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EVALUATION OF ANTI-INFLAMMATORY ACTIVITY OF NARAYAN GHRITA W.S.R.T. GASTRIC ULCER (IN VITRO)

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ABSTRACT

Background: Inflammation plays a significant role in the pathogenesis of gastric ulcers through the release of inflammatory mediators and disruption of gastric mucosal defense. Owing to the limitations and adverse effects associated with prolonged use of synthetic anti-inflammatory drugs, there is growing interest in safer herbal alternatives. Narayan Ghrita, an Ayurvedic formulation described in the *Amlapitta Adhyaya* of Bhaishajya Ratnavali, is traditionally indicated in gastric inflammatory disorders and may possess anti-inflammatory potential. **Aim:** To evaluate the in-vitro anti-inflammatory activity of Narayan Ghrita. **Materials and Methods:** Narayan Ghrita was prepared according to the classical reference using authenticated raw materials and subjected to physicochemical and organoleptic analysis as per Ayurvedic Pharmacopoeial standards. The anti-inflammatory activity was assessed using the Bovine Serum Albumin (BSA) denaturation assay. Different concentrations of the formulation were tested, and diclofenac was used as the reference standard. Percent inhibition of protein denaturation and IC_{50} value were calculated. **Results:** Narayan Ghrita demonstrated concentration-dependent inhibition of protein denaturation. Maximum inhibition of 67.18% was observed at 1800 µl concentration. The IC_{50} value was found to be 354.6 µl, indicating moderate to good anti-inflammatory activity. Analytical parameters confirmed the quality, stability, and standardization of the formulation. **Conclusion:** The

study establishes significant in-vitro anti-inflammatory activity of Narayan Ghrita and scientifically supports its traditional use in gastric inflammatory conditions such as Amlapitta and gastric ulcer.

INTRODUCTION

In the pathogenesis of gastric ulcers, inflammation plays pivotal role. It is most often associated with the use of nonsteroidal anti-inflammatory drugs and Helicobacter pylori infection. Recent studies suggest that ulcer development is strongly influenced by the body's immune and inflammatory responses. Activation of immune cells and the release of inflammatory mediators (cytokines such as TNF-α, IL-1β, IL-6, and IL- 8, eicosanoids (prostaglandins and leukotrienes), and other signalling molecules like nitric oxide (NO) and platelet-activating factor (PAF) [1]) disturb the normal protective mechanisms of the gastric mucosa, leading to tissue damage and delayed healing. Ongoing inflammation further disrupts the balance between injury and repair, allowing ulcers to persist or worsen.[2]

Hence, Inflammation plays a central role in the pathogenesis of most gastric disorders, making it a key target in therapeutic research. Over time, significant efforts have been directed toward the development of safer and more effective anti-inflammatory agents. Although non-steroidal anti-inflammatory drugs (NSAIDs) are widely prescribed for inflammatory conditions, their prolonged use is often associated with adverse effects and recurrence of symptoms after discontinuation. These limitations of synthetic drugs have led to increased interest in Herbal therapeutic options. Natural formulations are increasingly being explored due to their favourable safety profile and reduced toxicity, thereby offering a promising approach for the management of inflammatory conditions.[3]

From an Ayurvedic viewpoint, these pathological changes may be correlated with aggravation of *Prana Vayu*, *Apana Vayu*, *Saman Vayu*, *Pachak pitta* and *Kledaka kapha*. These *Dosha* along with impairment of Agni (*Jatharagni*-Digestive fire), leading to inflammatory conditions of the *Amasaya* (stomach), comparable to *Amlapitta* (Hyperacidity) and *Shoola (Annadrava shola or Parinam Shoola* – Gastric or Peptic Ulcer).[4]

In the management of such conditions, an ideal formulation should possess effective anti-inflammatory activity along with Pitta-shamana (alleviating) properties. Narayan Ghrita, described in the Amlapitta Adhyaya of Bhaishajya Ratnavali, is traditionally indicated for similar pathological states and therefore may have potential anti-inflammatory properties. It

is a simple formulation with uncomplicated manufacturing process and made using easily available raw ingredients, that are all either predominantly Tikta rasa pradhan, Pitta shamak, Deepak, and Pachak. The presence of these properties contributes to its therapeutic efficacy in Agni-vikriti (vitiation of digestive fire) related disorders, especially disorders of the Annavaha Srotas such as Amlapitta (gastritis) and associated gastric ailments.

