Research Protocol

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Ayurveda Section

Comparative Evaluation of Antidiabetic Activity of Five Geographical Indicators of *Haridra* (*Curcuma longa* Linn.) in Wistar Albino Rats: A Research Protocol

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ABSTRACT

Introduction: Diabetes mellitus is a rapidly progressing metabolic disorder with a high global prevalence, leading to severe complications. Ayurvedic literature extensively documents the use of *Haridra* (*Curcuma longa* Linn.) in the management of *Prameha* (Diabetes mellitus). Different geographical indicators of turmeric exhibit variations in their phytochemical composition and efficacy.

Need of the study: *Haridra* (*Curcuma longa* Linn.) is a traditionally used and classically described drug that has been utilised since ancient times for its various medicinal benefits. Different geographical indicators of turmeric have been used in India for centuries. According to various research studies, these varieties show differences in morphological characteristics, phytochemical profiles, and efficacy. Although *Haridra* is a proven drug for its antidiabetic activity, geographical variations have not been explored to date.

Aim: To compare the antidiabetic activity of five geographical indicators of *Haridra*—*Erode*, *Kandhamal*, *Lakadong*, *Sangli*, and *Waigaon*—in Wistar albino rats.

Materials and Methods: An analytical experimental study will be conducted in the Department of *Dravyaguna Vigyan*, Mahatma Gandhi Ayurveda College, Hospital and Research Centre (MGACH & RC), Salod, Wardha, Maharashtra, India, from May 2025 to September 2026. Mature rhizomes of *Haridra* (*Curcuma longa* Linn.) will be collected from fields located in *Erode*, *Kandhamal*, *Lakadong*, *Sangli*, and *Waigaon* during the appropriate season. Standard pharmacognostical and phytochemical characterisation will be performed on all samples using established methods such as macroscopy, microscopy, and High Performance Thin Layer Chromatography (HPTLC) fingerprinting, as prescribed in the Ayurvedic Pharmacopoeia of India (API). The animal experiment will be conducted using 48 Wistar albino rats of either sex. The rats will be divided into eight groups, including a control group with positive and negative controls and five experimental groups administered different geographical varieties of *Haridra*. Diabetes will be induced using Alloxan, and fasting blood glucose levels will be measured at regular intervals. Phytochemical analysis of curcumin and other bioactive compounds will be conducted to correlate with the observed pharmacological effects. Statistical analysis will be performed using descriptive and inferential statistics, including Student's paired t-test, one-way ANOVA, and Tukey's multiple comparison test. A p-value of <0.05 will be considered as statistically significant.

Keywords: Diabetes mellitus, Experimental, Geographical mapping, Hypoglycaemic activity, Phytochemicals, Turmeric

INTRODUCTION

Diabetes mellitus is a rapidly advancing chronic metabolic non communicable disease that progresses across generations and results in multiple complications. Approximately 422 million people worldwide have diabetes, with most residing in low- and middle-income countries. Additionally, diabetes causes 1.5 million deaths annually. Over the past few decades, both the number of cases and the incidence of diabetes have steadily increased [1]. In 2014, 8.5% of adults over the age of 18 years worldwide were estimated to have diabetes, according to the World Health Organisation (WHO). The global prevalence was projected to reach 9.3% (463 million) in 2019, 10.2% (578 million) by 2030, and 10.9% (700 million) by 2045 [2,3].

Ayurvedic literature extensively discusses *Prameha Roga* (Diabetes mellitus) and its treatment. References to this condition and its management are found in the *Brihatrayees*, *Laghutrayees*, and *Nighantus*. *Haridra* (*Curcuma longa* Linn.) is highlighted as a potent remedy in these classical texts, with various formulations and details of its effectiveness in managing *Prameha Roga* [4]. "*Meheshu Dhatri–Nishe*" is quoted in *Ashtanga Hridayam* (*Uttarsthan*, *Vajikarana Vidhi Adhyaya* 40/48), which mentions *Dhatri* (*Amalaki*—*Emblica officinalis* Linn.) and *Nisha* (*Haridra*—*Curcuma longa* Linn.) as *Agrya* (the best medicines) for *Prameha* [5,6].

Haridra (Turmeric) (*Curcuma longa* Linn., Family: Zingiberaceae) is used in religious rituals and as a condiment, dye, medicine, and cosmetic. India is one of the world's leading producers and exporters of turmeric [7]. Various geographical indicators of turmeric are cultivated in different regions of India. The five geographical indicators included in this study are *Erode*, *Kandhamal*, *Lakadong*, *Sangli*, and *Waigaon*.

