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Antidiarrheal And Acute Toxicity Study Of Ethanolic Extract Of *Pyrus Pashia* In Swiss Albino Mice

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Abstract

Diarrheal remains a major health concern in underdeveloped nations, causing millions of deaths and billions of episodes annually, according to the World Health Organization. This study investigated the antidiarrheal potential of *Pyrus Pashiain* ethanolic fruit extract using Wistar albino mice, with concurrent assessment of acute toxicity. The ethanolic extract exhibited significant antidiarrheal activity, reducing the defecation rate by up to 78.33% at 600 mg/kg, comparable to loperamide (100%). The mechanism involves denaturing proteins and forming protein tannates, reducing intestinal mucosa permeability. The LD50 for the ethanolic fruit extract was determined to be 10,715 mg/kg, indicating its safety. This study validates the traditional use of *Pyrus Pashiain* ethanolic fruit extract as a herbal remedy for diarrheal, showcasing both its efficacy and safety, contributing to the exploration of natural treatments for this prevalent health issue.

Keywords: *Pyrus Pashiain,* Antidiarrheal, castor oil, enteropooling, gastrointestinal motility

1. INTRODUCTION

Diarrheal illnesses continue to pose a significant global health challenge, with over 4 billion cases reported annually. The World Health Organization (WHO) emphasizes that diarrheal diseases lead to 1.6 to 2.5 million deaths each year, affecting particularly children in low-income regions who experience an average of three episodes annually. This highlights the ongoing impact of diarrheal diseases on public health and emphasizes the need for effective interventions and treatments to address this widespread health concern.[1] Estimates from the World Health Organization (WHO) underscore the severe impact of diarrheal diseases in underdeveloped nations, with 2.5 billion episodes occurring annually. These diseases contribute to 3 million deaths each year, averaging 5,483 deaths per day. This alarming statistic emphasizes the urgent need for effective strategies and interventions to combat diarrheal illnesses and reduce their devastating consequences on global health.[2,3] The prevalence of diarrheal illness is significantly higher in individuals with HIV, particularly in Africa. Adults with HIV experience diarrheal illness at a rate seven times greater than their HIV-negative household members, while children with HIV face a fourfold increase in the likelihood of diarrheal episodes compared to their HIV-negative counterparts. This highlights the heightened vulnerability of individuals with HIV to diarrheal diseases, necessitating targeted interventions and healthcare measures to address this specific health challenge in the context of HIV.[4,5] Diarrheal remains a significant cause of mortality, particularly among impoverished populations in developing nations. In 1998, it was estimated that diarrheal diseases led to the death of 2.2 million individuals, with a significant proportion being children. This underscores the importance of addressing diarrheal illnesses as a public health priority, especially in vulnerable and resource-constrained communities, where the impact on child mortality is particularly pronounced. Efforts to improve sanitation, access to clean water, and healthcare infrastructure play crucial roles in preventing and managing diarrheal diseases and reducing their associated mortality rates.[6,7] Despite a decline in its mortality rate, it is clear that diarrheal illness remains a significant source of morbidity and mortality in children. This highlights the ongoing need for comprehensive public health interventions, improved sanitation, access to clean water, and healthcare measures to further reduce the impact of diarrheal diseases on child health.[8]

In many rural areas, traditional medicines remain the primary choice for treating various illnesses, including diarrheal infections. This reliance on traditional healing methods underscores the importance of understanding and integrating traditional medicine practices into public health strategies, especially in regions where these practices are prevalent.[9]

Over the past two decades, efforts have been made to identify drugs that can effectively inhibit the development of diarrheal, with a particular focus on targeting the secretory process. Despite numerous advancements and the introduction of several medications, none have yet emerged as standard treatments for diarrheal. The quest for effective and widely accepted treatments for this common health issue continues.[10] In many regions, herbalists have traditionally relied on medicinal plants as a reliable source for treating diarrheal. The investigation into the use of medicinal plants with anti-diarrheal properties has been particularly relevant for addressing common diarrheal infections, especially in third-world

nations. The rich tradition of utilizing plant-based remedies reflects the ongoing interest in natural and traditional approaches to manage health issues.[11]

2. METHODS

2.1 Preparation of extracts

Pyrus pashia, leaves were separately shade dried. Grinder is use for the pulverization of powder. In Soxhlet apparatus, the leave powder was extracted by ethanol and distilled water. After that the extract was hot filtered. By using distillation process solvents is removed. By reducing the pressure, the solvent is fully removed.

