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PHARMACEUTICO-ANALYTICAL & EXPERIMENTAL STUDY OF *DARVYADI GHRITA* (MEDICATED BUTTER): A RESEARCH PROTOCOL

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ABSTRACT

Introduction: *Darvyadi Ghrita* (Medicated Butter) is a polyherbal formulation and is mainly indicated for the Diarrhoea. It is mentioned in the text Charak Samhita by Acharya Charaka. The ingredients of *Darvyadi Ghrita* (Medicated Butter) with their *rasapanchaka* (Properties)are very much competent to exhibit anti-bacterial and anti-diarrheal property from Ayurvedic perspective. **Need of the study:**

1. In developing countries like India morbidity and mortality rate related to diarrhoea are high, so there is a need of effective classical herbal formulation for it.
2. The microbial resistance is growing day-by-day, new antimicrobial drug development takes many decades and hepatotoxicity and nephrotoxicity is also an associated factor.
3. *Ghrita* (Clarified Butter) formulation was found effective in curing Bacterial Diarrhoea. *(Laxmi et.al,2021)*
4. Mostly Diarrhoea is caused by bacteria, therefore anti-bacterial & anti-diarrheal activity of *Darvyadi Ghrita* (Medicated Butter) will be evaluated.
5. No specific research has been conducted on anti-bacterial and anti-diarrheal activity of *Darvyadi Ghrita* (Medicated Butter).

Aim: To evaluate the Anti-bacterial and Anti-diarrheal activity of *Darvyadi Ghrita* (Medicated Butter). **Materials and methods:** This opened labelled protocol for prospective study will be conducted in the department of Rasa Shastra and Bhaisajya Kalpana, Rishikul Ayurvedic College, Uttrakhand. The study involves the pharmaceutical preparation, analytical characterization, antibacterial evolution and anti-diarrheal activity testing of *Darvyadi Ghrita* (Medicated Butter) using standard pharmacological methods and animal models.

KEYWORDS: *Atisara*, Anti-bacterial, Anti-diarrheal, *Darvyadi Ghrita* (Medicated Butter), Ayurveda.

INTRODUCTION

Ayurveda describes Diarrhoea as *"Atisara"*, which means *"ati"* (excess) and *"saranam"* (flowing); the condition of rapid and excessive discharge of watery stools.[1] Diarrhoea is the passage of three or more loose stools. It is characterized by increased gastrointestinal motility, secretion and decrease in the absorption of fluids and electrolytes.[2,3] Based on the duration, it is classified into 3 types: Acute Diarrhoea (duration < 2 weeks), persistent diarrhoea (duration from 2 to 4 weeks) and chronic diarrhoea (duration more than 4 weeks).[4] Acute diarrhoea is mainly caused by enteric pathogens including viruses, bacteria and parasites whereas in chronic diarrhoea most of the cases result from functional or inflammatory bowel disorders, malabsorption syndromes and drugs.[5,6] Pathogenic agents, such as *Cryptosporidium*, *Giardia lamblia* and enteropathogenic bacteria, are thought to be the causes of persistent diarrhoea.[7] Many patients with a sudden onset of diarrhoea have a self-limited illness requiring no treatment. However, in severe cases, dehydration and electrolyte imbalance are the main risks, particularly in infants, children and elderly patients.[8]

Acharya Charaka and Vagbhatta classify diarrhoea on the basis of *Sharir Dosha* and *Mansik Nidan* (Etiology) into six types viz; *Vatika* (Dosha related to air and space elements), *Paitika* (Dosha related to fire), *Kaphaja* (Dosha related to water and earth elements), *Sannipataja* (A condition where all three doshas are imbalanced), *Shokaja* (Grief or Emotional distress) *and Bhayaja* (Fear or Anxiety).[9,10] Acharya Sushruta mentioned *Amaja Atisara* (Diarrhoea due to incomplete digestion or metabolism) instead of *Bhayaja Atisara* (Diarrhoea due to fear or Anxiety).[11]

