Section A-Research paper
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Evaluation of Antidiarrheal Activity of Holarrhena Antidysentrica Bark Extract in Rats

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Abstract

Objectives:

The goal of the current investigation was to determine antidiarrheal effects of *Holarrhena antidysenterica* (Family-Apocynaceae) bark ethanolic extract against induced diarrhea in Wistar albino rats. **Materials and method:** The selected bark part of plant materials were evaluated using pharmacognostic parameters such as ash values, total ash value, soluble extract value etc. Then after standard plant materials was used for extraction process. The developed extract of plant was used for the investigation of diarrheal prevention action. The plant extract was evaluated for castor oil induced diarrhea. The estimation of diarrhea activity assessment was determined using 100, 200mg/kg body wt. orally in albino rat. The inhibition of diarrheal effect of the plant extract on castor oil induced diarrhea was measured by inhibition of dicharge volume of fluid. The diarrhea activity was determined using orally 100, 200mg/kg of plant extract and was noted the percentage inhibition of defecation was calculated for each group, change in fecal consistency, and body weight was recorded for each individual rat for 3 days. The diarrhea activity of plant extract was compare with standard drug Loperamide was given at 5mg/kg body.

Results and discussion: Due to castor oil administration there is accumulation of water and electrolytes in the intestinal loop. Loperamide has significantly reduced the mean volume of intestinal fluid in comparison to the control. The plant extract dose of 200mg/kg has significantly reduced the mean volume of intestinal fluid in comparison to control. The plant extract at a dose

of 200mg/Kg significantly decreased the propulsion of charcoal meal through the gastrointestinal tract in comparison to the control. **Conclusion:** Results of evaluation of *Holarrhena antidysenterica* extract having significant bioactive molecules. This bioactive molecule can be used for the treatment and management of diarrheal diseases.

Keywords: *Holarrhena antidysenterica*, inhibition of diarrheal, pharmacognostic parameters

1. INTRODUCTION

Holarrhena antidysenterica, commonly known as Kurchi or Kutaja, is a medicinal plant with a rich history of traditional use in various systems of medicine. The bark of this plant has garnered attention due to its potential therapeutic properties, particularly in gastrointestinal disorders. In recent years, extensive research has been conducted to evaluate the pharmacological effects and bioactive compounds present in *Holarrhena antidysenterica* bark extract. This discussion aims to explore the diverse therapeutic potential of Holarrhena antidysenterica bark extract and highlight its promising applications in modern medicine (1-5).

Diarrhea is one of the most common cause of death in infants and in children less than 5 years accounting to about 5-8 million. Diarrhoea is an alteration in normal bowel movement characterized by an increase in the water content, volume, or frequency of stools. One of the main processes of diarrhea is the secretory process. Hence there has been a need for the development of the drugs that inhibit this process.Diarrhea is a common gastrointestinal condition characterized by frequent, loose, and watery stools. It affects individuals of all ages and can be caused by various factors, including infections, dietary intolerances, medications, and underlying medical conditions. Diarrhea occurs when there is an imbalance in the normal processes of fluid absorption and secretion in the intestines. There are several mechanisms involved, including osmotic, secretory, inflammatory, and altered motility processes. Osmotic diarrhea occurs when unabsorbed solutes draw water into the intestines, leading to increased stool volume. Secretory diarrhea is the result of excessive fluid secretion into the intestines, often caused by infections or certain hormones. Inflammatory diarrhea is characterized by mucosal damage and inflammation, leading to impaired absorption and increased fluid loss. Altered motility can result in rapid transit of stool through the intestines, limiting water absorption and resulting in diarrhea (6-10). By evaluating herbal medicines which have antispasmodic effects, suppress gut motility and stimulate water absorption, we can find a potential anti-diarrheal agent. The major mechanisms by which the antidiarrheal drugs act is by decreasing the secretions or

reducing the gastrointestinal motility. According to the phytochemical screening report of a previous study, leaves of *Holarrhena antidysenterica* showed the presence of lignans and flavonoids. Both lignans and flavonoids possess various biological activities like antibacterial, spasmolytic and anti-inflammatory. *Holarrhena antidysenterica* was currently being used in the treatment of various disease conditions without standardization. The standardization of a crude drug is an integral part of establishing its correct identity. Before any crude drug can be included in an herbal pharmacopoeia, pharmacognostic parameters and standards must be established (11-13). The goal of the current investigation was to determine antidiarrheal effects of *Holarrhena antidysenterica* (Family-Apocynaceae) bark ethanolic extract against induced diarrhoea in Wistar albino rats

2. MATERIALS AND METHODS:

2.1 Collection, Identification and Authentication of Plant Specimens

The process of collecting, identifying, and authenticating plant specimens requires expertise and adherence to ethical guidelines, including obtaining necessary permits and permissions, respecting local regulations, and promoting sustainable collection practices. *Holarrhena antidysenterica* bark has been washed, cleaned and dried six days. The plant material was mixed into a coarse powder after the drying and kept for further investigations at room temperature.

