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IN-VIVO EVALUATION OF ANTI-DIARRHOEAL AND ANTI-EMETIC ACTIVITY OF PIPER LONGUM FRUITS IN RATS AND CHICKS

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Abstract

Diarrhea is a disease that causes approximately 5-8 million deaths per year around the world. Children under the age of five are particularly vulnerable to diarrhea, especially in developing countries. Emesis or vomiting condition defined as mainly go through of contents itself the upper gastro intestinal tract there by greatly causing irritation overstimulation and swell occur. The present study investigates the antidiarrheal and antiemetic properties of the methanolic fruit extract of Piper longum (Indian long pepper), a plant traditionally used for gastrointestinal disorders. Phytochemical screening revealed the presence of bioactive compounds such as alkaloids, flavonoids, saponins, phenols, and volatile oils. Acute toxicity studies established the safety of the extract up to 1600 mg/kg in mice. The antidiarrheal activity was assessed in Wistar rats using castor oil-induced diarrhea and loperamide-induced constipation models. Results demonstrated that Piper longum extract at 300 mg/kg significantly reduced fecal output and water content, indicating potent antidiarrheal action comparable to the standard drug loperamide. Antiemetic activity was evaluated using a chick emetic model induced by copper sulfate. The extract showed significant inhibition of retching episodes at both 75 and 150 mg/kg doses, with efficacy comparable to chlorpromazine, a standard antiemetic. The methanolic extract demonstrated significant laxative, antidiarrheal, and antiemetic activities, likely due to the presence of diverse phytochemicals. These findings suggest that Piper longum could be a potential therapeutic agent for managing diarrhea, constipation, and emesis, warranting further investigation into its active constituents and mechanisms of action.

Introduction

Diarrhea is a disease that causes approximately 5-8 million deaths per year around the world. Children under the age of five are particularly vulnerable to diarrhea, especially in developing countries [1]. To combat this disease, the WHO's diarrhea disease prevention programme was left unfinished, specifically, to investigate facets of conventional medicine practice and to assess health education and prevention strategies [2]. Emesis or vomiting condition defined as mainly go through of contents itself the upper gastro intestinal tract there by greatly causing irritation overstimulation and swell occur. Vomiting initiated by stimuli of G.I tract itself, electrical stimulation of chemoreceptor trigger zone also initiates vomiting. Mainly due to some of the conditions vomiting occurs. The conditions over G.T.I obstruct hepatitis, motion sickness, pregnancy, and also by uses of chemotherapeutic agents [3].

Long pepper (Piper longum) sometimes called Indian long pepper or pipli is a flowering vine in the family Piperaceae, cultivated for its fruits, which is usually dried and used as spices and seasoning. The fruit of the pepper consists of many minuscule fruits –each about the size of a poppy seed. The fruit contain the alkaloids, piprine , each contributes to their pungency. Volatile oil, resin, and the alkaloids piperine and piperlongumine can also be found in the fruits [4]. Hence the study was carried out to

evaluate the anti-diarrheal and anti-emetic activity of Piper longum methanolic fruit extract in rats and chicks.

MATERIALS AND METHODS

Plant material collection and extraction

Piper longum fruits were collected in the month of September 2020 in Jagganpet, East Godavari, Andhra Pradesh, India.

Piper longum fruit was collected and cleaned with purified water. The plant material was shade dried and coarsely powdered. The dried fruits were coarsely powdered by a mixer grinder after being shaded for two weeks. Separately, 300 g of powder was macerated in 2 litres of methanol for 4 days. The fruit extract was filtered and collected, further distilled and evaporated. Finally, the dried extract is obtained, and is stored in air tight container.

Phytochemical studies

The methanolic fruit extract of Piper longum was screened for chemicals. The fruits passed positive checks for volatile oils, gluten, protein, alkaloids, saponins, flavonoids, carbohydrates, and amygdalin [5].

Acute toxicity studies

Methanolic fruit extract of Piper longum was given orally to groups of mice (N=3) at doses of 50, 100, 200, 400, 800, and 1600mg/kg, and behavioural, neurological, and percentage mortality were observed 24 hours later. The dosage was given to the animals. According to the OECD guidelines 420, no pharmacological activities have been conducted on this plant species to date. As a result, doses for acute toxicity trials were calculated using data from other organisms in the same genus. 6. At 1600 mg/kg, the extract was determined to be free of mortality (OECD Guidelines 420, Acute Oral Toxicity – Fixed Dose Procedure, 2001) [6].