Therefore, the present experimental study focuses on the in-vitro evaluation of the anti-inflammatory activity of Narayan Ghrita. Through standardized experimental assay (Protein Denaturation Assay), the study aims to assess its potential role in modulating inflammatory responses, Thereby establishing a scientific basis for its therapeutic use in conditions associated with aggravated Pitta and inflammation-related gastric disorders.

AIM

Evaluation of in-vitro anti-inflammatory activity of *Narayan Ghrita.*

OBJECTIVES

To study in-vitro anti-inflammatory activity of *Narayan Ghrita.*

MATERIAL AND METHODS

नारायणघृतम्

जलैर्दशगुणैः क्काथ्यं पिप्पलीपलषोडश 1 पादशेषं हरेत् क्काथं क्काथतुल्यं घृतं पचेत् ।। १४१ ।।
रसप्रस्थं गुडूच्याश्च धात्र्याः षष्टिपलं रसम् 1 द्राक्षा धात्री पटोलञ्च विश्वं च कटुका वचा ।। १४२।।

पलप्रमाणकल्कञ्च दत्त्वा सर्पिः समुद्धरेत् । अम्लपित्तहरं खादेद् दाहच्छर्दिनिवारणम् ।।

असाध्यं साधयेत्सद्यो नाम्ना नारायणं घृतम् ।। १४३ ।।

-*Bhaishajyaratnawali* 53/ 141-143 (pg. 929)[5]

The content of Narayan Ghrita is as follows:

	Sr. No	Ingredients	Latin name	Part used (as per API)	Quantity
Drava-dravya	1.	*Pippali*	*Piper longum*	Fruit	768 gms
	2.	*Guduchi*	*Tinospora cordifolia*	Stem	768 ml
Drava-dravya + Kalka dravya	3.	*Amalaki*	*Emblica officinalis*	Fruit	2,880 ml (swaras) + 48gms (kalka)
Kalka	4.	*Draksha*	*Vitis vinifera*	Fruit	48 gms

dravya	5.	*Patola*	*Tricosanthes dioca*	Leaves	48 gms
	6.	*Shunthi*	*Zingiber officinalis*	Rhizome	48 gms
	7.	*Katuka*	*Picrorhiza kurroa*	Rhizome	48 gms
	8.	*Vacha*	*Acarus calamus*	Rhizome	48 gms
Sneha dravya	9.	*Goghrita*	-	-	1920 gms

All the ingredients of best quality were procured from the local market and were authenticated from the *Dravyaguna* Department of the college. *Narayan Ghrita* was prepared as per the classical reference mentioned in *Bhaishajya Ratnawali* in the Pharmacy of the college.

All relevant analytical tests of the final formulation were carried out in accordance with the standards mentioned in the Ayurvedic Pharmacopoeia of India (API) from a Certified Analytical Quality Control Lab.

Following which the *Narayan ghrita* was subjected to BSA (Bovine Serum Albumin) denaturation assay which is used to evaluate the anti-inflammatory potential of a test drug.

EXPERIMENTAL PROCEDURES FOR BSA DENATURATION ASSAY

1. The protocol was performed as per previously reported references (Anyasor et al., 2019; Baali et al., 2020).[6][7]
2. Different concentrations of the Test Samples were tested along with control.
3. 200 µl of 1% w/v BSA in PBS (pH 6.4) was added to 1800 µl of Test Samples.
4. All tubes were incubated at 37°C in for 20 min and then at 70°C in for 5 min.
5. Tubes were allowed to cool at room temperature.
6. The turbidity was measured at 660 nm by using Multiskan SkyHigh Plate Reader.
7. The Control represented 100% protein denaturation.
8. Diclofenac was used as a reference standard.
9. The percent inhibition of BSA denaturation was calculated using following equation:

Calculation

$$\text{Percent inhibition} = \frac{\text{Absorbance of control} - \text{Absorbance of test} \times 100}{\text{Absorbance of control}}$$

The IC_{50} value was determined from the graph equation.