2.2 Physicochemical Evaluation (12-14)

The powdered (bark) plant material of *Holarrhena antidysenterica* was evaluated by standard procedure for the determination of following physicochemical parameters.

2.3 Loss on drying

Loss on drying is the loss of mass expressed as percent m/m. About 5-6g of drug powder is accurately weighed in a Petri dish and kept in a hot-air oven maintained at ~105 ^{0}C for 4-5 hours. After cooling in dessicator, the loss in weight was recorded in each case. This procedure was repeated till the constant weight was obtained.

Loss on drying (%) = loss in weight X 100/ W

W= weight of the drugs in grams.

2.4 Determination of Ash Value

The determination of ash value in plant extracts is a common analytical procedure used to estimate the inorganic content or mineral matter present in the extract. The ash value is expressed as a percentage and provides information about the extract's purity, as well as its potential for contaminants or adulterants. Here's a general method for the determination of ash value in plant extracts. Calculation:

a. Calculate the ash value using the following formula:

Ash Value (%) = [(W2 - W1) / W1] × 100

Where,

W1 = weight of the empty crucible, W2 = weight of the crucible with ashed sample

2.5 Total Ash value

Take about 2 to 3g, accurately weighed powdered extract in a tarred platinum or silica dish previously ignited and weighed. Scatter the powdered drug on the bottom of the dish. Incinerate by gradually increasing the heat, not exceeding dull red heat until free from carbon, cool and weigh. If a carbon free ash cannot be obtained in this way, exhaust the charred mass with hot water, collect the residue on an ashless filter paper, add the filtrate, evaporate the residue and ignite at low temperature. Firstly, empty silica crucible was taken and put into the muffle furnace with the help of tong for ignition at 600^0C for 30 mins. Took out the silica crucible and weighed it then 2g of powdered drug was added to it. Then placed it in muffle furnace at 500-600^0C for 2-3 hours until it become white. Then weighed it and the percentage of total ash with air dried sample was calculated.

Total ash value = (z-x/y) × 100

Where,

X = weight of the silica crucible

Y = weight of the drug powder (g)

Z = weight of the silica cruicible with powder ash

2.6 Acid-insoluble ash

The ash was boiled for 10-15 minutes with ~ 30 ml of dilute hydrochloric acid and the insoluble matter was collected in a crucible. It was washed with hot water, ignited and weighed. The

percentage of acid-insoluble ash was calculated with reference to the air-dried drug.The ash obtained from above method was added to 25 ml of dil HCl. Boiled it for 5 mins. Then residue was collected on ash less filter paper. Then it was ignited in muffle furnace at 560^0C for 1 hour. The percentage of acid insoluble ash was calculated with reference to air dried sample.

Acid insoluble ash value % = (a /y) × 100

Where,

A = weight of the remaining residue

Y = weight of crude powder taken (g)

2.7 Water-soluble ash

Boiled the ash obtained from total ash value for 5mins in 25 ml water. The insoluble matter was poured into ash less filter paper. Then it was ignited at low temperature to constant weight. The weight of water soluble ash was determined by subtracting the weight of water insoluble ash from total ash value. The percentage of water soluble ash with reference to air died value was calculated.

2.8 Extractive Value

Procedure 5 gm of coarsely powdered air dried drug was macerated with 100 ml of solvent (chloroform, ethanol and water) in a closed flask for 24 hour, shaking frequently for six hours and allowed to stand for eighteen hours. It was then filtered rapidly taking precaution against loss of alcohol. 25 ml of the filtrate was evaporated to dryness in tared flat bottomed shallow dish, dried at 105^0C and weighed. The percentage of alcohol soluble extractive was calculated with reference to the air dried drug. Extractive value means the quantity of soluble components used for extraction in that specific solvent. Extractive value was determined using the formula

$$\text{Extractive value (\%)} = \frac{weight\ of\ residue}{weight\ of\ dry\ powder} \text{ x } 100$$

2.9 Determination of swelling index

Accurately weighed 1g of powdered was placed in measuring cylinder containing 50 ml of water and was kept aside for 24 hours and shaked it occasionally. The volume occupied by the sample was measured in 24 hours.

