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EVALUATION OF ANTIDIABETIC ACTIVITY OF *WITHANIA COAGULANS* L. DUNAL

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Withania *coagulans* dunal, Diabetes mellitus, Hypoglycemia, Glucose uptake by yeast cells method

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ABSTRACT: The present study estimated the antidiabetic activity of methanolic extract of plant fruits of plant *Withania coagulans* L. Dunal. *Withania coagulans* (family Solanaceae) is commonly known as "Paneer ke phool" in Hindi and "Indian cheese maker" or "Vegetable rennet" in English, distributed in drier parts of India. The primary aim of this study was to confirm antidiabetic action of *Withania coagulans* by using the *in-vitro* method of glucose uptake by yeast cells using methanolic fruit extract of the sample. It is reported that the Withanolides are responsible for its anti-hyperglycemic activity, which has been standardized by the TLC method using 50% Sulphuric acid and dragendroff's reagents as a spraying agent in this study. The current research found that *Withania coagulans* methanolic fruit extract improved glucose absorption in yeast cells by 0–90% at various glucose concentrations (5, 10, and 25 mM). Metformin was used as a standard reference. At all glucose concentrations, the highest concentration of WCME (5 mg/ml WCME) demonstrated maximum activity and showed the greatest increase (81.83 percent) in the presence of 25 mM glucose. In conclusion, this report suggests that the increase of glucose transport across the cell membrane might be the anti-hyperglycaemic mechanism of action of *Withania coagulans* fruits.

INTRODUCTION: Traditional medicine is "the knowledge, skills, and practices based on the theories, beliefs, and experiences indigenous to different cultures, used in the maintenance of health and in the prevention, diagnosis, improvement or treatment of physical and mental illness. The vast use of Traditional medicine may be due to many reasons like high cost and long-time taken to develop the new drug, toxicity, and non-renewable source of basic raw materials.

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Also inadequate, especially in managing certain chronic diseases, renewable source, cultivation and processing environment friendly, plants constitute a major source of new lead generations [1]. Currently, herbs are applied to treat chronic and acute conditions and various ailments and problems such as cardiovascular disease, prostate problems, depression, inflammation, and to boost the immune system.

Nowadays, diabetes is a rapidly increasing prevalence, characterized by hyperglycemia, glycosuria, polyuria, polydipsia, polyphagia *etc*., resulting in profound socioeconomic effects in both developed and underdeveloped countries. It is a major public health issue affecting more than 400 million people worldwide [2]. Diabetes is a chronic

carbohydrate, fat, and protein metabolism disorder characterized by increased fasting and postprandial blood sugar levels [3]. DM is caused either by deficiency of insulin secretion, damage of pancreatic β cells, or insulin resistance related to the non-use of insulin. Inclination to a sedentary lifestyle may be the major reason for the global rise in diabetic patients, which is expected to strike 366 million in 2030 in the elderly population (>65 years) [4]. Diabetes mellitus is a complex metabolic disorder resulting from insulin insufficiency or dysfunction.

Diabetes is generally divided into Two Types:

Type 1 Diabetes Mellitus: Diabetes is an autoimmune condition in which the body's immune system destroys the insulin-producing cells in the pancreas, causing blood sugar and glucose levels to rise. Urination is frequent, thirst is intense, hunger is intense, and weight loss is rapid.

Type 2 Diabetes Mellitus: This usually happens as we age or gain weight. Even though the body produces insulin, its sensitivity is reduced to a minimum in this situation and does not respond. Fatigue, vision disturbance, and slow wound and cut healing are all signs close to Type 1.

The current treatments for diabetes mellitus include the use of insulin and synthetic drugs. These synthetic drugs are valuable but restricted by their side effects, such as hypoglycaemia, damage to the liver, lactic acidosis, diarrhoea, abdominal pain, weight loss and loss of appetite. Substances and extracts obtained from various natural resources play a critical role in developing medicine and treating diabetes mellitus as a hyperglycemic issue. Plant WC is magical and life-saving in and of itself, as it exhibits a wide range of medicinal properties including antidiabetic, antioxidant, hypolipidemic, hepato-protective, cardiovascular and other benefits. In this project, the investigation is done to analyze the characteristics of WC and test its antidiabetic effectiveness using *in-vitro* methods.

Withania coagulans Dunal (family Solanaceae), is reported in Ayurveda for its biological potential. It is commonly known as "Paneer ke phool" in Hindi and "Indian cheese maker" or "Vegetable rennet" in English, distributed in drier parts of India. This shrub is common in East India, Nepal, and Afghanistan; in India, it occurs in Punjab, Rajasthan, Simla, Kumaun and Garhwal [5]. The effective treatment of diabetes depends on active constituents of medicinal plants capable of controlling hyperglycemia and its secondary complications.