Erode turmeric: Grown in the Erode district of Tamil Nadu, Erode turmeric is known for its rich aroma, vibrant colour, and high curcumin content. It is considered one of the finest varieties of turmeric due to its superior quality [8].

Kandhamal turmeric: This variety is cultivated in the *Kandhamal* district of Odisha. *Kandhamal* turmeric is recognised for its distinct aroma, bright colour, and high curcumin content. It is widely cultivated by local farmers and valued for its medicinal properties [9,10].

Lakadong village in the Jaintia Hills district of Meghalaya, India, is home to the highly prized *Lakadong* turmeric variety. This turmeric is renowned for its exceptionally high curcumin content, which gives it its vibrant yellow colour and strong therapeutic potential. Lakadong turmeric is valued for its purity and is widely sought after for its numerous health benefits, including its antioxidant and anti-inflammatory properties [11].

Sangli turmeric is primarily cultivated in the Sangli district of Maharashtra, India. This variety is known for its bright yellow colour and strong aroma and is frequently used in Indian cuisine to enhance taste and colour.

Waigaon turmeric is a variety grown in the *Waigaon* region of Maharashtra, India. It is recognised for its distinctive flavour, aroma, and vibrant colour [9,10].

REVIEW OF LITERATURE

In Ayurvedic literature, *Haridra* (*Curcuma longa* Linn.) is described as one of the most commonly used herbs for both internal and external applications. The word *Haridra* is derived from Sanskrit and is commonly known as turmeric. Taxonomically, it belongs to the Kingdom Plantae, Subkingdom Viridiplantae, Superdivision Embryophyta, Division Tracheophyta, Subdivision Spermatophytina, Class Magnoliopsida, Order Zingiberales, Family Zingiberaceae/ Scitaminae, Genus *Curcuma* L., and Species *longa* [12]. In the *Samhitas*, a detailed description of *Haridra* is available. In the *Brihatrayees*, *Acharya Charaka* describes *Haridra* in the *Prameha Chikitsa Adhyaya* and includes it in several *Mahakashayas* and formulations such as *Nishamalaki* and *Haridra Khanda*. *Acharya Charaka* also mentions that, for *Prameha*, *Haridra Churna* should be consumed with *Amalaki Swarasa* and honey as *Anupana* (adjuvants) [13]. *Acharya Sushruta* lists *Haridra* in three *Vargas*—*Haridradi*, *Mustadi*, and *Lakshadi Gana*—and describes its various therapeutic uses in conditions such as *Prameha*, *Vrana*, and *Visa* [14]. *Acharya Vagbhata*, in *Ashtanga Hridaya*, mentions *Haridra* as *Pramehahara* and lists it as an *Agrya Aushadha* (best medicine) for *Prameha* [15]. In the *Nighantus*, virtually all classical lexicons mention *Haridra* in various *Vargas*, detailing its synonyms, botanical descriptions, properties, and therapeutic uses [16].

Although turmeric rhizome powder is widely used as a spice across India, only a limited number of people are aware of its medicinal properties. Turmeric is considered one of the best drugs for managing diseases such as diabetes and skin disorders, owing to its multiple pharmacological activities. The active constituents in turmeric rhizomes—curcuminoids—reduce lipid peroxidation by maintaining higher levels of antioxidant enzymes such as superoxide dismutase, catalase, and glutathione peroxidase. The antioxidant activity of *Curcuma longa* is attributed to curcumin and its derivatives (demethoxycurcumin, bisdemethoxycurcumin, and diacetylcurcumin). Scientific studies have shown antidiabetic, hypolipidemic, and hepatoprotective effects of freeze-dried *Curcuma longa* rhizome powder dissolved in milk, suggesting that it may serve as an effective and safe dietary antidiabetic supplement [17].

Several research studies have been conducted on *Haridra* (*Curcuma longa* Linn.) and have shown that it is a potent antidiabetic drug in type 2 diabetes. Although studies on different geographical indicators of *Haridra* exist, they are largely limited to preliminary investigations such as phytochemical analysis and metabolite profiling [18-20]. Existing studies evaluating the antidiabetic activity of *Haridra* lack proper documentation regarding the geographical origin and authentication of plant samples. To date, no research has compared the antidiabetic activity of different geographical indicators of *Haridra* on multiple parameters in a systematic manner.

Therefore, this study aims to evaluate and compare the antidiabetic activity of five geographical indicators of *Haridra* (*Curcuma longa* Linn.) in Wistar albino rats.