In the modern scenario, the disease mentioned as *Atisara* in Ayurveda can be correlated to Diarrhoea which resembles the clinical feature of *Atisara.* Signs and symptoms associated with Diarrhoea may include loose watery stool, abdominal cramps, fever and blood in stool etc. The common causes of bacterial Diarrhoea include *Salmonella, Shigella, E Coli,* and *S. aureus* etc.[12]

Herbal medicines have been used as a medicament for curing diarrhoeal diseases and it is estimated that up to 80% of the population in developing countries depend on the conventional medicines for their primary healthcare.[13] There are an enormous number of herbal medicines around the world that are claimed to be effective in treating diarrhoea.[14] Medicinal plants are the auspicious source of new anti-diarrheal drugs. For this reason, the WHO has encouraged studies relevant to the treatment and prevention of diarrhoeal diseases using traditional medicinal practices.[15]

Currently available modern drugs are linked with adverse effects and contraindications.[16,17] Drug resistance is another challenge to think about antibiotics used in the treatment of diarrhoea.[18]

Panch-vidha Kashaya Kalpana (Five forms of meditional preprations) is appraised to be the basic preparation in *Ayurvedic* pharmaceutics from which various secondary preparations are derived.[19] There are so many dosage forms as medicament of Diarrhoea is advised such as *Churna* (Powder), *Kashaya* (Decoction), *Vati* (Tablet or Pill), *Asava* (Fermented liquor), *Rasa-Aushadhi* (Herbo-mineral or Meta or Mineral formulations), *Sneha* (Oleaginous or fat substance) *and Panaka* (Fruit juice).[20]

Sneha Kalpana (Oleaginous substance) is a group of products of medicated *Ghrita* (Clarified Butter) and *Taila* (Oil). It is used in therapeutics both topically and systemically. Thus, we can see a wide variety of uses of *Sneha Kalpana*(Oleaginous substance),[21] which are *Nasya Kalpana* (Nasal Instillation Therapy), *Abhyanga* (Oil Massage), *Anuvasana Basti* (Enema Therapy) etc.

Ghrita (Clarified Butter) is a secondary preparation comes under *Sneha Kalpana* (Oleaginous substance). It is one of the techniques widely used in the *Ayurvedic* pharmaceutical industry to dissolve fat soluble and water-soluble extractives into the medium of *Ghrita* (Clarified Butter).

Ghrita (Clarified Butter) is boiled with prescribed *Dravya* (Drug) and *Kalka* (Paste) of drugs taken in specific ratios and by exposing them to unique heating pattern and duration to fulfill certain pharmaceutical parameters, according to need of therapeutics.[22]

Ayurvedic literature have lots of unexplored or least tested medicines, *Darvyadi Ghrita* (Medicated Butter) is one of those *Ayurvedic* formulation used in the management of all types of *Atisara* (Diarrhoea) including *Sannipataja* (Conglomeration of vitiated tridosa) described in *Charak Samhita*.[23] All the ingredients of this formulation have anti-diarrheal and anti-bacterial properties in different texts.

REVIEW OF LITERATURE

I. Review of Ayurvedic Literature

Darvyadi Ghrita (Medicated Butter) is depicted by Acharya Charak in his text Charak samhita for the management of Diarrhoea.

a. The literature regarding to *Atisara* (Diarrhoea), *Darvyadi Ghrita* (Medicated Butter) and its ingredients will be compiled from different classical texts.
b. The literature regarding to preparation of *Darvyadi Ghrita* (Medicated Butter) will be compiled from different Ayurvedic texts.

II. Review of modern literature

a. The literature regarding Diarrhoea causing bacteria's will be compiled from different books, journals, outcome of survey of prestigious institute and seminar reports etc.
b. The literature regarding methods of Anti-bacterial study and Anti-diarrheal study will be reviewed from different books on microbiology, internet and various research articles etc.

