausiae demonstrated significant reductions in faecal output when compared to the standard groups. In conclusion it can be said that tannins and flavonoids present in the plant extracts may be responsible for the antidiarrhoeal activity.

Keywords: anti-diarrhoeal, Ficus dalhousiae, wistar, methanolic, experimental models.

INTRODUCTION

Diarrhoea is a very common ailment and national problem in many tropical countries and the cause of4-5 million deaths throughout the world annually[1,2]. Children are more susceptible to this disease

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which is the second leading cause of death of children under 5 years old[3]. Diarrhoea results from an imbalance between the absorptive and secretory mechanism in the intestinal tract accompanied by intestinal hurry, resulting in an excess loss of fluid in the faeces[4]. Diarrhoea is characterized as rapid movement of faecal matter through intestine resulting in poor absorption of water, nutritive elements and electrolytes producing abnormal frequent evacuation of watery stools. According to world health organization, it is the one of the most common cause of morbidity and mortality in many developing countries affecting mainly the infants and children[5]. It is often caused by enterotoxins which are produced by bacteria such as Escherichia coli, Salmonella typhi, Salmonella typhimurium, Clostridium difficile, Clostridium freundii, Aeromonas hydrophila, Campylobacter jejuni and Vibrio cholera to name a few. These bacteria cause the influx of water and ions to the intestinal lumen and thus increase the intestinal motility, thereby causing watery stools. Such secretory diarrhoea is treated by the administration of oral rehydration salts in children or adults to reduce the loss of essential electrolytes and maintain the body fluids osmolality[6]. The major causative agents of diarrhoea in humans include Shigella flexneri, Staphylococcus aureus, Escherichia coli, Salmonella typhi and Candida albicans[7,8].

Alternatively, many opiod drugs like Diphenoxylate, Loperamide, Diloxanide furoate for protozoal infections induced diarrhoea and dysentery, racecadotril, muscarinic receptor blockers like atropine sulphate etc; are available in the market for treating diarrhoea. But all of the existing drugs suffer from adverse effects like the induction of bronchospasm, vomiting by racecadotril; intestinal obstruction and constipation by loperamide[9]. Combination of antidiarrhoeal drugs with different mechanisms of action are often used for synergistic action. The value of these combination have not been studied experimentally and hence the present study was aimed at studying the antidiarrhoel activity of some drugs singly and in combination in models of hypermotility and hypersecretion of intestinal tract in experimental animals[10].

MATERIAL AND METHODS

Plant Material

The plant material was collected from Tirupathi and was authenticated by K.Madhava Shetty, Assistant Professor and Taxonomist from Sri Venkateshwara University, Tirupathi.

Extraction Process

The plant material was dried under shade, pulverized by a mechanical grinder passed through a #40 sieve and stored in tightly closed container for further use. The coarse powder of leaves is extracted in a soxhlet apparatus by using methanol as a solvent. After extraction, the solvent is removed by vacuum distillation. The semi solid mass was stored in a desiccator for further use. Preliminary

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phytochemical screening was done to identify the presence of alkaloids, steroids, flavonoids and tannins in Ficus dalhousiae leaf extract.

Phytochemical Screening

The preliminary phytochemical screening of the extracts was performed by the method described byKhandelwal[11]

Experimental Animals

Wistar Albino rats weighing around 150 to 200 grams of either sex were maintained at uniform laboratory conditions in standard steel cages, provided with food and water and were acclimatized for a week before the experiment.

Experimental Methods Castor oil induced diarrhoea

Induction of secretory diarrhea was done according to the method described by Karthik et al.[12] with slight modifications. The rats weighing around 150 to 200 grams were fasted for 18 hours. They were divided into five groups with six rats in each group (n=6). The first group of animals were served with castor oil (2 ml). The second group received standard drug, Loperamide (3 mg/kg) orally as suspension. The third, fourth and fifth group were treated with FDLME 100, 200 and 400 mg/kg respectively. After 60 minutes, the animals of each group received 2 ml of castor oil orally. Watery faecal material and frequency of defecation were noted upto 4 hours in transparent metabolic cages with plastic dishes weighed and placed at the base of the cages. The weight of plastic dish before and after the defecation were noted and compared with the control.

Magnesium sulfate induced diarrhoea

Diarrhoea was induced using the same method as that described in the previous experimental model with the only difference that magnesium sulfate was used at the dose of 3 mg/kg instead ofcastor oil. Again in this model, the faecal matter and the frequency of defecation were noted up to 4 hours in the transparent metabolic cages with pre weighed plastic dishes placed at the base of the cages. Weight of the plastic dishes were recorded before and after defecation and compared with the control group.

Phytochemical screening

Preliminary phytochemical screening of methanolic extract of Ficus dalhousiae showed the presence of alkaloids, steroids, tannins, saponins, terpenoids and flavonoids.