3. Preparation of crude Extracts

The freshly collected plant materials were washed, shadow dried and then dried in hot air oven at a temperature not more than 50°C. The dried powder of aerial parts of plant was extracted in soxlet apparatus with different solvent methanol using successive extraction method solvent used. The extraction was continued for 72 hours for each solvent at 40-45 ^{0}C. The extraction was started with hexane then petroleum ether and continued with chloroform and then lastly with methanol. After the completion of extraction, the solvent was evaporated, and residue obtained was then stored in dessicator for further study. At the beginning of this process, the barks are removed off of the tree and then dried and afterwards homogenised into fine powder. The measurement of powder was determined in contrast with natural solvents in a conical carafe. It was placed in the rotary shaker at 190-220 rpm for 24 hours. Use of dissolvable refinery gadgets to provide the last volume of a fourth of the first volume had been recovered. It is stored in close-air bottles for future tests at 40°C.

3.1 Phytochemical screening of extract (12-14)

Preliminary phytochemical screening was done using the specified protocols for the qualitative analysis of Alkaloids, carbohydrates, fixed oils, flavonoids, glycosides, phytosterol/terpenoids, saponins, and tannins/phenols. The screening tests as follows:

3.2 Experimental animals

The project was started after obtaining Institutional Animal Ethical Committee approval (IAEC /KMC/52/2012). Male albino rats of Wistar strain weighing 200-250 g were used in this study. They were fasted for 24 h, were kept in polypropylene cages and maintained at 27^{o}C. They were fed with standard rat feed procured from and water ad libitum was provided.

Animals were divided in four groups, each group containing six animals.

Group I-control (distilled water)

Group 2-loperamide 4mg/kg, p.o (standard)

Group 3- *Holarrhena antidysenterica* barks extract 100mg/kg, p.o

Groups 4- *Holarrhena antidysenterica* bark extract 200mg/kg, p.o

Fig 3.8 Wistar Rat

3.2.1 Induction of experimental diarrhea

Each animal was given 0.5 mL of castor oil 30 min following the drug treatment. Later they were placed in separate cages to observe for the mean weight of the feces for 4 h. Feces was collected with an absorbant paper placed in the cages. Results were expressed as percentage of inhibition of diarrhea.

3.2.1.2 Effect of the plant extract on castor oil induced intestinal fluid accumulation

After 30 min of 2 m L of castor oil administration, the rats were anaesthetized. The region of small intestine from pylorus to the cecum was dissected out and the contents were collected into a measuring cylinder to measure the volume of the fluid.

3.2.1.3 Effect of *Holarrhena antidysenterica* on isolated rat ileum

Rats were fasted overnight, only access to water was allowed. After sacrificing the rats, the abdomen was dissected out, around 2 cm of rat ileum was removed and mounted in an organbath containing tyrode solution, which was aerated with oxygen. Controlled conditions like temperature and pH was maintained. The effect of the extract on acetylcholine induced contraction of rat ileum was demonstrated on a smoked drum.

3.2.1.4 Charcoal meal test for intestinal propulsion

Rats fasted for 24 h were treated with respective drugs .After 30 min 1ml of 10% activated charcoal suspension in 100 ml of 5% aqueous gum acacia was given orally to each rat. The rats

were sacrificed and the abdomen was dissected. The distance travelled by charcoal plug from the pylorus to the cecum was measured and peristaltic index calculated.

Peristaltic index= [length traversed by charcoal meal X 100] /Total small intestine length

4. RESULTS AND DISCUSSION:

The physicochemical parameters provide valuable information about the physical and chemical characteristics of *Holarrhena antidysenterica*. The dark brownish color suggests the presence of certain pigments or chemical compounds responsible for the coloration. The semi-solid consistency indicates a certain degree of viscosity, which might be attributed to the presence of soluble components or moisture content. The yield value reflects the amount of powder obtained from a specific quantity of the starting material and can be used to assess the efficiency of the extraction or preparation process. The color of the *Holarrhena antidysenterica* powder was observed to be dark brownish. This visual attribute suggests the presence of pigments or chemical compounds responsible for the distinctive coloration. The dark brownish hue may indicate the existence of compounds with potential medicinal properties or bioactive components. In terms of consistency, the powder displayed a semi-solid nature. This implies that the powder possessed characteristics of both a solid and a liquid, indicating a certain degree of viscosity. The semi-solid consistency could be attributed to the presence of soluble components within the powder or even residual moisture content. The yield of the dried powder was determined to be 9.5 g per 100 g of the original dried material used. This yield value provides information about the efficiency of the extraction or preparation process. It indicates the quantity of powder obtained from a specific amount of the initial dried material, serving as a measure of the overall yield during processing. The physicochemical evaluation of *Holarrhena antidysenterica*, including its dark brownish color, semi-solid consistency, and yield of 9.5 g per 100 g of dried powder, offers essential insights into its physical and chemical properties. These observations serve as a foundation for further analysis and investigation of *Holarrhena antidysenterica* 's potential medicinal or therapeutic applications.

