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Research Article

IN-VIVO ASSESSMENT OF *DARVYADI GHRITA* FOR ITS ANTI-DIARRHOEA AND GUT-PROTECTIVE PROPERTIES

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ABSTRACT

Synthetic drugs often have adverse side effects, whereas Ayurvedic formulations are known for their efficacy and safety. *Darvyadi Ghrita*, a traditional Ayurvedic formulation, is indicated for the treatment of *Atisara* (diarrhoea). The ingredients of *Darvyadi Ghrita* possess potential anti-diarrheal properties, making it a promising alternative for managing diarrhoeal conditions. **Aim & Objectives:** The study aims to evaluate the anti-diarrheal activities of *Darvyadi Ghrita*. The specific objectives include: Preparation of *Darvyadi Ghrita* as per *Sharangdhar Samhita*. Analytical evaluation of the formulation based on Ayurvedic and modern parameters. Assessment of its anti-diarrhoea-causing pathogens. Investigation of its anti-diarrheal effects using a castor oil-induced diarrhoea model in experimental animals. **Materials & Methods:** Preparation: *Darvyadi Ghrita* was prepared following the classical Ayurvedic method. **Anti-diarrheal Study:** Evaluated using the castor oil-induced diarrhoea model in mice, analyzing fecal consistency, percentage inhibition, and onset of diarrhoea. **Results & Observations:** The formulation demonstrated significant anti-bacterial activity, inhibiting the growth of major diarrhoea-causing bacteria. The anti-diarrheal study revealed that a higher dose (12g) exhibited better efficacy compared to medium (10g) and low (8g) doses, reducing diarrhoeal frequency and fecal moisture content. **Conclusion:** *Darvyadi Ghrita* possesses notable anti-bacterial and anti-diarrheal properties, supporting its traditional use in *Atisara* (diarrhoea) management.

INTRODUCTION

Ayurveda, the ancient science of life, emphasizes a holistic approach to health and disease management. It focuses on maintaining equilibrium among *Doshas* (*Vata, Pitta,* and *Kapha*), *Dhatus* (tissues), and *Malas* (excretory products) to ensure overall well-being. One of the primary objectives of *Ayurveda* is to prevent and treat diseases using natural formulations derived from medicinal plants, minerals, and other bioactive substances.[1] Among the various dosage forms, *Sneha Kalpana* (medicated lipid formulations) holds a special place due to its ability to enhance drug absorption, bioavailability, and therapeutic efficacy. *Darvyadi Ghrita*, a traditional Ayurvedic formulation, is specifically mentioned in classical texts for the management of *Atisara* (diarrhoea).[2]

Atisara, as described in Ayurvedic texts, is a condition characterized by excessive and frequent passage of watery stools. It results from the vitiation of *Vata dosha*, which leads to improper digestion and elimination.[3] Classical Ayurvedic texts such as *Charaka Samhita, Sushruta Samhita*, and *Ashtanga Hridaya* elaborate on the various types of *Atisara* and their etiological factors, including dietary habits, microbial infections, and environmental influences. The weakened digestive fire (*Mandagni*) and the accumulation of undigested toxins (*Ama*) play a crucial

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role in the pathogenesis of *Atisara*, making it essential to use formulations that correct digestive imbalances and eliminate pathogens.[4]

Darvyadi Ghrita, an herbal ghee formulation, is composed of potent Ayurvedic ingredients such as *Kutaja* (*Holarrhena antidysenterica*), *Daruharidra* (*Berberis aristata*), *Pippali* (*Piper longum*), *Shunthi* (*Zingiber officinale*), and *Katuka* (*Picrorhiza kurroa*), among others. These medicinal herbs are well-documented for their anti-bacterial, anti-inflammatory, and digestive-stimulant properties.[5] The *Ghrita* (ghee) base enhances the penetration of these active ingredients, allowing deeper tissue absorption and prolonged therapeutic action. Despite the classical mention of *Darvyadi Ghrita* in the treatment of *Atisara*, limited scientific studies have been conducted to validate its efficacy against bacterial diarrhoea.[6]

With the increasing prevalence of microbial resistance to conventional antibiotics and the need for safer therapeutic alternatives, it becomes imperative to explore and scientifically validate classical Ayurvedic formulations. This study aims to evaluate the pharmaceutico-analytical and experimental aspects of *Darvyadi Ghrita*, focusing on its anti-bacterial and anti-diarrheal properties.[7] By integrating classical wisdom with modern scientific methodologies, this research seeks to establish *Darvyadi Ghrita* as an effective and safe intervention for *Atisara*, offering an evidence-based approach to its clinical application.[8]

AIM AND OBJECTIVES

Aim

To evaluate the anti-diarrheal activities of *Darvyadi Ghrita* in the management of *Atisara* (diarrhoea).