The natural active principles of *W. coagulans* contributing to anti-hyperglycemic activity were not determined. It was therefore, necessary to determine the active anti-hyperglycemic agent. It is reported that the withanolides responsible for its anti-hyperglycemic activity. In India two species of genus Withania, *Withania somnifera* and *Withania coagulans* are found [6]. Dry fruits of *Withania coagulans* Dunal (Family: *Solanaceae*) have shown antidiabetic potential. Its antidiabetic property is mentioned in ancient Ayurvedic literature, Charaka Samhita under Brihaniya Mahakashaya and Madhur skandha dravya.

FIG. 1: (A) *WITHANIA COAGULANS* (L.) DUNAL HABIT (B) FRUITS [8]

In northern India, traditional healers use dried fruits of *Withania coagulans* to treat diabetes and this plant is well-known for its ethnopharmacological applications. The aqueous extract of *Withania coagulans* Dunal dried fruits (WCDF) has been shown to possess an effective antidiabetic activity at a dose of 1 g/kg body weight in streptozotocin-induced diabetic rats without any discernible toxic

effect. Additionally, WCDF (*Withania coagulans* dunal fruit) aqueous extract (1 g/kg; per oral) has also been reported to have hypolipidemic activity in triton-induced hypercholesterolemia in albino rats [7].

Withania coagulans is enriched in withanolides, which are steroidal lactones. Withanolides are polyhydroxy C28 steroidal lactones that exist naturally. All withanolides have a six or five-membered lactone ring connected to an intact or rearranged ergostane skeleton in their basic structure [6]. The term "withanolide" is a structural term that has been used for "withan" from the genus Withania and "olide" is chemical term for a lactone.

Antimicrobial activity, anti-inflammatory activity, anti-tumour activity, hepatoprotective activity, anti-hyperglycemic activity, cardiovascular activity, immunosuppressive activity, free radical scavenging activity, and CNS depressant activity are all known properties of the plant. Fruit is used to treat wounds and is also used to treat asthma, biliousness and Stranjury. W. *coagulans* seeds are also diuretic and can help with piles inflammation.

Yeast Cell for Glucose Uptake Determination: The characteristics of the sugar-transport system in yeast have been receiving renewed attention in several laboratories [9]. Recent studies on the transport of non-metabolizable sugars [10] and certain metabolizable glycosides suggest that sugar transport across the yeast cell membrane is mediated by stereospecific membrane carriers [11].

The carrier hypothesis states that the cell membrane contains stereo-specific carriers that can diffuse in both directions and simultaneously in both combined and uncombined states. Three reactions are thought to be involved in the process: the substrate and carrier combining to create a carrier-substrate complex, the carrier-substrate complex diffusing across the cell membrane, and the carrier substrate complex dissociating into free substrate and carrier. The diffusion step is thought to be the rate-limiting response among these. The conformance of glucose uptake with the predictions of the carrier hypothesis adds strong support to the general significance of this mechanism in sugar uptake in yeast. The recent evidence that carrier reactions are also involved in the sugar efflux reactions in Escherichia coli [12] and in sugar transport in Tetrahymena supports the conclusion that this mechanism is as fundamental in microorganisms as in higher forms [9]. Therefore the basic aim of this study is to confirm antidiabetic action of *Withania coagulans* by using the in vitro method of glucose uptake by yeast cells using fruit extract of the sample.

MATERIALS AND METHODS:

Collection and Authentication of Plant Material: Plant materials of *Withania coagulans* were collected from the local market of Chopda, Tal – Chopda, Dist – Jalgaon. As many species have the same appearance, proper identification is needed by a botanist for further processing of plant samples. So, the herbarium of *Withania coagulans* was prepared and sent to Pune for authentication.

The plant was authenticated by Prof. D. L. Shirodkar, a botanist at Botanical Survey of India, Pune, (Maharashtra) Ref No: BSI/WRC/Iden. Cer./2022/1003220013248 and plant herbarium was deposited at Botanical Survey of India, Pune under voucher specimen no. NGBWC1.

Collection of Crude Drugs

Month of Collection: December:

Preparation of Crude Extract: The fruits of *Withania coagulans L. dunal* was collected and cleaned. It was dried under shade until the complete removal of moisture. The dried fruits were grinded into coarse powder using a pulveriser and passed through sieve no 80. This powder was stored in an air-tight container and used for extraction and further studies.

