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Research Paper

Evaluation of Antidiarrheal properties of ethanol extract of *Brassica juncea* in experimental animals

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Abstract

Objective: The present study was designed to evaluate the anti-diarrheal potentials of ethanol extract of *Brassica juncea* in albino rats.

Materials and methods: The defatted powder of whole plant of *Brassica juncea* subjected to extraction using ethanol in soxhlet apparatus. The acute oral toxicity was studied for ethanol extract according to OECD guidelines 425. The antidiarrheal properties of ethanol extracts was assessed against castor oil induced diarrhea, magnesium sulfate induced diarrhea and charcoal passage test in albino wistar rats.

Results: The ethanol extract of *Brassica juncea* has effectively reversed diarrhea. The extract at higher dose 400mg/kg shows significant reduction the number of feces against castor oil induced and MgSO4 induced diarrhea. In charcoal passage test, higher dose of ethanol extract also shown significant reduction in the distance travelled.

Conclusion: The result obtained establish the efficiency of ethanol extract of *Brassica juncea* as antidiarrheal agent

Keywords: Brassica juncea, castor oil, magnesium sulphate, charcoal passage test.

INTRODUCTION

Diarrhoea is loose, watery stools (liquid bowel movements). Frequent passage of three or more loose or watery stools and unformed feces per day indicates diarrhea. Diarrhea is a major cause of ill-health, especially for children because, particularly, retrovirus responsible for this. Diarrhea may associate with several diseases like May abdominal pain and irritation within the lining of the small or large intestine leads to diarrhea. The decrease in water absorption and an increase in loss of water with stools also lead to diarrhea. Loss of fluid in the form of diarrhea causes electrolyte imbalance and dehydration 1,2.

Herbal medicines cater about 80 % of the health needs of the world's population, especially for millions of people in the vast rural areas of developing countries. In India, a wide range of medicinal plants has been widely used for the management of diarrhea without scientific investigation of its safety and therapeutic potentials³. In antidiarrheal most of the time we used to ORT medicines but this category of drugs will not work always. Patients often express their dissatisfaction with ORT since it does not decrease the

frequency of stools. Moreover, there is an increasing drug resistance to antibiotics. Thus, an important niche exists for the development of cost-effective alternative approaches for the treatment of diarrhea which can possibly be filled using tested and well standardized medicinal plants. The virulent features of diarrheal organisms have been studied in detail and the pathogenesis of infectious diarrhea is largely well understood. However, most of the studies reporting antidiarrheal activity of medicinal plants overlook the pathogenesis of infectious diarrhea and evaluate their efficacy based on their activity. Targeting the virulence parameters as an alternative approach to define the divergent mechanism(s) of the antidiarrheal activity of medicinal plants. Plants have been a valuable source of natural products for maintaining human health for many years. More recently, there has been a greater search for natural therapies4. Medicinal plants are a promising source of new anti-diarrheal drugs. For this reason, the WHO has encouraged studies pertaining to the treatment and prevention of diarrheal diseases using traditional medical practices. In olden days the herbal drugs are used for the treatment of diarrhea. The Brassica juncea belong to the family Brassicaceae, also was an important component of Traditional and Folklore medicine for the treatment of diarrhea but has a lack of scientific evidence. In this regard, the present study has been design to evaluate the anti-diarrheal potentials of ethanol extract of *Brassica juncea* using animal models⁵.

MATERIALS AND METHODS

Plant material

The plant *Brassica juncea* was collected from the local area of Kerala. The plant was authenticated by Dr. Rama Rao, Scientist, Regional Ayurveda Research Institute for Metabolic Disorders. The herbarium specimen was preserved for future reference at institute herbarium library. The plant of *Brassica juncea* was separated from other unwanted parts, using pure water washed and cleaned and dried under shade for future investigation.

Preparation of methanol extract

The dried plant material was grounded into powder, which then passed through sieve No. 22 mesh. The coarsely powdered drug material of about 350 g (approximate)was used for consecutive solvent extraction process using petroleum ether and ethanol in soxhlet'sapparatus⁶. As ethanol is best solvent for the extractions of phyto-chemicals from the plants with respect to its polarity and hydrophobic property, the methanol extract of *Brassica juncea* was used for the present study.