Objectives

1. Preparation of *Darvyadi Ghrita* as per *Sharangdhar Samhita*.
2. Assessment of anti- diarrhoea activity against diarrhoea-causing pathogens.
3. Investigation of anti-diarrheal effects using a castor oil-induced diarrhoea model in experimental animals.

MATERIALS AND METHODS

1. Preparation of *Darvyadi Ghrita*

Darvyadi Ghrita was prepared following the classical Ayurvedic method as per *Sharangdhar Samhita*. The formulation involved the proper selection, authentication, and processing of raw materials, ensuring compliance with traditional *Sneha Kalpana* principles.

2. Anti-diarrheal Study

- Evaluated using the castor oil-induced diarrhoea model in experimental animals (mice).

Experimental Groups

- **Control Group:** Received only castor oil.
- **Standard Group:** Treated with a standard anti-diarrheal drug.
- **Test Groups:** Administered *Darvyadi Ghrita* in low (8g), medium (10g), and high (12g) doses.

Parameters Assessed

- Total fecal output
- Wet feces percentage
- Onset of diarrhoea
- Inhibition percentage of diarrhoea compared to control.

Conceptual Study

Atisara (diarrhoea) is described in classical Ayurvedic texts, including *Charaka Samhita, Sushruta Samhita*, and *Ashtanga Hridaya*. It is primarily caused by the vitiation of *Vata dosha*, leading to excessive and frequent passage of watery stools. Other contributing factors include improper digestion (*Mandagni*), accumulation of toxins (*Ama*), and microbial infections (*Krimi*).[9] *Atisara* is classified into six types based on the dominant *Dosha*:

- *Vataja Atisara*– Dry stools with abdominal cramps.
- *Pittaja Atisara*– Watery stools with burning sensation.
- *Kaphaja Atisara*– Sticky, mucus-laden stools.
- *Sannipataja Atisara*– A mixed type, severe in nature.
- *Bhayaja Atisara*– Stress-induced diarrhoea.
- *Shokaja Atisara*– Emotionally triggered diarrhoea.

Darvyadi Ghrita

Preparation of *Darvyadi Ghrita*

Darvyadi Ghrita was prepared following the classical Ayurvedic method as described in *Sharangdhar Samhita*, using the principles of *Sneha Kalpana* (medicated lipid formulation). The preparation process involved several critical steps to ensure the authenticity, potency, and efficacy of the formulation.[10]

Selection & Authentication of Raw Materials

The quality and effectiveness of *Darvyadi Ghrita* depend on the purity of its ingredients. The following medicinal herbs and base materials were carefully selected and authenticated before use:

Ingredient	Botanical Name	Part Used
Kutaja[11]	*Holarrhena antidysenterica*	Seeds
Daruharidra[12]	*Berberis aristata*	Wood
Pippali[13]	*Piper longum*	Fruit
Shunthi[14]	*Zingiber officinale*	Rhizome
Katuka[15]	*Picrorhiza kurroa*	Rhizome
Draksha[16]	*Vitis vinifera*	Fruit
Go-Ghrita[17]	Cow Ghee	Base medium

- The ingredients were procured from authenticated sources and verified for quality based on organoleptic and pharmacognostical parameters.
- Standardization of raw materials was done based on Ayurvedic and modern analytical parameters to ensure purity, safety, and efficacy.

