ANTIDIARRHEAL ACTIVITY OF A MYSTERIOUS HERB, GYNURA PROCUMBENSE IN ALBINO SWISS MICE

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Abstract

In order to evaluate the activity of extracts on a castor oil-induced diarrhea model, three groups of rats (3 animals per group) were injected with ethanolic extracts of Gynura Procumbens (300 and 600mg / kg body weight) orally in albino swiss mice. The other two groups received normal saline (3 mg / kg body weight) and loperamide (3 mg / kg body weight) as a positive control. The effect of the extract on intestinal passage and castor oil-induced intestinal fluid accumulation (intestinal reservoir) was evaluated. A significant and dose-dependent antidiarrheal activity was showed by the plant extract at oral doses of 300mg/kg and 600mg/kg body weight. The protective role of the extract at 600 mg / kg was comparable to that of loperamide (3 mg / kg) standard. No mortality and signs of visible weakness were observed in rats following administration of extracts at doses up to 1000 mg / kg. Our results showed that the extract of Gynura procumbens had significant antidiarrhoeic activity supporting its use in traditional herbal medicine practice.

Keywords: Antidiarrheal activity, Castor oil, Gynura procumbens, mortality, albino swiss mice etc.
Introduction
Gynura Procumbens are being recently treated as mysterious herb healing in diabetes, cancer, obesities and also under investigation of many disease management. It is a everyday salad and vegetable like spinech. No report till date regarding the effect of this herb in dirrohe management.

Diarrheal diseases are a major problem in Third World countries and are responsible for the death of millions of people each year [1]. Diarrhea is an alteration in normal bowel movement and is characterized by an increase in the water content, volume, or frequency of stools [2][3][4]. For discovery of new medicines plants have been long important history in pharmaceutical industry. Medicated plants are a promising active compound with therapeutic activity. International organizations, including the World Health Organization (WHO), have therefore inspired research on treatment and prevention of diarrheal diseases using traditional herbal medical practices [5],[ 6],[7].

The most common causes of diarrhea include Bacteria from contaminated food or water, Viruses such as the flu, norovirus, or rotavirus. Rotavirus is the most common cause of acute diarrhea in children. Parasites, which are tiny organisms found in contaminated food or water, Medicines such as antibiotics, cancer drugs, and antacids that contain magnesium. Diarrehea is also treated by replacing lost fluids and electrolytes to prevent dehydration. Depending on the cause of the problem, you may need medicines to stop the diarrhea or treat an infection. Adults with diarrhea should drink water, fruit juices, sports drinks, sodas without caffeine, and salty broths. As your symptoms improve, you can eat soft, bland food. Children with diarrhea should be given oral rehydration solutions to replace lost fluids and electrolytes.

Antidiarrheal drugs work in several way such as inhibition of intestinal Na+ K+ ATPase activity, thus reducing normal fluid absorption,activation of adenylate cyclase or mucosal cAMP-mediated active secretion, stimulation of prostaglandin formation, and platelet activating factor. Most recently nitric oxide has been claimed to contribute to the diarrheal effect of castor oil.

Globally diarrheal disease is the second leading cause of death in children under five, and responsible for the deaths of about 760,000 children every year. The disease is particularly dangerous for young children, who are more susceptible to dehydration and nutritional losses, especially those living in low and middle income countries [8]. Bangladesh is a riverine country, which is susceptible to severe flooding. Recently, there were extensive floods during the monsoons of 1988, 1998, and 2004. During these periods, 25-50% of Bangladesh was submerged, resulting in the destruction of infrastructure, contamination of water, and epidemics of diarrheal illness.

Food intolerances and sensitivities, which are problems digesting certain ingredients or foods. An example is lactose intolerance, Diseases that affect the stomach, small intestine, or colon, such as Crohn's disease , Problems with how the colon functions, such as irritable bowel syndrome.

According to the WHO and UNICEF reports, there are about 2.5 billion cases of diarrheal disease worldwide every year, and 1.9 million children below 5 years of age die from diarrhea each year, of whom most are from developing countries. Of all child deaths from diarrhea, 78% occur in African and Southeast Asian regions [9].

The present work was undertaken to investigate the potential in vivo antidiarrheal effect of the ethyl acetate extract of Gynura procumbens leaves extract in various experimental models of diarrhea in albino Swiss mice that are collected from animal house, Department of pharmacy of Jahangirnagar university.

Materials And Methods
Plant material
The leaves of Gynura procumbens plant were collected from the Mirpur-12(DOHS) in April 2018. After collection then plant leaves were identified by the National Botanical Garden of Bangladesh.

Preparation of ethanolic extract
The plant leaves were separated from the undesirable materials and dried at room temperature for two weeks. The plant leaves were ground into a coarse powder with the help of suitable grinder. About 350 gm of powder materials was taken in a clean and dried glass beaker and soaked in 600 ml of ethanol. The container with its contents was sealed and kept for a period of 10 days.