FIG. 2: POWDER OF DRIED FRUITS OF *WITHANIA COAGULANS*

Extraction of Withanolides from Fruits of *Withania Coagulans:* The plant material was ground into powder, and the extract was

prepared according to the methodology of Indian Pharmacopoeia (Anonymous, 1966) by cold maceration. 10 g of dried powdered *Withania coagulans* (Dunal) was taken in a 250ml conical flask and 100ml of methanol was added and kept at room temperature for 48 hrs. It was then filtered and subjected to distillation. The final extract was obtained and used for the following assay [13].

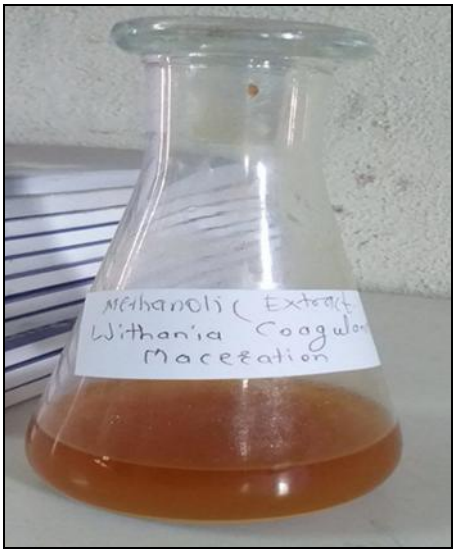

FIG. 3: EXTRACTION OF WCDFS BY PROCESS OF MACERATION USING SOLVENT ALCOHOL

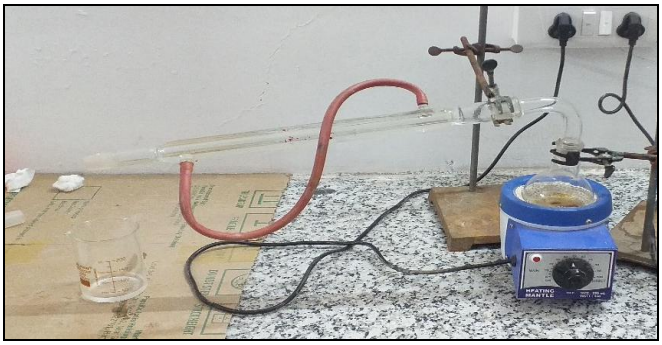

FIG. 4: DISTILLATION OF WC METHANOLIC EXTRACT

Standardization of Plant Extract and Its Antidiabetic Constituent Withanolide by Analytical Method:

Thin Layer Chromatography: The TLC was performed using the standard procedure on a 20×20 cm and 0.25 mm thick plate. A slurry of Silica Gel G was prepared in the ratio 1:2 (1 part Silica and 2 parts water). It was coated onto a clean grease free glass plate. The coat was approximately 0.25 mm thick. Plate was kept for air dry at RT for 10-20 minutes. Then plate was activated at 100-120 °C by placing in a hot air oven for 1hour. Meanwhile, the plates get activated; the mobile phase was prepared and kept for saturation covered in a TLC solvent chamber. After activation of the silica plate, methanolic extract was spotted onto the plate using a capillary. The chromatophore was placed in a saturated TLC solvent chamber and the chromatophore was allowed to develop. The solvent system used for Methanolic extract was Butanol: Water: Acetic Acid (7: 1: 2). The development was stopped shortly before the solvent front reached the top edge of the plate. The chromatophore was removed and allowed to dry for 10 minutes. The spots were observed under UV light.

Stationary Phase: Silica Gel G.

Mobile Phase: Butanol: Water: Acetic (7: 2: 1).

For the spot development, the chromatophores were sprayed with 2 separate freshly prepared detecting reagents: 50% Sulphuric acid in ethanol. Dragendroff `s reagent: and placed at 90 °C for charring for 15 minutes. The chromatophores were cooled and kept at room temperature for 24 hours for spot colour development. The Retention Factor (Rf) value for each spot was determined [14, 15].

***In-vitro* Pharmacological Evaluation of Withania *Coagulans* Fruit Extract for its Antidiabetic Activity as a Hypoglycemic Agents:**

***In-vitro* Evaluation of Glucose Uptake by Yeast Cells:**

Preparation of Yeast Cells: Commercial baker's yeast (Anheuser-Busch) was washed by repeated centrifugation in distilled water until the supernatant fluids were clear. The volume of the centrifuged yeast was determined after 5 min of centrifugation at 3,000 X g, and a 10% (v/v) suspension was prepared in distilled water. In most experiments, 2.0 ml of a 10% suspension were placed in 12-ml centrifuge tubes and centrifuged at 3,000 X g for 1 min. giving 0.2 ml of packed yeast per tube [9].