II. DRUG REVIEW

Table No. 1: INGREDIENTS OF *DARVYADI GHRITA* (MEDICATED BUTTER).

S. No	INGREDIENTS	PART USED	PROPERTIES	PHYTO- CONSTITUENTS
1	***Kutaja (Holarrhena antidysenterica* Linn.)**	Seed	Anti-diarrheal, Anti-spasmodic.[24]	Alkaloids, tannins, saponins, flavonoids.[25]
2	***Daruharidra (Berberis aristate* Dc.)**	Bark	Anti-diarrheal and Anti-microbial.[26]	Alkaloids,tannins,saponins, flavonoids.[27]
3	***Pippali (Piper longum* Linn.)**	Fruit	Anti-bacterial, Anti-inflammatory, Anti-spasmodic.[28]	Piperine, Asarinin.[29]

4	***Shunthi (Zingiber officinale* Rosc.)**	Rhizome	Anti-bacterial, Anti-oxidant and Anti-inflammatory.[30]	Alkaloids, tannins, saponins, flavonoids.[31]
5	***Katuka (Picrorrhiza kurroa* Royle ex Benth.)**	Rhizome	Anti-bacterial , Anti-microbial Anti-oxidant.[32]	Glycosides , Cucurbitacins.[33]
6	***Draksha (Vitis vinifera)***	fruit	Antibacterial activity Antioxidant, Anti-fungal.[34]	Flavonoids , stilbenoid.[35]
7	***Go-ghrita* (Clarified Butter)**	Animal product	Anti-microbial and expectorant .[36]	Triglycerides , di- glycerides, monoglycerides.[37]

Table No. 2: PROPERTIES OF INGREDIENTS.[38]

Ingredients	***Rasa* (taste)**	***Guna* (properties)**	***Virya* (potency)**	***Vipaka* (metabolism)**	***Karma/ doshaghnta***
***Kutaja (Holarrhena antidysenterica* Linn.)**	*Tikta* (Bitter), *Kashaya* (Astringent)	*Laghu* (Lightness), *Ruksha* (Dryness)	*Sheeta* (Cold)	*Katu* (Pungent)	*Tridoshashamaka* (Alleviate all the three dosha) *Stambhan* (Stagnation), *Krimighna* (Antihelmintic)
***Daruharidra (Berberis aristate* Dc.)**	*Tikta,* (Bitter) *Kashaya* (Astringent)	*Laghu* (Lightness), *Ruksha* (Dryness)	*Ushna* (Hot)	*Katu* (Pungent)	*Kapha- Pitta shamaka* (Kapha -Pitta Pacifying), *Shothhara* (anti-inflammatory) *Vednasthapan* (analgesic)
***Pippali (Piper longum* Linn.)**	*Katu* (Pungent)	*Laghu* (Lightness), *Tikshna* (Sharpness)	*Ushna* (Hot)	*Madhura* (Sweet)	*Kapha – Vatashamaka,* Triptighna (anti-satiative) Krimighna (antihelmintic) *Vataanulomana* (Carminative)
***Shunthi (Zingiber officinale* Rosc.)**	*Katu* (Pungent)	*Ruksha* (Dryness) *Guru* (Heavyness), *Tikshna* (Sharpness)	*Ushna* (Hot)	*Madhura* (Sweet)	*Kapha-Vatashamaka* (Kapha-Vata Pacifying) *Triptighna* (anti-satiative) Arshoghna (relieves piles) Vatanulomaka (Carminative)
***Katuka (Picrorrhiza kurroa* Royle ex Benth.)**	*Tikta* (Bitter)	*Ruksha* (Dryness), *Laghu* (Lightness)	*Sheeta* (Cold)	*Katu* (Pungent)	*Kapha- Vatashamaka,* (kapha-vata pacify) *Pramehaghna, kusthghna, Krimighna*
Draksha (Vitis vinifera)	*Madhura* (Sweet)	*Guru* (Heavyness), *Snigdha* (unctuous), *Mridu*	*Sheeta* (Cold)	*Madhura* (Sweet)	*Vata- Pittahara* (Vata-Pitthara Pacifying),*Vrishya* (aphrodisiac),*Medhya (intellect) Sandhankaraka* (fermentation)
***Go- Ghrita* (Clarified Butter)**	*Madhura* (Sweet)	*Guru* (Heavyness) *Snigdha* (unctuous) *Mridu* (Softness) *Tridoshashamaka* (Alleviate all the three dosha)	*Sheeta* (Cold)	*Madhura* (Sweet)	*Tridoshashamaka* (Alleviate all the three dosha)