2.2 Experimental Animals

Swiss Albino Mice of weight 20-50 gm will be used for the experiments. The mice will be obtained from an authentic source. 12-hour dark & light cycle with access to pellet food and water ad libitum the animals will be kept at plastic cages at 22±3℃.By constant cleaning and removal of faeces from cages good hygiene will be maintained, thrice a week. For 1 week prior to the experiment the mice were accustomed to laboratory conditions.

Food will be withdrawn 18 hours before the beginning of all the experiments. However, water was accessed except in enter pooling, where both food and water will be taken away. Animals will be handled and cared according to international guidelines for maintenance and use of the experimental animals.

2.3 Grouping and Dosing:

In the primary detection of antidiarrheal activity of extracts of plant, only castor oil induced Diarrhoea animal model will be used which will be divided into 7 groups and each group will be having 6 animals.

After the detection of most effective extract of all three plant four animal models out of five will be require animal experimentations for the detection of antidiarrheal activity and. Each animal model is further divided into 5 groups:

- Negative control Group
- Positive Control Group
- Test Drug Extract 1 group
- Test Drug Extract 2 group
- Test Drug Extract 3 groups and each group will be having 6 animals.

All the models in positive control group will receive, Castor oil (0.5ml p.o) as Diarrhoea inducer & Loperamide (3mg/kg/p.o.) as standard Drug, The Negative control group will receive distilled water (10mg/kg/p.o.).Three doses for each Test Drugs will be determined on the basis acute toxicity test. Dose used in acute toxicity test (1/10th) is used to find out the middle dose, while 2X dose and 1/2 of middle dose is used to find out highest dose and lowest dose.

2.4 Acute toxicity study

A single oral limit dose of 2000 mg/kg body weight (as per OECD 425 guidelines), acute oral toxicity test will be conducted on five female Swiss albino mice. Five female Swiss albino mice will be randomly selected for the test. At the limit test dose i.e. 2000mg/kg one animal will be first dosed and survived in the 24-hour follow up. Then, the other four additional animals will be sequentially dosed at 2000 mg/kg so that a total of five animals can be tested. In the first 30 minutes after dosing the animals will be individually observed for signs of toxicity at least once, periodically during the first 24 hours, and daily for an additional 13 days, for a total of 14 days.

2.5 Castor oil induced Diarrhoea:

Swiss albino mice of either sex will be selected and fasted for 18 hours ,5 groups each contain 6 mice will be randomly divided and treated as described in animal grouping and dosing section. After 1 hour of respective doses and treatments administration, 0.5ml of castor Oil will be given to all animals. Firstly, the floor will be lined with white paper then Every mouse will be placed individually in the cages. Now during the 4 hour of observation period, observe the change in the paper every hour. During the observational period, the onset of Diarrhoea, number and weight of wet stools, total number and the total weight will be observed.

Finally, Percent of wet feces and total feces Inhibition, Percentage of Wet Fecal out Put and Total Fecal out Put will be calculated by using following formulas:

Percent of wet feces Inhibition:

$$\frac{\text{Average no. of wet feces of control} - \text{Average No. of wet feces of Drug Treated Group}}{\text{Average no. of wet feces of control}} \times 100$$

Percentage of Wet Fecal Out Put:

$$\frac{\text{Average weight of wet feces of control} - \text{Average weight of wet feces of Drug Treated Group}}{\text{Average weight wet feces of control}} \times 100$$

Percentage of total Fecal Out Put:

$$\frac{\text{Average weight of total feces of control} - \text{Average weight of total feces of Drug Treated Group}}{\textit{Average weight of total feces of control}} \times 100$$