RESULT

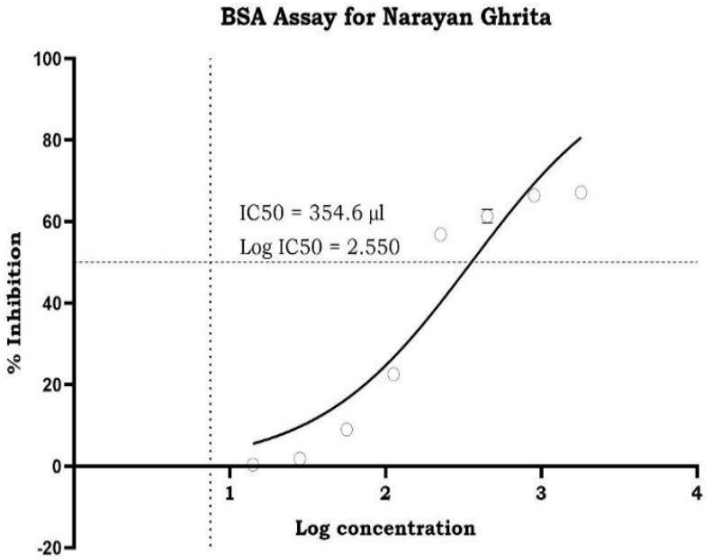
Analytical test results of Narayan Ghrita Organoleptic result

Test	Narayan Ghrita
Roop (Colour)	Yellow with brown tinge
Sparsha (Texture)	Oily separated from semi solid paste form
Gandha (Odour)	*Ghrita* mixed with *Amalaki* like
Shabda (Sound)	Not significant
Rasa (Taste)	*Tikta kashay*

Sr. no.	Test Parameter	Narayan Ghrita
1.	Moisture content%	0.3%
2.	Specific Gravity	0.91092
3.	Refractive Index	1.4601
4.	Peroxide value	0.72
5.	Acid value	1.96
6.	PH value	5.58
7.	Iodine value	27.01
8.	Saponification value	236.17
9.	Viscocity	10.69mpas

Results of BSA Denaturation Assay

Conc. (µl)	Log Conc.	Abs . 1	Abs . 2	Abs . 3	Average Absorbance	Percent Inhibition 1	Percent Inhibition 2	Percent Inhibition 3	Average Percent Inhibition (%)
Control	–	0.323	0.328	0.328	0.326	–	–	–	–
14.06	1.148	0.323	0.325	0.326	0.325	0.920	0.307	0.000	0.409
28.13	1.449	0.321	0.319	0.320	0.320	1.534	2.147	1.840	1.840
56.25	1.750	0.301	0.295	0.294	0.297	7.669	9.509	9.816	8.998
112.50	2.051	0.251	0.254	0.252	0.252	23.006	22.086	22.699	22.597
225.00	2.352	0.136	0.140	0.146	0.141	58.282	57.055	55.215	56.851
450.00	2.653	0.121	0.125	0.132	0.126	62.883	61.656	59.509	61.350
900.00	2.954	0.108	0.110	0.110	0.109	66.871	66.258	66.258	66.462
1800.00	3.255	0.106	0.107	0.108	0.107	67.485	67.178	66.871	67.178
Log IC50			2.550						
IC50 Value			354.6 µl						



DISCUSSION

Narayan Ghrita was prepared according to the proportions mentioned in the reference of Bhaishajya Ratnawali and ghrita paka was carried out for 5 days according to the reference mentioned in Bhaishajyaratnawali in 5th adhyay under the snehapaka kala that if the siddhi is to be done by using kalka or Kashaya then the siddhi should be carried out for 5 days.

Narayan Ghrita subjected to analytical tests as per the paraments mentioned in the CCRAS general guidelines for drug development of ayurvedic formulations. The evaluation of physicochemical parameters including moisture content, specific gravity, refractive index, peroxide value, acid value, pH, iodine value, saponification value, and viscosity provides essential information regarding the quality, stability, and compositional integrity of the formulation. These parameters collectively indicate purity, oxidative and hydrolytic stability and lipid characteristics, thereby ensuring proper standardization, safety, and reproducibility of the herbal formulation.