Objectives:

Primary objective:

1. To assess the antidiabetic activity of *Erode* turmeric in diabetes-induced Wistar rats.
2. To assess the antidiabetic activity of *Kandhamal* turmeric in diabetes-induced Wistar rats.
3. To assess the antidiabetic activity of *Lakadong* turmeric in diabetes-induced Wistar rats.
4. To assess the antidiabetic activity of *Sangli* turmeric in diabetes-induced Wistar rats.
5. To assess the antidiabetic activity of *Waigaon* turmeric in diabetes-induced Wistar rats.

Secondary objective:

1. To study and compare the physicochemical and phytochemical parameters of five geographical indicators of *Haridra* (*Curcuma longa* Linn.).
2. To study the organoleptic, macroscopic, and microscopic characteristics of the five geographical indicators of *Haridra*.
3. To analyse the HPTLC fingerprint profiles of the five geographical indicators of *Haridra* (*Curcuma longa* Linn.) using curcumin as the marker compound.

Hypotheses:

Null Hypothesis (H_0): There is no variation in the antidiabetic activity among the five geographical indicators of *Haridra* (*Curcuma longa* Linn.) in Wistar albino rats.

Alternate Hypothesis (H_1): There is a statistically significant difference in the antidiabetic activity among the five geographical variants of *Haridra* (*Curcuma longa* Linn.)—*Erode*, *Kandhamal*, *Lakadong*, *Sangli*, and *Waigaon*—with at least one variety demonstrating notably higher efficacy in Wistar albino rats.

MATERIALS AND METHODS

An analytical experimental study will be conducted in the Department of *Dravyaguna Vigyan*, Mahatma Gandhi Ayurveda College, Hospital and Research Centre (MGACH & RC), Salod, Wardha, Maharashtra, India, from May 2025 to September 2026. This study has been approved by the Institutional Animal Ethics Committee (IAEC) (Reference No.: DMIHER/IAEC/24-25/24, dated 24/08/2024).

Inclusion criteria: Healthy wistar albino rats of either sex (male or female) and weighing 150-300 grams will be included in the study.

Exclusion criteria: Diseased wistar albino rats, pregnant female rats, rats weighing less than 150 grams or more than 300 grams, Wistar albino rats that have been recently used in other studies will be excluded from the study.

Source of Data

Experimental data: Mature rhizomes of *Haridra* (*Curcuma longa* Linn.) will be collected from various geographical locations—*Erode*, *Kandhamal*, *Lakadong*, *Sangli*, and *Waigaon*—across India during the appropriate season, in accordance with traditional Ayurveda scriptures and WHO Good Collection Practices (GCP) guidelines [21]. The samples will be authenticated by submitting voucher specimens to the Foundation for Revitalisation of Local Health Traditions (FRLHT), Bengaluru; the Botanical Survey of India (BSI); a recognised botany department; or an authorised expert from the Department of *Dravyaguna*.

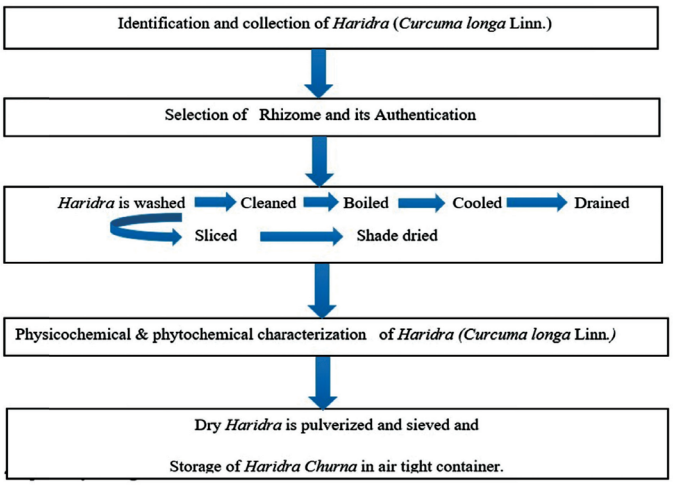
Forty-eight Wistar albino rats of either sex will be used in the study.

- The *Haridra Churna* will be prepared at Dattatray *Ayurveda Rasashala*, DMIHER (DU), Wardha.
- The animal experimental study will be carried out at the Central Preclinical Research Facility (Animal House), Jawaharlal Nehru Medical College, DMIHER (DU), Sawangi (M), Wardha, Maharashtra.

[Table/Fig-1] shows flowchart of drug preparation.

The study will be conducted on healthy Wistar albino rats of either sex, weighing 150-300 g. The animals will be divided into eight groups, each containing six rats [22], totalling 48 rats [Table/Fig-2]. The rats will be acclimatised, and diabetes will be induced

intraperitoneally with Alloxan (100 mg/kg body weight). Blood Sugar Levels (BSL) will be monitored, and following successful induction of diabetes, the intervention drug will be administered for 21 days. Random blood glucose levels will be measured and recorded on days 1, 4, 8, 12, 16, and 21 to ensure treatment continuity and monitor for side-effects.