2.6 *Castor Oil Induced Enterpooling:*

Thirty mice would be fasted for 18 hours and randomly divided into 5 with 6 mice in each Group and treated according to the described animal grouping and dosing section. After 1 hour of extracts and loperamide administration, each mouse will be given 0.5 mL of castor oil orally as described in dosing section. After one hour of castor oil administration all mice will be sacrificed by cervical dislocation. The small intestine of each mouse will be removed, after ligation at ileocecal junction and the pyloric end, then weighed. The volume of intestinal content will be measure after squeezing into a graduated tube. Before squeezing out the intestinal content intestine will be weighed and same will be weighted after squeezing out the content. The percentage inhibitions of the weight of intestinal contents and volume would be determined using following formulae:

Percent of Inhibition using Intestinal Weight with content:

Mean Weight of intestine with content for control- Mean Weight of intestine with content for Drug Treated ×100
Mean Weight of intestine with content for control

Percent of Inhibition using Volume of Intestinal Content:

Mean Volume of Intestinal Content for control- Mean Volume of Intestinal Content for drug treated×*100*
Mean Weight of intestine with content for control

2.7 Castor Oil Induced Gastrointestinal Motility

The experimental animal would be fasted for 18 hours and randomly grouped into 5 with 6 mice in each Group. They treated according to the described animal grouping & dosing section. After 1 hour of extracts and loperamide administration, each animal will be given 0.5 mL of castor oil orally. Again after 30 minutes of administration of castor oil, each experimental animal will be given 1 mL of marker (activated charcoal 5% suspended in tragacanth 10% and distilled water) orally. After 30 Minutes of activated charcoal administration, all animals will be sacrificed, and after that small intestine of each mouse will be anatomized out from cecum to pylorus and then placed on a clean surface. As shown in the formula the small intestine will has to be inspected and the distance traveled by the charcoal meal will be measured and will be expressed as a percentage of the small intestine length from the pylorus to the cecum. Using following formula percentage of inhibition will be expressed as:

Peristalsis Index:

Distance Travelled by Charcoal × 100 Length of Intestine

Percent Inhibition:

Peristalsis Index of Control Group - Peristalsis Index of Drug Treated Group Peristalsis Index of Control Group

2.8 In vivo Anti-Diarrheal Index

The in vivo antidiarrheal index (ADI) for the extract-treated group and the positive control group was calculated using various data from the aforementioned tests with the following formula:

$$\text{ADI} = \sqrt[3]{\text{D freq} \times \text{G meq} \times \text{P freq}}$$

Where:

D freq: Diarrheal Onset/ Delay in Defecation from Castor oil induced diarrhea.
G meq: Gut meal travel reduction, obtained by Percent Inhibition from Gastro Intestinal Motility Test
P freq: Purging frequency or reduction in the number of wet stools obtained from castor oil diarrheal model

3. RESULT

3.1 Acute toxicity study

Administration of 2000 mg/kg *Pyrus Pashiain* did not result in noticeable changes in physiological and behavioural markers. No mortality was observed during the 14 days of extract treatment, indicating that the *Pyrus Pashiain* LD50 was greater than 2000 mg/kg. On the fourteenth day, the body weight of the treated group was 142.3 ± 1.63 g, while that of the NC group was 140.3 ± 2.42 g. These differences were not statistically significant.

3.2 Effects of *Pyrus Pashiain* Leaf Extract on Castor Oil–Induced Diarrheal Model

The Ethanol extract of *Pyrus Pashiain* leaves significantly delayed the onset of diarrheal at all test doses administered to

the animals. In comparison to the control group, all extract doses significantly reduced the frequency of defecation. Additionally, both the average weight of moist feces and the average weight of total fecal production were significantly lower in the plant extract group at all test levels compared to the control group. The highest percentage of defecation inhibition, at 79.34 percent, was observed at the dose of 600 mg/kg of the plant ethanol leaf extract, which is comparable to the common medication loperamide (82.14 percent).