Table: Physicochemical Evaluation

S.N.	**Physicochemical evaluation**	***Holarrhena antidysenterica***
1	Colour	Dark Brownish

2	Consistency	Semi solid
3	Yield	9.5g/100g of dried powder

Figure: Powder of *Holarrhena antidysenterica*

Various Extracts Characteristic

Dissolvable separated plant removes were weighed to decide the dissolvable free dry mass of the concentrates delivered from the dissolvable extraction measure. Each extractive's rate yield from 50 grams of plant material (exposed to Soxhlet extraction) is processed for all restorative plant removes. *Holarrhena antidysenterica* bark were ground into a fine powder, which had a little harsh lingering flavor.

Phytochemical Screening

For the treatment of a few diseases, Phytodrugs are utilized. Because of normalization, each bundle of medication provided contains the legitimate measure of dynamic fixing and will have the ideal helpful impact. On the other hand, the discoveries of physicochemical assessment of rough powder of *Holarrhena antidysenterica* and were investigated, remembering misfortune for drying, all out debris, dissolvability, and substantial metal examination**.**

S.N.	**Parameters**	**Values (W/W)**
1	Loss on Drying	6.5%
2	Total Ash	8.5%

3	Petroleum Ether Soluble Extraction	12.5%
4	Ethanol Soluble Extraction	20.5%
5	Water Soluble Extractive	24.5%

During the physicochemical evaluation of *Holarrhena antidysenterica*, several parameters were assessed to gain insights into its composition. The following values were obtained for various parameters expressed as a percentage of the weight:

(A) Loss on Drying:

The loss on drying was found to be 6.5%. This parameter indicates the amount of moisture present in the sample. A lower percentage suggests lower moisture content, which is desirable for maintaining the stability and shelf life of the product.

(B) Total Ash:

The total ash content of *Holarrhena antidysenterica* was determined to be 8.5%. Total ash represents the inorganic residue left behind after complete combustion of the sample. It provides an estimate of the mineral content and can serve as an indicator of the purity and quality of the botanical material.

(C)Petroleum Ether Soluble Extraction:

The petroleum ether soluble extraction yielded a value of 12.7%. This parameter measures the amount of extractable components soluble in petroleum ether. It provides insights into the presence of lipophilic or non-polar compounds, such as certain essential oils or fatty acids.

(D) Ethanol Soluble Extraction:

The ethanol soluble extraction resulted in a value of 20.5%. This parameter quantifies the amount of extractable constituents soluble in ethanol. It indicates the presence of polar or hydrophilic compounds, including phenolic compounds, alkaloids, or glycosides, which are often associated with potential pharmacological activities.

The phytochemical screening

The phytochemical screening of the ethanol extract of Nirgundi revealed the presence of carbohydrates, proteins, alkaloids, phenolic compounds & tannins, flavonoids, and cardiac glycosides. However, gums and mucilages, saponins, terpenoids/triterpenoids, glycosides, and fixed oils and fats were not found in the extract. These findings provide important insights into the chemical constituents of *Holarrhena antidysenterica* and form a basis for further investigation into its therapeutic potential and medicinal applications.

Table: Phytochemical Screening of Extract of *Holarrhena antidysenterica*

S.N.	Phyto-Chemicals	Observation
1	Carbohydrates	+
2	Proteins	+
3	Alkaloids	+
4	PhenolicCompounds &Tannins	+
5	Flavonoid	+
6	Cardiac Glycosides	+
7	Gums and mucilages	-
8	Saponins	-
9	Terpenoids/Triterpenoids	-
10	Glycosides	-
11	Fixed oils and fats	-

3.2.2 Holarrhena antidysentrica (15-19)

3.2.2.1 Effect of alcoholic extract of *Holarrhena antidysenterica* on Castor Oil Induced Diarrhoea in rats

In a study evaluating the effect of *Holarrhena antidysenterica* bark extract on fecal output, different groups of subjects were administered various treatments. The mean weight of feces after 4 hours and the corresponding percentage inhibition for each group are as follows:

1. Control (distilled water): The control group, treated with distilled water, had a mean weight of feces of 29.50. The percentage inhibition in this group is not provided.

2. Loperamide: The loperamide-treated group exhibited a significantly reduced mean weight of feces, measuring 12.65. This resulted in a percentage inhibition of 55.40%, indicating a substantial decrease in fecal output compared to the control group.

3. *Holarrhena antidysenterica* bark extract 100mg/kg: The group treated with 100mg/kg of *Holarrhena antidysenterica* bark extract showed a mean weight of feces of 15.26. The percentage inhibition in this group was calculated to be 28.53%, indicating a moderate reduction in fecal output compared to the control group.