Table No. 3: *RASA PANCHAKA* (5TASTES) OF CONSTITUENTS IN *GHRITA MURCHHANA* (PROCESSING OF GHEE).[39]

DRUG	***RASA* (Taste)**	***GUNA* (Properties)**	***VIRYA* (Potency)**	***VIPAKA* (Metabolism)**
***Haritaki* (Terminalia chebula Retz.,)**	*Kashaya Pradhan pancha rasa* (Astringent)	*Laghu* (Lightness), *Ruksha* (Dryness)	*Ushna* (Hot)	*Madhura* (Sweet)
***Bibhitaki* (Terminalia bellirica Roxb.)**	*Kashaya* (Astringent)	*Laghu* (Lightness), *Ruksha* (Dryness)	*Ushna* (Hot)	*Madhura* (Sweet)
***Amalaki* (Phyllanthus**	*Amla pradhan Pancha rasa*	*Laghu* (Lightness), *Ruksha*	*Sheeta* (Cold)	*Madhura*

emblica)	(Sour)	(Dryness)		(Sweet)
***Haridra* (Curcuma longa Linn.)**	*Tikta* (Bitter), *Katu* (Pungent)	*Laghu* (Lightness), *Ruksha* (Dryness)	*Ushna* (Hot)	*Katu* (Pungent)
***Mustaka* (Cyperus rotundus)**	*Tikta* (Bitter), *Katu*, (Pungent), *Kashaya* (Astringent)	*Laghu* (Lightness), *Ruksha* (Dryness)	*Sheeta* (Cold)	*Katu* (Pungent)
***Matulunga* (Cytrus medica Linn.)**	*Amla* (Sour)	*Laghu* (Lightness), *Snigdha* (Unctuous)	*Ushna* (Hot)	*Amla* (Sour)

B. MATERIALS AND METHODS

1. PHARMACEUTICAL STUDY

1. **Procurement**: The samples of each raw drug will be procured from local drug store.
2. **Authentication:** Procured sample will be authenticated in P.G. Department of Dravyaguna, Rishikul Campus, UAU Haridwar.
3. **Method of preparation:** *Darvyadi ghrita* (Medicated Butter) will be prepared in Rasa Shastra and Bhaisajaya Kalpana department of Rishikul campus, UAU Haridwar.

- *Sneha Kalpana* (Oleaginous substance) is the preparation prepared by using one part of '*Kalka Dravya* (Fine paste of medicinal plant),4 parts of '*Sneha Dravya*'(Lipid substances) and 16 parts of '*Drava Dravya*'(Fluid substances).(Sa . Ma.Kha. 9/1)
- *Kalka Dravya* (Fine paste of medicinal plant) will be triturated with needed quantity of water to obtain *Kalka* (Paste).
- *Murchhita Ghrita* (Processed ghee) will be taken in a clean wide mouthed stainless-steel vessel heated over mild fire.
- *Kalka Dravya* (Fine paste of medicinal plant) with required quantity of water (*Drava*) will be added to the *Ghrita*,(Clarified butter) keep boiling with frequent stirring.
- After *Sneha Siddha Lakshanas* (Assessment of fatty substances) the *Ghrita* (Clarified butter) is filtered using muslin cloth into a clean stainless-steel vessel.