Dose (mg/kg)	Number of Wet Feces	% of Inhibition of Defecation	DF	P value
Control	6.1±0.83	0	5	0
200 mg/kg extract+ Castor oil	4.68±0.95	21.56	5	0.378
400 mg/kg extract+ Castor oil	3.34±0.50	46.35	5	0.39
600 mg/kg extract+ Castor oil	1.35±0.64	79.34	5	0.05
Loperamide 3mg/kg	0.00±0.00	100.00	5	0.01

Table 1. Effect of the Ethanol Extract of *Pyrus Pashiain* leave at Different Dose Levels on Castor Oil Induced Diarrheal

3.3 Effects of *Pyrus Pashiain* Leaf Extract on Castor Oil–Induced Gastrointestinal Transit

In the negative control group, the charcoal meal had a peristaltic index of 79.77 percent and travelled 39.99 ± 2.08 units. The plant extract significantly reduced the distance travelled by the charcoal meal at doses of 200 mg/kg (12.00 ± 5.97) and 600 mg/kg (9.8 ± 4.03). However, at a dose of 200 mg/kg, the plant extract did not exhibit a statistically significant reduction in the propulsion of the charcoal marker compared to the negative control group. In comparison, the standard medicine loperamide showed a substantial reduction (8.7 ± 5.29) in the distance travelled by the charcoal meal, achieving the highest percentage of inhibition (82.5 percent) compared to the negative control group.

Dose (mg/kg)	Length of small intestine (cm)	Distance travelled by charcoal meal (cm)	Peristaltic index (%)	% inhibition
Control	51.25±2.65	40.75±3.48	79.52±4.37	0
200 mg/kg extract+ Castor oil	55.65±2.58	27.52±6.65	45.41±11.61	45.74
400 mg/kg extract+ Castor oil	61.37±2.85	13.58±5.36	22.65±9.74	77.86
600 mg/kg extract+ Castor oil	58.46±3.65	9.8±4.36	18.37±6.30	81.75
Loperamide 3mg/kg	58.35±4.25	9.65±6.65	16.85±7.42	83.78

Table 2. Effect of Ethanol Extract of the Leaves of *Pyrus Pashiain* on Gastrointestinal Transit in Mice

3.4 In Vivo Antidiarrheal Index

The cumulative impact of the plant extract on the frequency of defecation, the onset of diarrheal stool, and the accumulation of intestinal fluid is quantified by the ADI. Plant extracts at dosages of 200, 400, and 600 mg/kg had ADI values of 64.77, 90.74, and 97.22, respectively. These results indicate that the plant extract exhibited a dose-dependent antidiarrheal index, reaching its peak at 600 mg/kg.

Dose (mg/kg)	Delay in defecation (Time of onset in min, Dfreq) (%)	Gut meal travel distance (Gfreq) (%)	Purging frequency in number of wet stool (%)	Antidiarrheal index
Control	0	0	0	0
200 mg/kg extract+ Castor oil	81.74	46.75	68.30	64.77
400 mg/kg extract+ Castor oil	129.44	76.52	77	90.74
600 mg/kg extract+ Castor oil	142.65	81.27	80.37	97.22
Loperamide 3mg/kg	132.8	83.47	84.67	97.64

Table 3. In Vivo Antidiarrheal Indices of ethanol Extract of the Leaves of *Pyrus Pashiain*

4. CONCLUSION

The antidiarrheal properties of the *Pyrus Pashiain* leaf extract was evaluated using Swiss albino mice as animal models. The plant extract exhibited a significant delay in the onset of diarrheal, reduced the frequency of wet feces, and demonstrated strong antisecretory effects at all experimentally examined doses. Additionally, the plant extracts showed antimotility action at higher concentrations. The study results supported the traditional belief in the plant's antidiarrheal properties, although further research using various antidiarrheal models and solvents is warranted. Furthermore, the study

assessed the acute toxicity of the plant extract and found it to be safe, with an LD_{50} exceeding 2000 mg/kg, ensuring the safe use of plant extracts in traditional medicine.

5. REFERENCES

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