4. *Holarrhena antidysenterica* bark extract 200mg/kg: The group administered 200mg/kg of *Holarrhena antidysenterica* bark extract displayed a mean weight of feces of 14.25. The percentage inhibition for this group was determined to be 38.70%, indicating a significant decrease in fecal output compared to the control group.

Diarrhoea was seen in all the treated animals following administration of castor oil for next 4 h. The mean weight of the feces as significantly reduced in the loperamide group 12.65 compared to control 29.50. A similar reduction in weight of the feces was observed with *Holarrhena antidysenterica* extract in doses of 100 and 200 mg/kg i.p.

Table: Effect of alcoholic extract of *Holarrhena antidysenterica* on Castor Oil Induced Diarrhoea in Rats

S.N.	**Groups**	**Dose 100 mg/kg i.p.**	**Dose 200 mg/kg i.p.**
1	Control (distilled water)	29.50	
2	Loperamide	12.65	
3	*Holarrhena antidysenterica* bark extract 100mg/kg	15.26	28.53%
4	*Holarrhena antidysenterica* bark extract 200mg/kg	14.25	38.70%

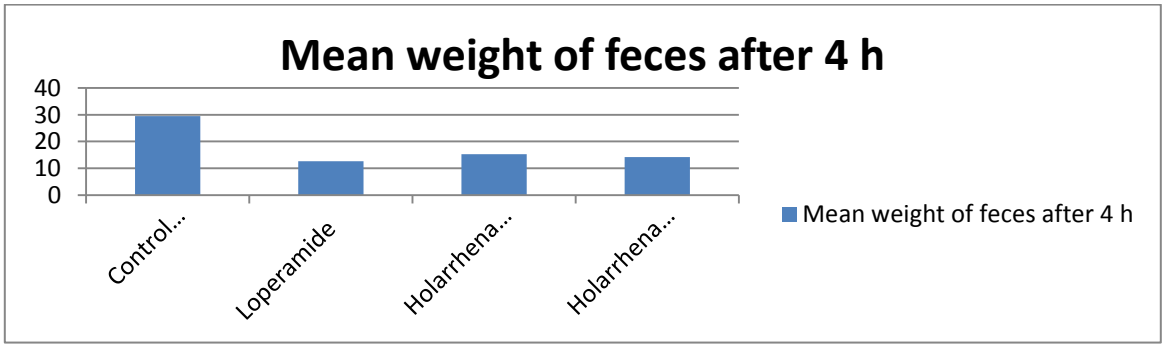

Figure: Effect of alcoholic extract of *Holarrhena antidysenterica* on Castor Oil Induced Diarrhoea in Rats

3.3 Effect of alcoholic extract of *Holarrhena antidysenterica* on castor oil induced enteropooling in rats

In a study examining the effects of *Holarrhena antidysenterica* bark extract on intestinal fluid volume, different treatment groups were administered varying doses of the extract. The mean volume of intestinal fluid (in milliliters) after treatment and the corresponding percentage inhibition for each group are as follows:

1. Control (distilled water): The control group, treated with distilled water, had a mean volume of intestinal fluid of 2.25 ml. The percentage inhibition in this group is not provided.

2. Loperamide: The loperamide-treated group exhibited a significantly reduced mean volume of intestinal fluid, measuring 1.02 ml. This resulted in a percentage inhibition of 76.40%, indicating a substantial decrease in intestinal fluid volume compared to the control group.

3. *Holarrhena antidysenterica* bark extract 100mg/kg: The group treated with 100mg/kg of *Holarrhena antidysenterica* bark extract displayed a mean volume of intestinal fluid of 1.85 ml. The percentage inhibition in this group was calculated to be 20.53%, indicating a mild reduction in intestinal fluid volume compared to the control group.

4. *Holarrhena antidysenterica* bark extract 200mg/kg: The group administered 200mg/kg of *Holarrhena antidysenterica* bark extract showed a mean volume of intestinal fluid of 1.12 ml. The percentage inhibition for this group was determined to be 50.70%, indicating a significant decrease in intestinal fluid volume compared to the control group.