Experimental animals

The study used albino and wistar rats weighing between 150 and 250 g of either sex, as well as male chicks weighing 35 to 52g. The animals were held in colony cages at a constant temperature of20 ± 25°C, a 12-hour light/dark period, and a relative humidity of 50 ± 5%, with free access to food and water. Before the procedure, the animals were acclimatized to the laboratory atmosphere for at least a week. Food was derived overnight and during the experiment time, but not water. Many of the tests were conducted during daylight hours (9 :00 -16:00 h). The animal experiment was carried out with the consent and recommendations of the Institutional Ethics Committee (IEC) REG. NO. 1269/a/10/CPCSEA.

Fecal output in naïve rats

Rats weighing 150-250 g were included in the study. The quality of the stools expelled within24 hours of either drug or vehicle administration was examined. The percentage diarrhea was calculated as the ratio of the amount of animals containing unformed stools to the number of animals examined when the manure became unformed, i.e., muddy or watery. To prevent the feces from drying out, all of the feces were gathered shortly after each evacuation and placed in a covered vessel prepared for each animal. The feces obtained during each8-hour cycle were dried for more than 8 hours at 70ºC in a ventilated oven after the wet weight was weighed to determine the duration of operation of each compound [7]. The disparity between the fecal wet weight and the dry weight was used to measure the fecal water value.

The animals were divided into 4 groups of 3 each.

Group I: Vehicle

Group II: Loperamide (10mg/kg)

Group III: Low dose of Piper longum (150mg/kg)

Group IV: High dose of Piper longum (300mg/kg)

Antidiarrheal activity:

Castor oil induced diarrhea in rats

Wistar rats weighing 150-250 g were used in the experiment. Diarrhea (unformed feces) was found after castor oil (0.75 ml/animal) was administered. Since diarrhea was normally observed 1 hour after dosing in preliminary trials, the influence of each medication was measured by the decrease in diarrhea occurrence over the 4-hour cycle following castor oil dosing. This time is four times the time it takes to cause diarrhea, allowing one to assess the medications' long-term effects. During the 4-hour cycle, all feces were collected and the fecal water content was measured as mentioned above. 1 hour before castor oil administration, drugs or vehicles were given orally [8].

Loperamide induced Constipation in Rats:

The animals were placed into metabolic cages and given a clean filter paper to gather stools atthe bottom of the cage. They were also fasted for 18 hours. The prescription, the normal, and the vehicle were given to various classes, and 1 hour later, loperamide was given to all of them (loperamide 5 mg/kg was given orally to cause constipation) [9]. Finally, all groups' species input was consumed for 8 hours. The animals were divided into 4 groups of 3 each.

Group I: Vehicle

Group II: Sodium Picosulphate (5mg/kg)

Group III: Low dose of Piper longum (150mg/kg)

Group IV: High dose of Piper longum (300mg/kg)

Antiemetic activity (chick emetic model)

Copper sulphate induced emesis

Chick emesis was used to test the anti-emetic function. Each chick was stabilized for 10 minutes in a big beaker. Chlorpromazine and the extract were dissolved in 0.9 %saline containing 5% DMSO and 1%tween80, respectively, and given to the test animal at doses of 75 and 150mg/kg b.w. After 10 minutes, each chick received 50mg/kg b.w. copper sulphate orally, and the amount of reches was counted for the next 10 minutes [10]. The animals are divided into 4 groups of 3 each.

Group I: Vehicle

Group II: Chlorpromazine (150mg/kg)

Group III: Low dose of Piper longum (75mg/kg)

Group IV: High dose of Piper longum (150mg/Kg)

The percentage inhibition was determined using the formula below.

Inhibition (%) = [(A-B)/A] x100

Where A=frequency of retching in control group

B = frequency of retching in test group

Statistical analysis

Data was analyzed by Graphpad INSTAT® version 3.0 software and presented as Mean± S.E.M values. Statistical tests used were one- way analysis of variance (ANOVA) followed by Dunnet's multiply comparison test. The levels of statistical significance ranged from $p<0.05$ to $p<0.001$.

Results and Discussion

Phytochemical studies

Flavonoids, phenols, alkaloids, hormones, cardiac glycosides, saponins, terpenes, volatile oils, starch, and resins have been found in the methanolic fruit extract of Piper longum in phytochemical experiments.

Faecal output in naïve rats:

The methanolic fruit extract of Piper longum increases fecal weight and water content. At 300mg/kg, the greater percentage of fecal water content was observed, indicating unrestricted bowel movement and significant findings as compared to expectations. However no diarrhea was observed (Figure 1).