2. ANALYTICAL STUDY

Analytical Parameters of *Ghrita* (Clarified butter)

1. Description
- Colour
- Odour
- Taste
- Consistency

2. Viscosity
3. Refractive index
4. Saponification value
5. Peroxide value
6. Iodine value
7. Acid value
8. Microbial contamination
- Total bacterial count
- Total fungal count

9. Test for heavy metals
- Lead
- Cadmium
- Mercury
- Arsenic

10. Test for specific pathogens
- *E-coli*
- *Salmonella sp.*
- *S. aureus*
- *Shigella sp.*

11. Identification TLC/HPTLC/HPLC/GLC (As Per availability)

3. ANTIBACTERIAL STUDY

a. Antibacterial study for pathogens will be employed by using Well diffusion Method and suitable media will be used as per requirement of the pathogens in different fraction of formulations.

b. Ciprofloxacin 500 mg will be used as standard drug to compare the anti-bacterial activity of *Darvyadi Ghrita* (Medicated Butter).

4. ANTI-DIARRHEAL ACTIVITY TEST

EXPERIMENTAL ANIMALS

- **No. of groups: 5**
- **Total no. of animals: 30 (N=6)**
- **Test animals: Swiss albino mice**
- **Test substance: *Darvyadi Ghrita* (Medicated Butter)**
- **Administration of Test Dose: Single Test Dose**
- **Route of the administration: Per oral**
- **Dose: As required.**

CASTOR OIL INDUCED DIARRHOEA MODEL

PROCEDURE

- Mice will randomly be assigned into 5 groups of 6 animals each to perform Anti-diarrheal activity using the model: The control group, the standard group and three test groups.
- Animals will be kept fasting for 12 to 18 hours and placed individually in cages with blotting paper on the floor below and changed hourly.
- Animals then will receive either vehicle or treatment samples.
- Diarrhoea will be induced by administering 0.5 ml of castor oil per oral route to each mouse just one hour after the previous treatments.

OBSERVATION

- Animals will be observed individually after dosing at least once during first hour, periodically during the first 24 hours, with special attention given during the first four hours.

- Time of onset of diarrhoea, a total number of fecal outputs (frequency of defecation) and weight of feces excreted by the animal will be recorded.

CALCULATIONS

- Finally, the percentage of diarrhoeal inhibition, percentage weight of wet fecal output and percentage weight of total fecal output will be determined with respect to their formula.

$$\% \text{ Inhibition} = \frac{\text{Average number of WFC} - \textit{Average number of WFT}}{\textit{Average number of WFC}} \times 100$$

Where, WFC = average number of wet feces in the control group and WFT = average number of wet faeces in the test group.

$$\% \text{ of wet fecal output} = \frac{\textit{Mean weight of wet feces of each group}}{\textit{Mean weight of wet feces of the control}} \times 100$$

$$\% \text{ Of total fecal output} = \frac{\textit{Mean fecal weight of each group}}{\textit{Mean weight of the control}} \times 100$$

Table no. 4: Experimental Study Groups.

No. of Groups	Name of groups	No. of animals	Drug used for study	Dose
Group- A	Control Group	6 mice	Normal saline and castor oil	10ml/kg
Group- B	Standard Group	6 mice	Loperamide and Castor oil	Loperamide 3mg/kg Castor oil 0.5 ml
Group- C	Test Group	6 mice	*Darvyadi ghrita* (Medicated butter) (low dose) and castor oil	Animal dose of *Ghrita* (Clarified butter) calculated by Barnes and Paget rule Castor oil 0.5ml
Group- D	Test group	6 mice	*Darvyadi ghrita* (Medicated butter) (medium dose) and castor oil	Animal dose of *Ghrita* (Clarified butter) calculated by Barnes and Paget rule Castor oil 0.5ml
Group- E	Test group	6 mice	*Darvyadi ghrita* (Medicated butter) (high dose) and castor oil	Animal dose of *Ghrita* (Clarified butter) calculated by Barnes and Paget rule Castor oil 0.5ml

*Calculation of doses in mice (by Barnes and Pagets rule).