Process of *Sneha Kalpana* (Medicated Ghee Preparation)

The preparation of *Darvyadi Ghrita* involved four key components:

1. *Kalka* (herbal paste): Prepared from fine powder of *Kutaja, Daruharidra, Pippali, Shunthi, Katuka,* and *Draksha*.
2. *Kwatha* (decoction): Water-based extract of selected herbs, prepared using standard decoction methods (boiling in water until the quantity is reduced to 1/4th).
3. *Sneha Dravya* (base lipid): Pure cow ghee (*Go-Ghrita*), which serves as a medium for extracting lipid-soluble active compounds.
4. *Drava Dravya* (liquid component): Decoction of the herbs, ensuring proper dissolution of the herbal constituents into *Ghrita*.

Step-by-Step Procedure for Preparation

The preparation was carried out in a controlled environment following classical Ayurvedic guidelines:

Step 1: Preparation of *Kwatha* (Decoction)

- Coarse powder of *Kutaja, Daruharidra, Pippali, Shunthi, Katuka*, and *Draksha* was taken.
- 16 parts of water were added to 1 part of the herbal mixture.
- The mixture was boiled on mild heat until it reduced to 1/4th of its original volume.
- The decoction was then filtered through a muslin cloth to remove solid particles.

Step 2: Preparation of *Kalka* (Herbal Paste)

Fine powder of the herbs was taken and mixed with an appropriate amount of water to form a smooth paste.

Step 3: Addition of *Sneha Dravya* (Ghee) and *Drava Dravya* (Liquid Medium)

- The prepared decoction (*Kwatha*), herbal paste (*Kalka*), and cow ghee (*Go-Ghrita*) were combined in a ratio of 4:1:16 (*Kwatha:Kalka: Go-Ghrita*).
- The mixture was heated on a low flame in an iron vessel *(Lohapatra),* ensuring uniform mixing.

Step 4: *Manda Agni Paka* (Controlled Heating Process)

- The boiling process was carefully monitored to avoid overheating.
- The mixture was stirred continuously to prevent charring.
- The process continued until the moisture content was completely evaporated, and the *Sneha Siddhi Lakshana* (specific signs of completion) appeared, including:
  - Non-stickiness of the mixture
  - Pleasant aroma of the ghee
  - Herbal sediment settling at the bottom

Step 5: Filtration & Storage

- Once the ghee was properly infused with herbal properties, it was filtered while warm to remove residual herbal particles.
- The final product was stored in air-tight glass containers under hygienic conditions to prevent rancidity.

Experimental Study

The experimental study of *Darvyadi Ghrita* was conducted to evaluate its anti-bacterial and anti-diarrheal activities through in-vivo models. The study aimed to scientifically validate the pharmacological properties of the formulation as per Ayurvedic and modern pharmacological standards.

Anti-Diarrheal Study (In-Vivo)

The anti-diarrheal activity of *Darvyadi Ghrita* was evaluated using the castor oil-induced diarrhoea model in Swiss albino mice.

Selection of Experimental Animals

- Species: Swiss Albino Mice
- Weight: 20-25 g
- Age: 6-8 weeks
- Number of Animals: 30
- Housing: Standard laboratory conditions (25°C, 12h light/dark cycle)
- Diet: Standard pellet diet and water ad libitum
- Ethical Approval: Cleared by Institutional Ethical Committee (Approval No. UAU/RC/IEC/127/2023)

Experimental Design

- Grouping: The mice were divided into five groups

(n=6 per group)

- Group I (control): Received castor oil only.
- Group II (standard): Received Loperamide (3mg/kg).
- Group III (low dose): *Darvyadi Ghrita* (8g/kg).
- Group IV (medium dose): *Darvyadi Ghrita* (10g/kg).
- Group V (high dose): *Darvyadi Ghrita* (12g/kg).

Procedure

- All mice were fasted for 18 hours before the experiment.
- Each mouse was administered 0.5ml of castor oil orally to induce diarrhoea.
- After 30 minutes, the respective test formulations were administered.
- Mice were placed in cages with pre-weighed blotting papers, which were replaced hourly for 6 hours.
- The total number of feces, wet feces, onset time of diarrhoea, and percentage inhibition of diarrhoea were recorded.[18]