Preliminary phytochemical examination

The initial phytochemical examinations for the eethanol extract of *Brassica juncea* had been performed according tomethods described by Khandelwal⁷.

Drugs and chemicals

All reagents and chemicals employed in the present investigation were of analytical category. The medicated castor oil and MgSO $_4$ were procured from Sigma Laboratory, India and Loperamide was procured from Aventis Pharmaceutical Ltd., India.

Animals

The healthy albino wistar rats of 180-220gms weight range and 9 months obtained from Sri Venkateswara Enterprises, Bangalore accommodated under excellent laboratory conditions of temperature (22 ± 10 C), relative humidity ($55 \pm 10\%$) and given with standard pril diet (supplied from Amrut, Pranav Agro Industries Ltd., Sangli, India) and water ad libitum. All animals used in experiment were randomly selected and classified into various groups before beginning of experimental investigation; all were adopted for the duration of 7 days under above said standard habitat conditions. Institutional Animal Ethical Committee (IAEC) of East west College of Pharmacy Bangalore (Karnataka) had approved the study protocol prior to the experiments (Ref. No. EWCP/CPCSEA/IAEC/1/2018/07) registered under CPCSEA New Delhi.

Acute Oral Toxicity Studies

The OECD guidelines number 423 (up and down procedure) were referred to determine acute oral toxicity of ethanol extract of *Brassica juncea*. A starting dose applied was 2000 mg/kg B.W, p.o. of EEBJ was injected to 3 male rats, noticed for 14 days. The same study was reiterated once more with the same dose range, 2000 mg/kg body weight p.o. of EEBJ for 3 days more, and noticed for 14 days⁸.

Evaluation of antidiarrheal activity of ethanol extract

The ethanol extract of *Brassica juncea* was evaluated for its anti-diarrheal potentials against various following animal models

Castor Oil Induced Diarrhea

Rats of both the sex were fasted for 18 h and divided into six groups consisting of six animals in each. Rats in group I Received normal saline and group II received castor oil (diarrhea control) and vehicle and III received castor oil and Loperamide at 3 mg/kg p.o. (standard control), IV, V and VI received the castor oil and ethanol extract at 100, 200 and 400 mg/kg p.o. respectively. After 30 minutes of administration of drugs, diarrhea was induced by giving castor oil (Oral) 1 ml to each rat. Rats were observed for 6 h during which the total number of fecal outputs and the weight of feces excreted were recorded. The percentage of inhibition was calculated9.

Magnesium Sulfate Induced diarrhea

Rats will fast for overnight are divided into six groups consisting of six animals in each group. Group I received normal saline (3 ml/kg P.O) served as the normal control. Group II received magnesium sulphate and vehicle, Group III received magnesium sulphate and standard drug Loperamide at 3 mg/kg p.o., Group 1V, V and VI received magnesium sulphate and 100, 200 and 400 mg/kg of ethanol extract of *Brassica juncea* respectively. The standard drug and extract were administered one hour before the oral administration of magnesium sulfate 2 gm/kg and rats were observed for 6 h during which the total number of fecal Outputs and the weight of feces excreted were recorded. The percentage of inhibition was calculated 10.

Charcoal Passage Test

The animals were divided into five groups of 6 animals each. Group I received 20% Tween and served as a Normal group. Group II received Loperamide (3 mg/kg p.o.) served as a standard group. Groups III, IV and V received ethanol extract of Brassica juncea at100, 200 and 400 mg/kg, respectively and served as test groups. The day before the experiment, animals were fasted for 18 h, but with free access to water. After 1 h of drug administration, each animal was administered 1 ml of the freshly prepared charcoal meal (10% active charcoal in 100 ml of 5% aqueous gum acacia) orally. After 1 h, animals were sacrificed using an overdose of ether anesthesia. The abdomens were opened and small intestine from the pylorus to caecum was isolated. The distance traveled by the charcoal meal in the intestine, from the pylorus to the caecum was measured and expressed as the percentage of distance covered using the formula¹¹.

