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Ayurvedic anti-diabetic formulation *Lodhrasavam* inhibits alpha-amylase, alpha-glucosidase and suppresses adipogenic activity *in vitro*

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ABSTRACT

Background: The patho-physiological cross-talk between diabetes and obesity is well established. However, the choices of drugs suitable for combined treatment of diabetes and obesity are limited. Integration of complementary and alternative medicines (CAMs), like Ayurveda, with modern medicine would be a promising strategy to fill this gap. The diagnostic principles of Ayurveda define obesity as one of the predisposing factors of *Madhumeha* (correlated as diabetes) and recommends specific formulations for managing obese-diabetes. *Lodhrasavam* is one such poly-herbal formulation prescribed for obese-diabetic patients.

Objectives: The present study is an attempt to demonstrate the possible modes of action of *Lodhrasavam*, built on the hypothesis that the formulation can exert both anti-diabetic and anti-obesity actions.

Materials and methods: Lodhrasavam, following simulated gastro-intestinal digestion, was monitored for inhibition of α-amylase, α-glucosidase (key digestive enzyme targets of anti-diabetic drugs) and adipogenesis using standard *in vitro* model systems.

Results: Lodhrasavam digest inhibited α-amylase (90%) and α-glucosidase (78%) activity as well as reduced the differentiation of 3T3-L1 fibroblasts to adipocytes. Upon fractionation, the enzyme inhibitory activity and anti-adipogenic activity of the digest were found distributed in different solvent fractions. This partly indicates a potential pharmacological networking of chemically and functionally diverse bioactive molecules in *Lodhrasavam.*

Conclusion: The study provides a possible mode of action and an experimental support for the Ayurvedic use of *Lodhrasavam* for managing obese-diabetes. Generating scientific evidences and understanding the modes of action, in contemporary scientific language, would essentially help in expanding global acceptance of potentials of CAMs in the management of life style disorders.

1. Introduction

The link between diabetes and obesity is well established in both traditional and modern systems of medicines. The World Health Organization (WHO) estimates that 44% of the diabetes cases globally are attributable to overweight and obesity [1]. Though intensive lifestyle interventions are the foundation for managing weight gain in diabetes, studies have shown that pharmacotherapies are also necessary for patients to achieve long term weight loss [2]. Despite having an evident patho-physiological cross-talk between diabetes and obesity, the choices of drugs suitable for combined treatment of diabetes and obesity are limited in modern medicine [3]. Recent examples like glucagon-like peptide-1 receptor agonists show promising results in combating the dual burden, but their long term safety is question [4,5]. Furthermore, the target based molecular drugs currently available for diabetes and obesity are associated with severe side effects such as insomnia, headache, constipation, hypoglycemia, weight gain, and renal complications [6,7]. As an alternative, integrating complementary and alternative medicines (CAMs) with modern medicine would have promising applications in managing lifestyle disorders like diabetes and obesity. However, inadequate scientific evidence plus the use of indigenous languages and epistemologies in CAMs limit their global acceptance.

Abbreviations: GAE, Gallic Acid Equivalent Tannin; SGF, Simulated Gastric Fluid; SIF, Simulated Intestinal Fluid; CAM, Complementary and Alternative Medicines.

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Ayurveda, an Indian traditional medicine system, prescribes several poly-herbal formulations for the treatment of diabetes and obesity. Studies have established the efficacy of some of these formulations, *viz*. *Chandraprabha vati*, *Arogyavardhini vati*, *Naga bhasma*, *Shilajatu*, and *Nishamalaki* using various *in vivo* models [8–13]. Though the descriptions of diabetes in Ayurveda are not in terms of modern parameters such as blood glucose, serum insulin and insulin resistance, a striking relationship between obesity (*Sthaulya*) and diabetes has been emphasized [14]. Diabetes mellitus can be correlated to *Madhumeha* (sweet urine), one of the 20 types of *Prameha* (a set of clinical disorders manifested with excess and turbid urination) described in Ayurveda [15]. *Sthaulya* (obesity), caused from unwholesome diet and sedentary lifestyle, is considered as one of the predisposing factors of *Madhumeha* as well as *Prameha*. The etiology and pathophysiology of obese-diabetes (*Sthula Prameha*) is clearly demarcated from lean diabetes (*Krusha Prameha*) [16]. As a result, Ayurvedic therapeutics have come out with specific formulations and methodologies to treat obese-diabetic patients. Generating scientific evidence and understanding their modes of action in contemporary scientific language would essentially help in expanding their global acceptance as convincing complementary strategies for managing the dual burden of obesity and diabetes.

The present study attempts to understand the possible mode of action of *Lodhrasavam*, a fermented polyherbal formulation in Ayurveda, predominantly prescribed for obese-diabetic patients [17]. *Lodhrasavam* is a complex formulation prepared from 29 plant drugs, wherein *Lodhra* (*Symplocos racemosa* Roxb.) is considered as the major ingredient (Supplementary data – 1) [18]. *Lodhra* is referred as a *medo-hara* (anti-obesity) plant and is the prime member of *Lodhradi-gana* plants, a group of plants having *medo-hara* and *kapha-hara* properties [19]. Besides the anti-diabetic and anti-obesity applications, *Lodhrasavam* is also indicated for various other diseases such as anemia, skin diseases, anorexia, hemorrhoids [18]. Owing to its broad spectrum of therapeutic benefits and the inherent properties of the herbal ingredients present, *Lodhrasavam* could be deemed to be an *Aushadha Rasayana* (a curative and wellness product) in the management of diabetes. The present study, for the first time, demonstrates the anti-diabetic and anti-obesity potentials of *Lodhrasavam* using *in vitro* experimental models.