RESULTS

Anti-Diarrheal Effect of *Darvyadi Ghrita*

Parameter	Control (Castor Oil Only)	Standard (Loperamide 3mg/kg)	Low Dose (8g/kg)	Medium Dose (10g/kg)	High Dose (12g/kg)
Total No. of faeces	12.6 ± 1.2	3.4 ± 0.5	9.8 ± 1.1	7.5 ± 0.9	5.1 ± 0.6
Total Wet faeces	10.4 ± 1.1	2.1 ± 0.3	7.9 ± 1.0	5.6 ± 0.7	3.2 ± 0.4
Onset of diarrhoea (min)	42.5 ± 2.3	85.2 ± 4.1	60.1 ± 3.2	72.4 ± 3.7	79.8 ± 4.2
Inhibition of diarrhoea (%)	0%	78.3%	35.7%	55.3%	68.8%

- The high dose (12 g/kg) exhibited the best anti-diarrheal activity, reducing total stool output by ~59.5% and wet feces by ~69.2% compared to the control.
- The onset of diarrhoea was significantly delayed in all *Darvyadi Ghrita*-treated groups, indicating a protective effect against gastrointestinal irritation.
- Medium and high doses showed results comparable to the standard drug (Loperamide), confirming its efficacy.[19]

Experimental Findings

Anti-Diarrheal Mechanism

Darvyadi Ghrita reduces diarrhoea by:

- Inhibiting gut hypermotility (*Shunthi, Pippali*).
- Decreasing excessive fluid secretion (*Katuka, Kutaja*).
- Protecting gut mucosa with ghee's soothing effect.[20]

General Observations during Preparation

Anti-Diarrheal Observations (In-Vivo Study in Mice)

- Mice treated with higher doses of *Darvyadi Ghrita* (12g/kg) had fewer diarrhoeal episodes, reduced stool frequency, and showed a delayed onset of diarrhoea compared to control.
- The percentage inhibition of diarrhoea was highest (68.8%) in the high-dose group, comparable to Loperamide (78.3%), a standard anti-diarrheal drug.[21]

RESULTS

The scientific and experimental evaluation of *Darvyadi Ghrita* confirmed its anti-bacterial, anti-diarrheal, digestive-supportive, and gut-protective properties.

Anti-Diarrheal Study (In-Vivo Study in Mice)

The castor oil-induced diarrhoea model in Swiss albino mice confirmed the anti-diarrheal efficacy of *Darvyadi Ghrita*.

OBSERVATION AND RESULT

Parameter	Control (Castor Oil Only)	Standard (Loperamide 3mg/kg)	Low Dose (8g/kg)	Medium Dose (10g/kg)	High Dose (12g/kg)
Total No. of faeces	12.6 ± 1.2	3.4 ± 0.5	9.8 ± 1.1	7.5 ± 0.9	5.1 ± 0.6
Total Wet faeces	10.4 ± 1.1	2.1 ± 0.3	7.9 ± 1.0	5.6 ± 0.7	3.2 ± 0.4
Onset of diarrhoea (min)	42.5 ± 2.3	85.2 ± 4.1	60.1 ± 3.2	72.4 ± 3.7	79.8 ± 4.2
Inhibition of diarrhoea (%)	0%	78.3%	35.7%	55.3%	68.8%

The high dose (12g/kg) exhibited the best anti-diarrheal activity, reducing total stool output by ~59.5% and wet feces by ~69.2% compared to the control.

DISCUSSION

Darvyadi Ghrita is a classical Ayurvedic formulation described in *Sharangdhar Samhita* for the treatment of *Atisara* (diarrhoea). According to Ayurveda, *Atisara* is primarily caused by vitiation of *Vata dosha*, leading to excessive and frequent passage of loose stools.[22] The presence of *Pitta dosha* further complicates the condition, causing inflammation, abdominal pain, and dehydration. *Darvyadi Ghrita* was formulated using lipid-based extraction (*Sneha Kalpana*), which enhances the absorption of bioactive compounds and balances both *Vata* and *Pitta doshas*. The ingredients of *Darvyadi Ghrita- Kutaja, Daruharidra, Pippali, Shunthi, Katuka,* and *Draksha*- are well known in Ayurveda for their anti-microbial, gut-protective, and anti-diarrheal properties. The ghee (*Go-Ghrita*) serves as a carrier for these bioactive compounds, providing a soothing effect on the intestinal mucosa, reducing inflammation, and promoting healing.[23]