Protein denaturation is a common cause of inflammation, and substances that prevent this denaturation are considered to have anti-inflammatory activity. *Narayan Ghrita* was tested for its ability to inhibit protein denaturation.

The results demonstrated a concentration-dependent inhibition of protein denaturation, indicating the ability of *Narayan Ghrita* to stabilize proteins against heat-induced damage. A gradual increase in percentage inhibition was observed with increasing concentrations of the test sample, reaching a maximum inhibition of approximately 67% at 1800 µl. This dose-

dependent response suggests the presence of bioactive constituents capable of suppressing inflammatory pathways.

The IC_{50} value of 354.6 µl further confirms the moderate to good anti-inflammatory activity of *Narayan Ghrita*. A lower IC_{50} value reflects higher potency, and the obtained value indicates that the formulation effectively inhibits protein denaturation at relatively lower concentrations. The sigmoidal nature of the dose–response curve supports the reliability and consistency of the experimental findings.

The observed anti-inflammatory activity may be attributed to the presence of various herbal constituents in *Narayan Ghrita*, which are traditionally known for their *Pitta-shamaka, Agni Deepak* and *Amlapittahar* properties. Bioactive compounds such as flavonoids, phenolics present in these herbs and fatty acid components of ghrita, are known to stabilize proteins, reduce oxidative stress, and inhibit inflammatory mediators.

From an Ayurvedic perspective, inflammation is closely associated with aggravated *Pitta dosha*, and formulations like *Narayan Ghrita* are indicated in conditions involving Pitta imbalance and gastric inflammatory conditions. The results of the present study provide scientific validation for this traditional claim by demonstrating measurable anti-inflammatory activity through an in-vitro model.

It is also noteworthy that the maximum concentration of Narayan Ghrita evaluated in the present in-vitro study was 1800 µl, which is considerably lower than the therapeutic dose described in classical Ayurvedic texts. According to Ayurveda, the recommended dose (Mātrā) of Narayan Ghrita is 1 Pala (approximately 48 ml). When compared quantitatively, this traditional therapeutic dose is substantially higher than the experimental concentration used in the present assay.

Despite this lower experimental concentration, Narayan Ghrita exhibited a maximum inhibition of approximately 67% in the BSA denaturation assay. This observation suggests that the formulation possesses strong intrinsic anti-inflammatory potential, even at comparatively low doses. Therefore, it can be reasonably inferred that administration at therapeutic dosage levels, as prescribed in Ayurveda, may exert enhanced anti-inflammatory effects in in vivo or humans, owing to greater bioavailability and sustained pharmacological action.

However, it must be emphasized that in-vitro findings cannot be directly extrapolated to clinical efficacy, as in-vivo or human responses are influenced by factors such as absorption, metabolism, and bioavailability. Nevertheless, the present results provide a strong experimental basis supporting the traditional therapeutic claims of *Narayan Ghrita* and justify further evaluation through in vivo studies and clinical trials.

CONCLUSION

The present study demonstrates that Narayan Ghrita possesses significant in-vitro anti-inflammatory activity as evidenced by its concentration-dependent inhibition of protein denaturation. The formulation showed a maximum inhibition of approximately 67% with an IC_{50} value of 354.6 µl, indicating effective stabilization of proteins against inflammatory damage.

The observed activity may be attributed to the synergistic action of its herbal constituents, known for their *Pitta-shamana, Agni-Deepana and Pachan* properties. Physicochemical evaluation confirmed the quality, stability, and standardization of the formulation.

Although the experimental concentration used was much lower than the classical therapeutic dose mentioned in Ayurvedic texts, Narayan Ghrita exhibited notable anti-inflammatory potential, suggesting strong intrinsic activity. These findings support its traditional use in gastric inflammatory conditions such as Amlapitta and Gastric ulcer. However, further in-vivo and clinical studies are required to substantiate its therapeutic efficacy and mechanism of action.

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