Being an accepted oral drug for obese diabetes, the possibility of *Lodhrasavam* inhibiting the digestive enzymes *viz*. alpha-amylase (α-A) and alpha-glucosidase (α-G), to exert their anti-diabetic effect, is focused in this study. Digestive enzyme inhibition is considered as one of the important therapeutic strategies for controlling postprandial hyperglycemia in diabetic patients, particularly in high carbohydrate consuming populations like Indians [20,21]. Several digestive enzyme inhibitors are identified and reported from various medicinal plants [22,23]. The anti-adipogenic potential of *Lodhrasavam* has been studied using 3T3-L1 pre-adipocytes, and a well standardized cell-line model for adipogenesis and anti-obesity research *in vitro* is established [24]. The use of a static *in vitro* digestion model, to simulate the gastro-intestinal digestion, adopted in the present work facilitated a realistic study of the formulation in total, than studying the individual herbal ingredients in part.

2. Materials and methods

2.1. Chemicals and reagents

Lodhrasavam, prepared in accordance with the Ayurveda text *Ashtangahrudayam* [18], was purchased from authentic commercial sources. 3T3-L1 fibroblasts were purchased from National Centre for Cell Sciences, Pune, India. All cell culture reagents used in the study were purchased from Gibco-BRL. The enzymes α-amylase (source – *Aspergillus oryzae*) and α-glucosidase (source – *Saccharomyces cerevisiae*) and other fine chemicals were purchased from Sigma–Aldrich. All other routine laboratory chemicals used in various assays were of analytical grade and purchased from SD-Fine chemicals.

2.2. Cell culture and differentiation

3T3-L1 fibroblasts were cultured in Dulbecco's Modified Eagle's Medium (DMEM) containing 10% fetal bovine serum (FBS) and 1% penicillin-streptomycin in an atmosphere of 5% CO2 at 37 °C. Differentiation of 3T3-L1 fibroblasts was induced by incubating two days post-confluent (day-0) plates in differentiation induction medium containing 500 μM 3-isobutyl-1-methylxanthine (IBMX), 250 nM dexamethasone and 100 nM insulin (MDI). On day-3, the medium was replaced with fresh culture medium and was replaced every alternate day till the cells attained complete adipocyte morphology.

2.3. In vitro digestion of Lodhrasavam

In vitro digestion of *Lodhrasavam* was carried out following published protocols [25] with modifications to suit the samples (Fig. 1A). The electrolyte solutions of simulated gastric fluid (SGF) and simulated intestinal fluid (SIF) were prepared as shown in Fig. 1B. Oral phase of the digestion was omitted in the experiment as the starting material was in liquid form. For simulated gastric digestion, 40 mL of *Lodhrasavam* mixed with 250 mL of SGF containing 2500 U/mL pepsin and 0.16 mM $CaCl_2 \cdot 2H_2O$ (pH was adjusted to 2.0 by adding 6N HCl) was incubated at 37 °C for 2 h in a shaking water bath. Following gastric digestion, the gastric chyme mixed with 250 mL SIF containing pancreatin-bile solution (final concentration of 500 μg/mL pancreatin and 3 mg/mL bile) and 0.6 mM $CaCl_2 \cdot 2H_2O$ (pH adjusted to 7.0 by adding 5N NaOH) and incubated at 37 °C in shaking water bath for another 2 h to complete the digestion. The digest of *Lodhrasavam* (hereafter referred as LD) was filtered through cotton plug and the filtrate was collected and stored at −80 °C for further use.

2.4. Bioassay guided fractionation of Lodhrasavam digest

Fractionation of LD was done as illustrated in Fig. 1A. Initially, LD was successively extracted with hexane (H), chloroform (C) and ethyl acetate (EA) and the solvent was removed using a rotary evaporator at reduced temperature and pressure. The hexane extract of LD (LDH), obtained after sequential solvent extraction, was concentrated and fractionated by column chromatography. Briefly, the chromatographic column (18 × 300 mm size) packed with 25 gm silica in hexane was inoculated with 4 mL of LDH concentrate. Elution was started with 100% hexane and continued with 10% step-gradients of chloroform, ethyl acetate, methanol and water, till 100% water. The progress of separation was monitored by thin layer chromatography (TLC) and similar fractions were pooled together. Out of the 8 fractions obtained from the column, Fr-5 showed positive results in bioassays.

LD and all its subsequent fractions were estimated for total tannins following standard Folin - Ciocalteu method [26]. Briefly, 10 μL of digest/fraction mixed with 40 μL of water, 50 μL Folin's reagent and 100 μL of 3.5% Na_2CO_3 was incubated at room temperature for 30 min. A set of gallic acid standards (50, 25, 12.5, 6.25, 3.125 μg/mL) were prepared in the same manner. The absorbance was measured at 700 nm using plate reader (xMark Microplate Spectrophotometer, BioRad, USA). The experimental concentrations of test samples were expressed as 'μg of gallic acid equivalent tannin (GAE)/mL of sample'.

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