[Table/Fig-1]: Detail of drug preparation.

Groups	Name of groups	Intervention/ drugs	No. of animals	Dose	Anupana (vehicle)	Study duration
Group-1	Normal [Negative] control	--	6	--	--	21 days
Group-2	Standard (Positive) Control	Metformin	6	265.71 mg/kg	Water 1 mL	21 days
Group-3	Vehicle Control	*Madhu* (Honey)	6	1 mL	--	21 days
Group-4	Test group 1	*Erode* Turmeric	6	265.71 mg/kg	*Madhu* 1 mL	21 days
Group-5	Test group 2	*Kandhamal* Turmeric	6	265.71 mg/kg	*Madhu* 1 mL	21 days
Group-6	Test group 3	*Lakadong* Turmeric	6	265.71 mg/kg	*Madhu* 1 mL	21 days
Group-7	Test group 4	*Sangli* Turmeric	6	265.71 mg/kg	*Madhu* 1 mL	21 days
Group-8	Test group 5	*Waigaon* Turmeric	6	265.71 mg/kg	*Madhu* 1 mL	21 days

[Table/Fig-2]: Containing grouping and posology.

All animal experiments will be conducted in compliance with CPCSEA (Committee for Control and Supervision of Experiments on Animals) guidelines [23].

The animals will be assigned randomly using simple randomisation, employing random number tables or Excel software, in accordance with ARRIVE guidelines [24].

Animal Housing and Husbandry [25]

All rats will be healthy and kept in a standard laboratory environment.

They will be housed in polypropylene cages and maintained under standard conditions of temperature, humidity, and light cycles. Rats will receive a standard pellet diet and water ad libitum. They will be fasted overnight before experimentation.

Animal Care and Monitoring [26]

All animals will be acclimatised before the study and handled carefully to avoid pain or distress. During blood withdrawal, only 0.5-1 mL of blood will be collected. The FDA guideline will be used for dose calculation in experimental studies, applying the rat conversion factor [27].

Outcomes

Random blood glucose test: This test will be performed on the same group of rats. Rats will remain on a standard pellet diet and water throughout the study. *Haridra Churna* (*Curcuma longa* Linn.) will be administered as per the prescribed dose. Blood samples will be collected at random times during the day, and blood glucose levels will be estimated on days 1, 4, 8, 12, 16, and 21.

Drug analysis:

Organoleptic parameters:

- *Shabda* (Sound)
- *Sparsha* (Touch)
- *Rupa* (Colour)
- *Rasa* (Taste)
- *Gandha* (Odour)

Physico-phytochemical analysis [28]: Physico-chemical parameters will be assessed as per API standards.

Pharmacognostical evaluation:

A) **Macroscopic study:** Observing the characteristic features of *Haridra* rhizome (*Curcuma longa* Linn.).

B) **Microscopic study:** A transverse section of the *Haridra* rhizome will be taken, and its microscopic features will be observed under a digital microscope.

C) **Powder characteristics:** Powder microscopy will be performed using dried turmeric rhizome powder, and structures will be observed under a digital microscope.

Physiochemical [29]:

1. **Foreign matter:** Determines any extraneous biological or chemical material present in *Haridra churna*.
2. **Loss on drying:** Measures the reduction in mass of the sample.
3. **Total ash value:** Determines the inorganic residue remaining after complete combustion.
4. **Water-soluble ash:** Portion of the total ash that dissolves in water.
5. **Acid-insoluble ash:** Portion of the ash that remains insoluble in dilute HCl.
6. **Acid-soluble ash:** Portion of the ash that dissolves in dilute HCl.
7. **Extractive values:**
   a) Alcohol-soluble extractive value: Percentage of material extractable in alcohol.
   b) Water-soluble extractive value: Determines how much of the drug content dissolves in water.
8. **Determination of pH:** Measures the acidity or alkalinity of the aqueous solution.

Preliminary phytochemical study: Qualitative tests will be performed for steroids, carbohydrates, glycosides, flavonoids, alkaloids, and tannins using standard methods.

Chromatographic study-HPTLC: HPTLC fingerprinting will be performed on a single plate, and comparative chromatograms will be analysed using appropriate methods.

STATISTICAL ANALYSIS

Statistical analysis will be conducted using descriptive and inferential statistics, including Student's paired t-test, one-way ANOVA, and Tukey's multiple comparison test. Statistical Package for the Social Sciences (SPSS) version 27.0 will be used for analysis, and p-value<0.05 will be considered statistically significant.

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