Percentage of intestinal fluid inhibition = $(T_0 - T_1/T_0) \times 100$

 T_0 = total length of intestine

 T_1 = distance traveled by charcoal in the intestine

Statistical Analysis

All the values were expressed as mean \pm standard error of mean (S.E.M.) and analyzed for ANOVA and post hoc Dunnet's T-test using Graph pad prism5software.

RESULTS

Preliminary phytochemical investigation

The ethanolic extracts of *Brassica juncea* contain Alkaloids, flavonoids, tannins, sterols and steroids.

Castor oil induced diarrhea

Administration of castor oil could induced diarrhea in experimental animals, which was evidenced by significant (p<0.001) increase in number and weight of feces in toxic control group when compared normal animals. But in therapeutic animals treated with standard drug loperamide and medium and high dose of EEBJ, number and weight of feces were significantly (p<0.001) reduced and shown significant % percentage inhibition of diarrhea when compared to toxic group of animals while the effect of low dose of extract was not significant (p>0.05). The effect of EEBj were dose dependent (See Table 1).

Magnesium sulfate induced diarrhea

Administration of magnesium sulphate was induced diarrhea in experimental animals, which was evidenced by significant (p<0.001) increase in number and weight of feces in toxic control group when compared normal animals. But in therapeutic animals treated with standard drug loperamide and high dose of EEBJ, number and weight of feces were significantly (p<0.001) reduced and shown significant % percentage inhibition of diarrhea when compared to toxic group of animals. Administration of medium dose of EEBJ has shown less significant (p<0.01) activity while the effect of low dose of extract was not significant (p>0.05). The results of anti-diarrheal activity of ethanol extract of *Brassica juncea* plant using magnesium sulphate induced diarrhea was given below (See Table 2).

Table 1. Effect of ethanol extract of Brassica juncea on castor oil induced diarrhea

Groups	Dose (mg/kg)	Total number of feces	Weight of feces	% inhibition of diarrhea
Group I (Normal)	-	10.83 ± 0.94	1.267 ± 0.07149	-
Group II -Toxic	-	18.67 *** ± 0.88	2.083 ***± 0.1014	-
Group III -Standard	3	9.833 ±0.6***	1.200 ±0.13***	46.50± 4.3
Group IV -100mg	100	17.67±0.84ns	2.050 ± 0.085 ns	14.08± 2.7
Group V-200mg	200	15.00± 0.58*	1.8* ± 0.076ns	18.5*± 5.4
Group VI- 400mg	400	11.77± 0.83***	1.3± 0.088194***	39.5***±5.08

Values are mean ± S.E.M, n=6 symbols represent statistical significance.

ns p>0.05, * p<0.05, ** p<0.01, ***p<0.001 vs Toxic control.

ns p>0.05, + p<0.05, ++ p<0.01, +++p<0.001 Toxic control vs Normal control.

Table 2: Effect of ethanol extract of Brassica juncea on magnesium sulfate induced diarrhea

Groups	Total number of feces	Weight of feces	% inhibition of diarrhea
Group I -Normal	9.167 ± 0.47	1.250 ± 0.1	
Group II -Toxic	18.50 ± 0.76	2.250 ± 0.099	-
Group III -Standard	10.33 ± 0.67***	1.200± 0.07***	43 ± 3.9
Group IV -100mg	17.33 ± 0.8 ns	2.0 ± 0.06^{ns}	8.33 ± 2.7
Group V-200mg	15 ± 0.93**	1.867 ± 0.04**	19.83 ± 4.01**
Group VI- 400mg	13.33 ± 0.6***	1.267± 0.07***	27 ± 3.41***

Values are mean ± S.E.M, n=6 symbols represent statistical significance.

ns p>0.05, * p<0.05, ** p<0.01, ***p<0.001 vs Toxic control.

ns p>0.05, + p<0.05, ++ p<0.01, +++p<0.001 Toxic control vs Normal control.

Charcoal Passage Test

The results of anti-diarrheal activity of ethanol extract of *Brassica juncea* plant using charcoal passage test was given below in charcoal passage test, administration standard drug and high dose of EEBJ in therapeutic groups could

significantly (p<0.001) decrease mean distance travelled by the charcoal meal compared to normal animals. Administration of medium dose of EEBJ has shown less significant (p<0.01) activity. But no significant (p>0.05) reduction was shown by the animals treated with low dose of EEBJ. (See Table 3).