The whole plants chemical constituents of Gynura procumbens were isolated and purified by the
different types of techniques such as silica gel column chromatography, Sephadex LH-20 gel column chromatography, medium pressure column chromatography, and semi-preparative HPLC, and their structures were elucidated by chemical properties and spectroscopic analyses. Results: Sixteen compounds were identified to be (1) quercetin , (2) apigenin , (3) luteolin , (4) kaempferol , (5) astragaline , (6) kaempferol-5-O-(6″-O-acetyl)-β-D-glucopyranoside , (7) negletein , (8) 4-methoxycinnamic acid , (9) benzyl-O-β-D-glucopyranoside , (10) 2-phenylethyl-β-D-glucopyranoside , (11) 3,5-dicaffeoylquinic acid methyl ester , (12) 3,5-dicaffeoylquinic acid ethyl ester , (13) 3,4-dicaffeoylquinic acid methyl ester , (14) 4,5-dicaffeoylquinic acid methyl ester , (15) protocatechuic acid, and (16) eugenol glucoside.[10] Animal Albino Swiss mice (weighing 25 - 30 g) of both sexes (male and female), were stayed in standard metal cages with proper bedding. They were provided with food and water and also allowed two-weeks as adaptation period prior to start of our research by our desire extract. During study feeding niddle is used to feed the crude extract. The mice were all screened initially by giving 1 ml of castor oil and only those showing diarrhea were selected for the final experiment.

Standard drug and control solution preparation

Weight of the standard drug was 2mg ( Active) but power weight was 200mg. For preparation of 5mg standard solution we taken 500mg power and dissolve it into 3ml distilled water. Normal saline (NaCl 0.9% or .9g/100ml) prepared for control mice.

Preparation of extract solution

300mg/kg and 600 mg/kg extract were prepared by distilled water. For each extract 3 mice were allocated, 300mg/kg extract was prepared by taking 21.600 mg and dissolved in 3ml distilled water. On the other hand 600mg/kg extract was prepared by taking 43.200 mg and dissolved in 3ml distilled water. Average weight of the mice was 24 gm.

Test Procedure of antidiarrheal Test

The method will be adopted form Galvez et al; (1993). Test samples, control and Loperamide were given orally by means of a feeding needle. The mice were fed with the samples, control and Loperamide 40 minutes after the oral administration of castor oil at a dose of 1 ml per mouse. Individual animals of each group were placed in separate cages having adsorbent paper beneath and examined for the presence of diarrhea every hour in three hours study after the castor oil administration. Number of stools or any fluid material that stained the adsorbent paper were counted at each successive hour during the 3 hours period and were noted for each mouse. The latent period of each mice also counted. At the beginning of each hour new papers were placed for the old ones. During an observation period of 3 hr, the total number of faecal output including diarrheic faeces excreted by the animals was recorded. A numerical score based on stool consistency was assigned as follows: normal stool=1, and watery stool=2[11].

Results and Discussion

In the traditional herbal medicine system, Gynura procumbens is used in the management of antidiabetic and others diseases management by traditional medicine practitioners in Asian subcontinent (especially India, Pakistan, china, Bangladesh and Africa). This study tried to evaluate the antidiarrheal activity of plants. Our results showed that this extract significantly inhibited castor oil induced diarrhea in albino swiss mice. Several mechanisms have previously been proposed to explain the diarrhea effect of castor oil. These include the inhibition of intestinal Na + K + ATPase activity, ie the decrease in normal fluid absorption[12], the activation of adenylate cyclase or mucosal cAMP mediated activation secretion [14], stimulation of prostaglandin formation [13], and platelet activity Factor 23 is included. Most recently, it is claimed that nitric oxide contributes to the diarrhea effect of castor oil [15]. However, it is well documented that castor oil causes diarrhea due to its most active ingredient ricinoleic acid (12-hydroxy-9-cis-octadecenoic acid is a fatty acid) through hypersecretion reaction [16] [17][18]. Therefore, it can be considered that the anti-diarrhea effect of the extract is mediated by the anti-secretion mechanism. This was also evident from the suppression of castor oil induced liquid accumulation by extracts. The results were equivalent to loperamide, the standard drug.

Conclusion

In this research, It demonstrates that the ethanol extract of Gynura procumbens leaves pharmacologically or therapeutically active
substances with prominent anti-diarrhea activity in animal models. The present data provided a scientific support for the traditional use of this plant as diarrheal remedy that we can use pharmaceutically. However, a more detailed phytochemical analysis would be necessary to isolate, identification and characterize the active compounds involved in anti-diarrheal action, and to understand the precise mechanism of action of these actions.

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Table 1: Effect of the Ethanolic extract of Gynura Procumbens on castor oil induced diarrhea in mice.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>First diarrhea in minute (latent time)</th>
<th>Total number of feces</th>
<th>% inhibition of defecation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>2</td>
<td>11</td>
<td>--</td>
</tr>
<tr>
<td>Loperamide</td>
<td>43</td>
<td>3</td>
<td>72.73%</td>
</tr>
<tr>
<td>Gynura procumbens 300mg/Kg</td>
<td>15</td>
<td>7</td>
<td>36.36%</td>
</tr>
<tr>
<td>Gynura procumbens 600mg/Kg</td>
<td>40</td>
<td>5</td>
<td>54.54%</td>
</tr>
</tbody>
</table>

Percent inhibition of defecation in mice was calculated by using the following equation: % inhibition = \(\frac{(Mo - M)}{Mo}\) x 100; where, Mo = Mean defecation of control and M = Mean defecation of test sample.

Figure 1: Graphically representation % of defecation of the mice.