PARAMETERS OF DETERMINING ANTI-DIARRHEAL ACTIVITY

- The time elapsed between administration of the drug and excretion of the first diarrheic feces.
- Total number of fecal outputs.
- Total weight of fecal outputs.
- No. of Diarrheic feces excreted by the animals in 4 hour.
- Total weight of the Diarrhoeal stools in that period of time.
- Percentage of Inhibition.
- Consistency.

DISCUSSION

Diarrhoea is a prevailing problem of the contemporary era, due to irregular and unhealthy habits relating to *Ahara* (Food) and *Vihara* (Lifestyle). *Atisara* (diarrhoea) finds a place as a salient disease in an individual's life as everyone suffers from it at least once in lifetime. It is usually caused by *staphylococcus aureus*, *streptococcus aureus*, *vibrio cholera*, *salmonella typhimurium* and *Escherichia coli*. Different Acharyas have illustrated numerous types of *Atisara* (Diarrhoea) i.e Vatika (Air), Paitika (Bile, Fire), Kaphaja (Phlegm), Sannipataja (Conglomeration of vitiated tridosha), Shokaja (Grief), Amaja (Incomplete digestion) and Bhayaja (Fear). Acharya Charaka explained *Darvyadi Ghrita* (Medicated butter), a polyherbal formulation that combats with *Atisara* (Diarrhoea). The main components of the formulation are *Kutaja* (Holarrhena Antidysenterica Wall.), *Daruharidra* (Berberis Aristata DC.), *Pippali*(Piper Longum Linn.) and *Go-Ghrita* (Cow's

Butter). The common *rasa* (Taste) of the components are *Katu* (Pungent), *Tikta* (Bitter) and *Kashaya*(Astringent) which have probable mode of action of *Agni Deepana* (Appetizer), *Pachana* (Digestion) and Pacifies *Tridoshas* (Three subtle life energies i.e Vata, Pitta, Kapha) as well as provide *Bala* (Strenght and Ability of the body) to the body. In addition to the *Rasas* (Taste), the drugs also possess *Stambhana* (Stagnation) property that inhibit the *Atisara* (Diarrhoea). As per Acharya Charaka, *Katu*(Pungent), *Vipaka*(Metabolism) accounts for the *Mala Baddhta* (Constipation) property i.e. it binds the *Mala*(Waste product produce by the body) in the body.As to a thorough assessment of other sources, *Kutaja* (Holarrhena Antidysenterica Wall.) has shown inhibition of the growth of diarrhoeal pathogens like S. Typhimurium, V. Cholera, E. Coli and S. typhi. It also shows significant decrease in the levels of inflammatory cytokines. The root and bark of *Daruharidra* (Berberis aristata DC.) have shown clinical efficacy in curing acute diarrhoeal disease. *Pippali* (Piper longum Linn.) showed a higher inhibition zone against microorgansims that originate diarrhoea.

CONCLUSION

In the present era, synthetic drugs are accompanied by their side effects, while herbal drugs are far away from the said notion. *Darvyadi Ghrita* (Medicated Butter) is one of the Ayurvedic formulations indicated for Diarrhoea. On the analysing the ingredients, it demonstrates that all the drugs present in *Darvyadi Ghrita* (Medicated Butter) possess anti-bacterial, anti-diarrhoeal properties. Their anti- bacterial property is significantly effective in controlling various pathogens causing diarrhoea and therefore helps speed up the treatment of *Atisara* (Diarrhoea). Thus, proving its anti-diarrhoeal effect, *Darvyadi Ghrita* (Medicated Butter) can be Practiced popularly for the allevation of *Atisara* (Diarrhoea).

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