Due to castor oil administration there is accumulation of water and electrolytes in the intestinal loop. Loperamide has significantly reduced the mean volume of intestinal fluid in comparison to

the control. The plant extract dose of 200mg/kg has significantly reduced the mean volume of intestinal fluid in comparison to control. (Table 2)

Table: Effect of alcoholic extract of *Holarrhena antidysenterica* on castor oil induced Enteropooling in Rats

S.N.	Groups	Dose 100 mg/kg i.p.	Dose 200 mg/kg i.p.
1	Control (distilled water)	2.25	
2	Loperamide	1.02	76.40%
3	*Holarrhena antidysenterica* bark extract 100mg/kg	1.85	20.53%
4	*Holarrhena antidysenterica* bark extract 200mg/kg	1.12	50.70%

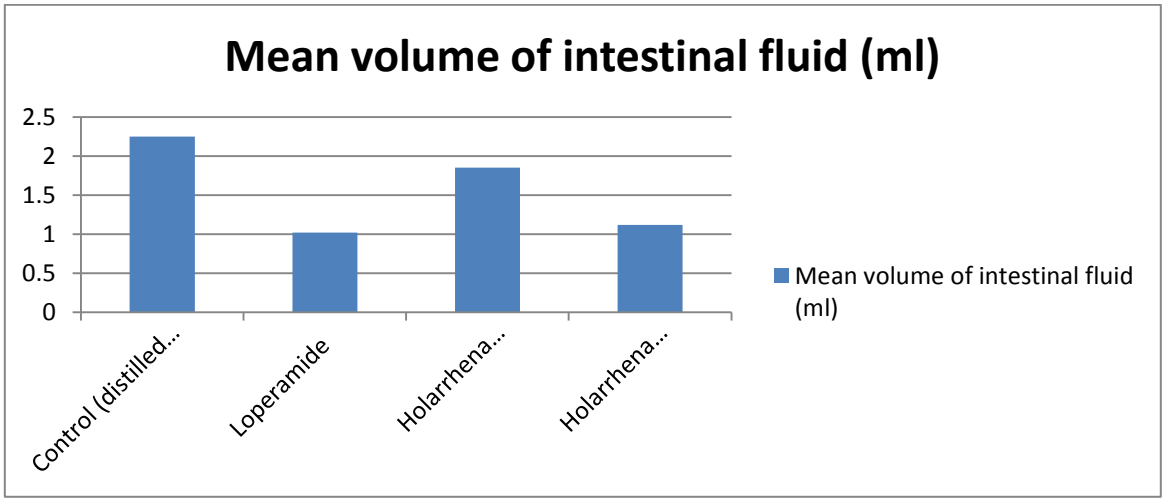

Figure: Effect of alcoholic extract of *Holarrhena antidysenterica* on castor oil induced Enteropooling in Rats

3.4 Effect of *Holarrhena antidysenterica* bark extract on gastrointestinal transit

In a study assessing the peristaltic index, different treatment groups were administered various substances. The peristaltic index, representing the strength and frequency of intestinal contractions, was measured for each group. The results for each group are as follows:

1. Control (distilled water): The control group, treated with distilled water, had a peristaltic index of 50.55.
2. Loperamide: The group treated with loperamide exhibited a reduced peristaltic index of 30.25. This indicates a decrease in the strength and frequency of intestinal contractions compared to the control group.
3. *Holarrhena antidysenterica* bark extract 100mg/kg: The group treated with 100mg/kg of *Holarrhena antidysenterica* bark extract displayed a peristaltic index of 35.55. This suggests a moderate impact on the strength and frequency of intestinal contractions.
4. *Holarrhena antidysenterica* bark extract 200mg/kg: The group administered 200mg/kg of *Holarrhena antidysenterica* bark extract showed a peristaltic index of 27.25. This indicates a significant reduction in the strength and frequency of intestinal contractions compared to the control group.

The plant extract at a dose of 200mg/Kg significantly decreased the propulsion of charcoal meal through the gastrointestinal tract in comparison to the control. (Table 3)

Table: Effect of alcoholic extract of *Holarrhena antidysenterica* on gastrointestinal transit

S.N.	**Groups**	**Dose 100 mg/kg i.p.**	**Dose 200 mg/kg i.p.**
1	Control (distilled water)	2.25	50.55
2	Loperamide	1.02	30.25
3	*Holarrhena antidysenterica* bark extract 100mg/kg	1.85	35.55
4	*Holarrhena antidysenterica* bark extract 200mg/kg	1.12	27.25