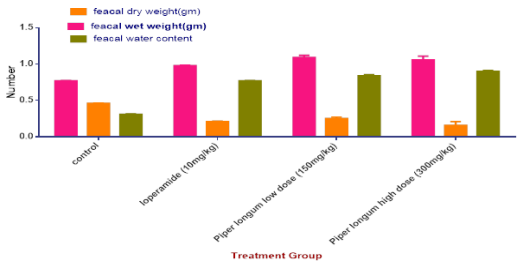

Figure 1: Effect of Methanolic Fruit Extract of Piper longum on Faecal Output in Naïve rats

Castor oil induced diarrhoea in rats

In castor oil caused diarrhea in rats, the methanolic fruit extract of Piper longum exhibits anti diarrheal activity shown by decrease in the total amount of feces of relative to the control group after the administration of castor oil. The important effect was observed by Piper longum (300mg/kg) and the results are compared with that of normal (Figure 2).

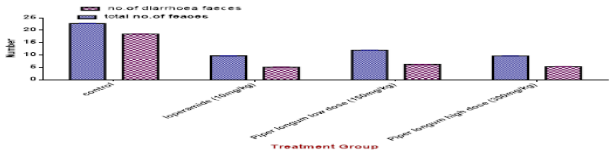

Figure 2: Effect of methanol Fruit extract of Piper longum in Castor oil induced constipation in Rats.

The water keeping ability of insoluble solids, which are mostly dietary fibre, and the overall water in lumen are linked to fecal consistency. There are two categories of feces: ample water holding solids that are slightly unbound to free water (stools appear thick) and water hold solids that attach to all water present. Water absorption and secretion was mostly affected by certain antidiarrheal medications and laxatives.

Piper longum reduced diarrheal droppings in a castor oil-induced diarrheal model similarly to the positive control group (loperamide, an antimotility agent). Castor oil is converted to the active ingredient recinolic acid in the small intestine, which increases intestinal fluid volume. Changes in electrolyte and water transport induce diarrhoea, as well as massive contractions in the transverse and distal colon.

In this research, it was shown that piper longum treatment prevents constipation in loperamide- treated rats. Lop-induced constipation is, in reality, a well-known and commonly used form of spastic constipation. In the intestine, loperamide inhibits water release and peristalsis. which causes a delay in stool evacuation and luminal transit in the intestine. As a result, these conditions have a direct impact on the reduction of feed and water consumption in lop-exposed rats. As a result, dietary intake and water absorption are important considerations to remember when assessing constipation (Table 1). The chick emetic model was used to screen Piper longum's potential antiemetic behavior based on its folkloric application in the treatment of vomiting. The medulla oblongata contains a vomiting center/chemoreceptor control zone (CTZ). Active activation of the motor pathway or indirect stimulation of the chemoreceptor stimulus zone causes emesis. Piper longum had antiemetic activity similar to chlorpromazine for copper sulfate-induced emesis, which could be mediated by inhibition of the chemoreceptor trigger region (Table 2).

Table 1: Effect of methanolic Fruit extract of Piper longum in Loperamide Induced Constipation

Treatment	Dose (mg/kg)	Weight of feces (gm)
Control	Normal saline	0.943±0.002
Sodium picosulphate	5	3.856±0.851
Piper longum	150	1.84±0.005*
Piper longum	300	2.56±0.023*

Values are expressed as Mean±SEM; n=3 (number of animals in each group);*$p<0.001$ was significant when comparisons are made with that of standard

Table 2: Effect of Methanolic Fruit Extract of Piper longum in Chick Emetic model

Groups	Dose (mg/kg)	Mean no. of retches	%Inhibition of emesis
Control	Normal saline	68.12±0.023	___
Chlopromazine	150	13.43±0.09	80.28
Piper longum	75	18.06±0.15	77.48*
Piper longum	150	11.3±0.15	83.4*

Values are expressed as Mean±SEM; n=3(number of animals in each group);*p<0.001was significant when comparisons are made with that of standard.

Conclusion

P. longum has a strong protective effect against copper sulphate induced emesis in young chicks, likely due to receptor antagonism, according to preliminary screening. This research backs up the traditional use of P. longum for emesis and GIT disorders. Finally, the methanolic extract of Piper longum has antidiarrheal function, as shown by decrease in overall faecal production and diarrheal declines. As a result, this research backs up the use of the plant in conventional settings to treat diarrhoea. Due to the inclusion of phytochemical constituents such as alkaloids, phenols, flavonoids, and saponins, methanolic fruit extract of Piper longum has laxative, anti-diarrheal, and anti-emetic activity. In addition, our research suggests that piper longum therapy may be a therapeutic drug candidate for the prevention or treatment of constipation and emesis. Furthermore, active constituent separation and purification are being carried out in order to compile accurate data for evaluating the exact mode of action at various doses.

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Conflicts of interest

The authors declare no conflicts of interest.

Finding

Nil

Ethical Approval

Not Applicable

Inform Consent

Not Applicable

Author Contribution

Both Authors contributed equally

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