Effect of FDLME on castor oil induced diarrhoea

In this experiment, the test group FDLME 400 mg showed maximum inhibitory effect with 76%

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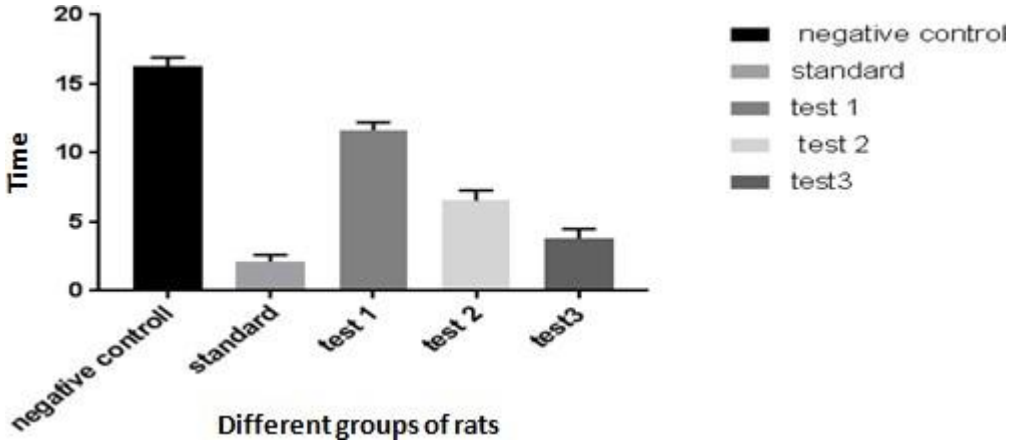
with number of diarrhoel faeces noted as (3.83 ± 0.70) as compared with the standard drug, Loperamide with 86.5% with the number of faeces (2.16 ± 0.47). Other test groups FDLME 100 mg and FDLME 200 mg showed significant inhibitory effect with 27.1% and 59.3% respectively and the number of diarrhoeal faeces were recorded as (11.66 ± 0.56) and (6.5 ± 0.76) respectively.

Table 1: Number of diarrhoeal faeces observed in treated groups

Treatment	**Dose (mg/kg)**	**Number of diarrhoealfaeces**	**Inhibition of diarrhoea(%)**
Negative control	Castor oil (2ml)	16.3 ± 30.66	-------_
Loperamide	3mg	2.16 ± 0.47	86.5 %
FDLME (100mg/kg) + castor oil (2ml)	100mg	11.66 ± 0.56	27.1 %
FDLME(200mg/kg) + castor oil (2ml)	200mg	6.5 ± 0.76	59.3 %
FDLME(400mg/kg) + castor oil (2ml)	400mg	3.83 ± 0.70	76 %

Values are expressed as ± S.E.M (n=6). P<0.01, when compared to the control.



Effect of FDLME on magnesium sulfate induced diarrhoea

In this experiment, the test group FDLME 100 mg/kg showed 57% inhibition to diarrhoea with number of diarrhoeal faeces as (13.16 ± 0.60). The test groups FDLME 200 mg/kg showed a

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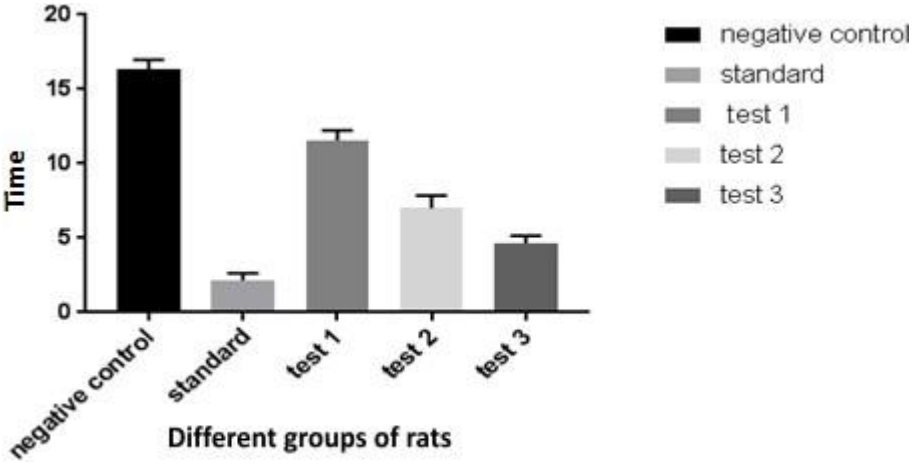
significant diarrhoeal inhibition with 77% where gradual decrease in the number of diarrhoeal faeces (8.66 ±0.88) was observed. The test group FDLME 400 mg/kg showed a maximum diarrhoeal inhibition at 84% with minimum number of diarrhoeal faeces noted as (6.66 ± 1.38).