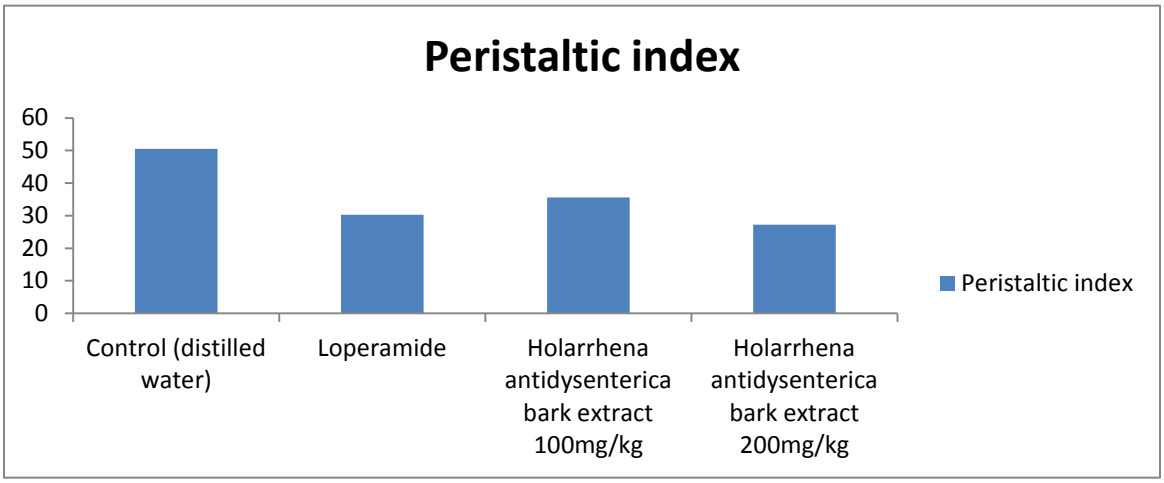

Figure: Effect of alcoholic extract of *Holarrhena antidysenterica* on castor oil induced Enteropooling in Rats

4.1 Effect of alcoholic extract of *Holarrhena antidysenterica* on Castor Oil Induced Diarrhoea in rats –

In a study evaluating the effect of *Holarrhena antidysenterica* bark extract on fecal output, different groups of subjects were administered various treatments. The mean weight of feces after 4 hours and the corresponding percentage inhibition for each group are as follows:

1. Control (distilled water): The control group, treated with distilled water, had a mean weight of feces of 29.50. The percentage inhibition in this group is not provided.

2. Loperamide: The loperamide-treated group exhibited a significantly reduced mean weight of feces, measuring 12.65. This resulted in a percentage inhibition of 55.40%, indicating a substantial decrease in fecal output compared to the control group.

3. *Holarrhena antidysenterica* bark extract 100mg/kg: The group treated with 100mg/kg of *Holarrhena antidysenterica* bark extract showed a mean weight of feces of 15.26. The percentage inhibition in this group was calculated to be 28.53%, indicating a moderate reduction in fecal output compared to the control group.

4. *Holarrhena antidysenterica* bark extract 200mg/kg: The group administered 200mg/kg of *Holarrhena antidysenterica* bark extract displayed a mean weight of feces of 14.25. The percentage inhibition for this group was determined to be 38.70%, indicating a significant decrease in fecal output compared to the control group.

Diarrhoea was seen in all the treated animals following administration of castor oil for next 4 h. The mean weight of the feces as significantly reduced in the loperamide group 12.65

compared to control 29.50. A similar reduction in weight of the feces was observed with *Holarrhena antidysenterica* extract in doses of 100 and 200 mg/kg i.p. Due to castor oil administration there is accumulation of water and electrolytes in the intestinal loop. Loperamide has significantly reduced the mean volume of intestinal fluid in comparison to the control. The plant extract dose of 200mg/kg has significantly reduced the mean volume of intestinal fluid in comparison to control. The plant extract at a dose of 200mg/Kg significantly decreased the propulsion of charcoal meal through the gastrointestinal tract in comparison to the control.

Conclusion:

In conclusion, the physicochemical evaluation of *Holarrhena antidysenterica* bark extract reveals valuable information about its physical and chemical properties, including its dark brownish color, semi-solid consistency, and yield. These characteristics suggest the presence of pigments and bioactive compounds, indicating its potential for medicinal applications. The phytochemical screening identifies various compounds present in the extract, such as carbohydrates, proteins, alkaloids, phenolic compounds, tannins, and flavonoids, further highlighting its potential therapeutic value. In the study on castor oil-induced diarrhea in rats, the *Holarrhena antidysenterica* bark extract exhibited promising effects. The extract at doses of 100mg/kg and 200mg/kg demonstrated a significant reduction in fecal output, comparable to the anti-diarrheal drug loperamide. Additionally, the extract decreased the accumulation of water and electrolytes in the intestinal loop and inhibited the propulsion of the charcoal meal through the gastrointestinal tract. These findings suggest that *Holarrhena antidysenterica* bark extract may possess anti-diarrheal properties, potentially due to its impact on intestinal fluid volume, motility, and absorption. Further research is warranted to explore the specific bioactive components responsible for these effects and to investigate its potential application in the management of diarrhea-related conditions in humans.