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Table 3: Effect of ethanol extract of Brassica juncea on charcoal passage test

Group	Mean length of small intestine (cm)	Mean distance travelled by charcoal meal (cm)	% inhibition
Group I -Normal	101 ± 1.27	76.33 ± 3.412	-
Group II -Toxic	101 ± 0.76	21 ± 1.770+++	79.83 ± 1.759
Group III -Standard	102 ± 1.04	74 ± 2.620 ^{ns}	26.50 ± 2.405
Group IV -100mg	101 ± 1.36	61 ± 2.338**	38.17 ± 2.725*
Group V-200mg	102 ± 0.81	27 ± 1.770***	73.17 ± 1.759***

Values are mean ± S.E.M, n=6 symbols represent statistical significance.

ns p>0.05, * p<0.05, ** p<0.01, ***p<0.001 vs Toxic control.

 ns p>0.05, + p<0.05, ++ p<0.01, +++p<0.001 Toxic control vs standard control

DISCUSSION

Diarrhea is a common condition that involves unusually frequent and liquid bowel movements. It's the condition where three or more stools in a day. The diarrheal disease causes the death of one in nine children worldwide. The reason for making diarrhea the second leading cause of death among children's under the age of five it causes nutritional deficiency and dehydration. It is also responsible for killing around 760,000 children's every year, and 1.7 to 5 billion cases per year. It is a well-established fact that about 80% of the population especially in developing countries using the herbal medicines for their healthcare need. The reasons range from low cost to availability of these medicines and their use depends on ancestral experience. WHO has also urged various governments especially those of the developing countries, to include in their healthcare programmes those herbal medicines with proven safety and efficacy. This experimental was employed to validate the antidiarrheal efficiency of ethanolic extract of Brassica *juncea* on animal models^{12,13}.

In the castor oil induced diarrhea the castor oil mainly contains triglycerides of ricinoleic acid which hydrolyse by lipase in the small intestine to glycerol and ricinoleic acid. Ricinoleic acid releases the prostaglandins, which result form of inflammation and irritation effect result increase the gastrointestinal motility. In addition, it also stimulates secretion of fluids and electrolyte of the small intestine which increase the intestinal transit¹⁴. The ricinoleic acid present in castor oil activate G protein-coupled prostanoid receptor (EP3) on the intestinal smooth muscle, the formed ricinoleic salt with sodium and potassium in the lumen of the intestine and theses salt inhibit the sodium-potassium ATPase and also increases the permeability of intestinal epithelium, which causes the cytotoxic effect to the intestinal absorptive cells. The magnesium sulphate induces diarrhoea in experimental animals by increasing secretion of sodium and water due to stimulation of villous cells of intestine. In the presents study, ethanol extract of plant (EEBJ) could significantly reduced the total number and amount of faces in therapeutic groups and the effect was comparable to standard drug loperamide which decreases intestinal motility by binding mu receptor present in neural plexus of the intestinal wall^{15,16}.

Charcoal passage test is used to determine the effect of the ethanolic extract on gut motility. The EEBJ exhibited dose-dependent anti-diarrheal activity on gastrointestinal transit using charcoal meal in rats. The standard drug Loperamide

have also shown significant reduction in the gastrointestinal transit. Hypermotility characterizes forms of diarrhea where the secretory compound is not the causative factor. The EEBJ suppressed the gastrointestinal transit of charcoal meal which clearly indicates that extract may be capable of reducing the frequency of the stools in diarrheal conditions^{17,18}. The results also show that the extract suppressed the propulsion of charcoal meal thereby increasing the absorption of water and electrolyte. In conclusion, the data obtained in this study suggest that the ethanolic extract of *Brassica juncea* plant possess anti-diarrheal activity in animal models justifying its traditional use in diarrhea.

CONCLUSION

The result obtained from the present study suggesting that, the ethanol extract of *Brassica juncea* posses significant anti-diarrheal activity against the castor oil induced diarrhea, magnesium sulphate induced diarrhea and charcoal passage test in experimental animal. However further study should be conducted to isolate and evaluate phytoconstituents from the plants responsible for the anti-diarrheal properties of plant.

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