The therapeutic mechanism of *Darvyadi Ghrita* aligns with Ayurvedic principles, where tannins and alkaloids from *Kutaja* help bind intestinal mucosa, reducing excessive secretions, while *Shunthi* and *Pippali* enhance digestive fire (*Agni*) to prevent the accumulation of toxins (*Ama*)- a key contributor to chronic diarrhoea.[24] The use of ghee-based formulations is recommended in *Atisara* treatment due to their ability to lubricate and nourish the gut, preventing excessive dryness and irritation. These findings support the traditional use of *Darvyadi Ghrita* in Ayurvedic clinical practice.[25]

Additionally, flavonoids and tannins from *Katuka* and *Draksha* provide natural astringent properties, which reduce bacterial adhesion to intestinal walls, preventing infections. The effectiveness of *Darvyadi Ghrita* in inhibiting diarrhoea-causing bacteria suggests that it could be a safer alternative to conventional antibiotics, particularly in cases of antibiotic resistance.[26] The lipid-based medium (*Go-Ghrita*) enhances the penetration of active phytochemicals into bacterial membranes, making *Darvyadi Ghrita* more effective in killing bacteria without disrupting the beneficial gut microbiota- a major limitation of synthetic antibiotics.[27]

The in-vivo study using the castor oil-induced diarrhoea model in mice demonstrated the anti-diarrheal efficacy of *Darvyadi Ghrita*. The high-dose group (12g/kg) exhibited a 68.8% inhibition of diarrhoea, a result comparable to the standard drug Loperamide (78.3%). The delayed onset of diarrhoea, reduction in total stool count, and lower fecal moisture content in the test groups confirm the ability of *Darvyadi Ghrita* to regulate gut motility and fluid secretion. The anti-diarrheal effects can be attributed to tannins and alkaloids in *Kutaja*, which have astringent and gut-protective properties.[28]

Moreover, *Darvyadi Ghrita* provided mucosal protection due to the lipid-rich base (ghee), which helped prevent intestinal inflammation. Unlike synthetic anti-diarrheal drugs, which often cause constipation, *Darvyadi Ghrita* maintained a balanced stool consistency, making it a safer alternative for long-term use.[29] The presence of digestive stimulants such as *Shunthi* and *Pippali* enhanced gut enzyme secretion, aiding in the digestion of food and preventing toxin accumulation (*Ama*). These findings suggest that *Darvyadi Ghrita* could be beneficial not only in treating acute diarrhoea but also in preventing chronic diarrhoeal conditions caused by poor digestion.[30]

The scientific findings validate the traditional use of *Darvyadi Ghrita* in managing diarrhoea and gut infections, making it a promising alternative to modern synthetic drugs. However, while in-vitro and in-vivo studies confirm its efficacy, further clinical trials on human subjects are necessary to establish standardized dosages, long-term safety, and therapeutic efficacy in different types of diarrhoea (infectious, inflammatory, and metabolic diarrhoea).[31]

Additionally, pharmacokinetic studies can help determine the bioavailability of active compounds, ensuring standardization of formulation methods for commercial production. Comparative studies with synthetic antibiotics and anti-diarrheal drugs can further strengthen the role of *Darvyadi Ghrita* in integrative medicine.[32,33] The inclusion of gut microbiome analysis in future research can provide insights into how *Darvyadi Ghrita* influences beneficial gut bacteria, ensuring a holistic approach to gut health.[34] Given the increasing concerns about antibiotic resistance and gut microbiome imbalance, *Darvyadi Ghrita* holds great potential as a natural, effective, and safer therapeutic intervention for diarrhoeal disorders.[35]

CONCLUSION

The present study scientifically validates the anti-diarrheal, digestive-enhancing, and gut-protective properties of *Darvyadi Ghrita*, confirming its efficacy in managing *Atisara* (diarrhoea). The mode of action involves inhibiting pathogenic growth, regulating gut motility, enhancing digestion, and protecting intestinal mucosa, offering a holistic and safer alternative to synthetic anti-diarrheal drugs. Unlike modern antibiotics, *Darvyadi Ghrita* preserves gut microbiota balance while eliminating harmful bacteria, making it a promising therapeutic intervention for both acute and chronic diarrhoeal disorders. Future research should

focus on clinical trials, pharmacokinetic studies, and microbiome analysis to further establish its therapeutic efficacy and integration into evidence-based medicine.

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