REFERENCES:

1. Bozzetti F. Nutritional issues in the care of the elderly patient. Crit Rev Oncol Hematol. 2003; 48:113-21.

2. Benzie I. F. F, Wachtel-Galor S. Biomarkers in long-term vegetarian diets. Adv Clin Chem. 2009; 47:170-208.

3. Harman D. Free radical theory of aging. Mutat Res. 1992; 275:257-66.

4. Beckman K. B, Ames B. N. The free radical theory of ageing matures. Physiol Rev. 1998; 78:47-81.

5. Holmes G. E, Bernstein C, Bernstein H. Oxidative and other DNA damages as the basis of aging: A review. Mutat Res. 1992; 275(3006):305–15.

6. Finkel T, Holbrook N. J. Oxidants oxidative stress and the biology of ageing. Nature. 2000; 408:239-47.

7. Ernst E, Schmidt K, Wider B. CAM research in Britain: The last 10 years. Complement Ther Clin Pract. 2005;11:17-20.

8. Patil, Vikas & Bhangale, Shandavi & Chaudhari, Kundan & Kakade, Rajanikant & Thakare, Vinod & Bonde, Chandrakant & Patil, Vijay. (2012). Evaluation of the antidiarrheal activity of the plant extracts of Ficus species. Zhong xi yi jie he xue bao = Journal of Chinese integrative medicine. 10. 347-52.

9. Reddy, Shivakumar. (2015). Evaluation of antidiarrheal activity of ethanolic extract of taxus baccata in wistar rats.

10. Solanki, Himanshu & Shah, Dushyant & Patel, Chirag & Jani, Girish. (2017). Evaluation of anti-diarrheal activity of probiotic by castor oil induced diarrhoea in Swiss albino mice model. Current Bioactive Compounds. 13. 1-1. 10.2174/1573407213666170221155223.

11. Rajput, Mithun & Nair, Veena & Chauhan, Akansha & Jawanjal, Hitesh & Dange, Vikramsingh. (2011). Evaluation of Antidiarrheal Activity of Aerial Parts of Vinca major in Experimental Animals. Middle East J. Sci. Res.. 7.

12. Teferi, Meseret & Abdulwuhab, Mohammedbirhan & Yesuf, Jibril. (2019). Evaluation of In Vivo Antidiarrheal Activity of 80% Methanolic Leaf Extract of Osyris quadripartita Decne (Santalaceae) in Swiss Albino Mice. Journal of Evidence-Based Integrative Medicine. 24. 2515690X1983334. 10.1177/2515690X19833340.

13. Sharma, Mrinal & Singh, Navjeet. (2018). A Review on Pharmacological Aspects of Holarrhena antidysenterica. 10.21276/sajp.2018.7.12.5.

14. Jamadagni, P. S., Pawar, S. D., Jamadagni, S. B., Chougule, S., Gaidhani, S. N., & Murthy, S. N. (2017). Review of Holarrhena antidysenterica (L.) Wall. ex A. DC.: Pharmacognostic, Pharmacological, and Toxicological Perspective. Pharmacognosy reviews, 11(22), 141-144. https://doi.org/10.4103/phrev.phrev_31_16.

15. Kavitha, D., Shilpa, P. N., & Devaraj, S. N. (2004). Antibacterial and antidiarrhoeal effects of alkaloids of Holarrhena antidysenterica WALL. Indian journal of experimental biology, 42(6), 589–594.

16. Sharma, D. K., Gupta, V. K., Kumar, S., Joshi, V., Mandal, R. S., Prakash, A. G., & Singh, M. (2015). Evaluation of antidiarrheal activity of ethanolic extract of Holarrhena antidysenterica seeds in rats. Veterinary world, 8(12), 1392–1395. https://doi.org/10.14202/vetworld.2015.1392-1395.

17. Ganapathy, P. S., Ramachandra, Y. L., & Rai, S. P. (2011). In vitro antioxidant activity of Holarrhena antidysenterica Wall. methanolic leaf extract. Journal of basic and clinical pharmacy, 2(4), 175-178.

18. Sinha, Snehadri & Sharma, Aishwarye & Reddy, P. & Rathi, Brijesh & Prasad, N.V.S.R.K. & Vashishtha, Amit. (2013). Evaluation of phytochemical and pharmacological aspects of Holarrhena antidysenterica (Wall.): A comprehensive review. Journal of Pharmacy Research. 6. 488–492. 10.1016/j.jopr.2013.04.004.

19. Kumar, N., Singh, B., Bhandari, P., Gupta, A. P., & Kaul, V. K. (2007). Steroidal alkaloids from Holarrhena antidysenterica (L.) WALL. Chemical & pharmaceutical bulletin, 55(6), 912–914. https://doi.org/10.1248/cpb.55.912