Table 2: Number of diarrhoeal faeces observed in treated groups

Treatment	**Dose (mg/kg)**	**Number of diarrhoeal faeces**	**Inhibition of diarrhoea (%)**
Negative control	Castor oil (2ml)	20 ± 1.06	----
Loperamide	3mg	2.16 ± 0.47	89 %
FDLME (100mg/kg) + castor oil (2ml)	100mg	13.16 ± 0.60	57%
FDLME(200mg/kg) + castor oil (2ml)	200mg	8.66 ±0.88	77%
FDLME(400mg/kg) + castor oil (2ml)	400mg	6.66 ± 1.38	84%

Values are expressed as ± S.E.M (n=6). P<0.01, when compared to the control.



DISCUSSION

The use of medicines derived from plants has been commonly used as folk medication in the treatment of diarrhoea. People in the developing countries still rely on this type of treatment

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system[13]. Among the various medicinal plants, antidiarrhoeal activity was found in plants which possess phytoconstituents like alkaloids, tannins[14], flavonoids[15] and triterpenes[16]. From the earlier studies, it has been reports that Ficus bengalensis containing tannins and flavonoids possessed antidiarrhoeal activity and the underlying mechanism appears to be spasmolytic and anti-enteropooling property by which it produced relief from diarrhoea[17]. Antidiarrhoeal activity of flavonoids has been ascribed to their ability to inhibit intestinal motility and hydroelectrolytic secretion[18]. The inhibitory effect of flavonoids on intestinal motility in a dose related manner was earlier reported[19,20]. Triterpenes have also been reported to show considerable antidiarrhoeal activity[21]. Tannins present in many plants denature protein to form the protein tannate, which makes the intestinal mucosa more resistant and reduces secretion[22]. Previous study have shown that antidysenteric and antidiarrhoeal properties of medicinal plants are due to the presence of tannins, alkaloids, flavonoids, saponins, reducing sugars, sterols and triterpenes[23]. Diarrhoea can be induced by castor oil through the production of active metabolite ricinolic acid[24]. Castor-oil induced diarrhoea is described to be an appropriate model that characterizes secretory diarrhoea[25]. Though several mechanisms have been proposed to explain the diarrhoeal effect of castor oil, it is well known that diarrhoea is initiated by its metabolite ricinoleic acid through a hypersecretory response which activates intestinal smooth muscles through EP3 prostaglandin receptors[26]. Ricinoleic acid also reduces active Na^+ and K^+ absorption and decrease Na^+K^+ ATPase activity in the small intestine and colon[27]. The liberation of ricinoleic acid results in irritation and inflammation of intestinal mucosa, leading to release of prostaglandins, which results in stimulation of secretion and thereby prevents the reabsorption of Nacl and H_2O and resulting in diarrhoea[28]. More precisely, castor oil elevates the biosynthesis of prostaglandin[29-31] which results in irritation and inflammation of the intestinal mucosa to stimulate the motility and secretion[31-33]. Several other mechanisms had been earlier proposed to explain the diarrhoeal effect of castor oil which includes inhibition of intestinal Na^+K^+ATPase activity, thus reducing normal fluid absorption[34].

Suppression of the intestinal fluid accumulation by the extract might also suggest the inhibition of gastrointestinal function[35]. Castor oil model, therefore, incorporates both secretory and motility diarrhoea[36]. Magnesium sulphate is known to increase the permeability of electrolytes at the level of the intestinal mucosa, alongside the secretion of cholecystokinin in the duodenum leading to hyper secretion which inhibits fluid reabsorption[37]. Loperamide used in this study as reference drug acts byinhibiting the peristaltic activity, through indirect effect on circular and longitudinal muscle of the intestinal wall, also by stimulating the absorption of water and electrolytes by enterocytes by increasing the intestinal transit time of the bowel content[38].

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Ficus dalhousiae methanolic leaf extract showed dose dependent inhibition of frequency of defecation in both experimental models, castor oil induced diarrhoea and magnesium sulphate induced diarrhoea. There was a maximum diarrhoeal inhibition observed with the test group FDLME 400 mg/kg which showed minimum number of faeces. Although Ficus dalhousiae leaf methanolic extract showed significant inhibition in all test groups (100 mg, 200 mg and 400 mg).

CONCLUSION

Ficus dalhousiae leaf methanolic extract has shown a significant antidiarrhoeal effect in albino wistar rats in both, castor oil induced and magnesium sulphate induced diarrhoea model. It is evident from our study that FDLME contains pharmacologically active ingredients that are responsible to show antidiarrhoeal activity.

CONFLICT OF INTEREST

The authours declare that they have no conflicts of interest.

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