Sugar-uptake Procedure: Sugar-uptake experiments were begun by adding 5.0 ml of sugar solution to the packed yeast, which was then rapidly brought into suspension by stirring with two wooden applicator sticks. The tubes were shaken on an inclined platform in a temperature-controlled water bath. After the experimental period, the tubes were centrifuged at 3,000 X g for

1 to 2 min. The packed yeast was then washed by centrifugation with three 5-ml portions of 0.9% NaCl for 4 C. The cells were centrifuged for 1 min for each wash, the whole procedure taking about 5 to 6 min. Burger et al. (1959) showed that loss of intracellular sugar at 4 C is negligible. This was confirmed by the fact that intracellular sugar content determined in washed yeast and in unwashed yeast, corrected for extracellular space by using several nonpenetrating substances, gave the same results.

When non-metabolizable sugars were analyzed, the washed cells were resuspended in 5.0 ml of distilled water and placed in a boiling water bath for 20 min. If metabolizable sugars were being analyzed, the cells were washed with ice-cold NaCl containing 10-3 M iodoacetic acid (IAA); 1.0 ml of 95% ethanol was added to each tube immediately after the last wash while the cells were still at 4 C. In all cases, the extracts were separated from the cells by centrifugation, diluted appropriately, and assayed. Various extracts (1 – 5 mg/ml) were added to 1 ml of glucose solution (5, 10 and 25 mM) and further incubated for 10 min at 37 °C. 100 µl yeast suspension is added in the reaction mixture to start the reaction. After 60 min of incubation at 37° C, centrifugation is done at 3800 rpm for 5 min and the amount of glucose in the supernatant is measured at 520 nm by UV-Vis spectrophotometer (JASCOV-530, JAPAN). The effective concentration (EC50) is obtained from the percentage activity curve. The (%) increase in glucose uptake is calculated according to the following formula:

% Increase in glucose uptake = (Abs control – Abs sample) × 100 / Abs. of control

RESULT & DISCUSSION:

Standardization of Plant Extract and Its Antidiabetic Constituent Withanolide by Analytical Method: Thin layer chromatography of Methanolic Extract of *Withania coagulans* L. Dunal (fruit) performed for preliminary identification of Steroids. Two separate steroid detecting agents, 50 percent Sulphuric acid in ethanol and Dragendroff's reagent, were used to partially identify steroids. On each of the chromatographic plates, a separate spot was created. After spraying with 50 percent Sulphuric acid in ethanol and using the solvent system Butanol: Water: Acetic Acid (7: 1: 2), one spot was observed on the plate with an Rf value of 0.932. Similarly, following spraying with Dragendroff's reagent with an Rf value of 0.810 and using the same solvent solution, 1 spot was spotted on the plate.

TABLE 1: TLC OF *W. COAGULANS* FOR PRELIMINARY IDENTIFICATION OF STEROIDS

S. no.	Solvent system	Detecting reagents	D1 (cm)	D2 (cm)	Rf value
1	Butanol: Water: Acetic Acid (7: 1: 2)	50% Sulphuric acid in ethanol	12	12.9	0.932
		Dragendroff`s Reagent	9.1	11.1	0.810

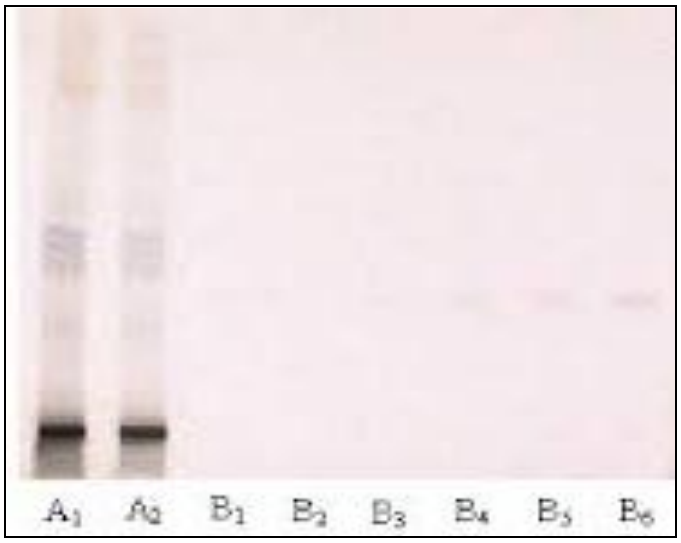

FIG. 5: IMAGE OF TLC PLATE AT 366NM AFTER DERIVATIZATION Note: A1, A2 represent methanolic extract, B1 to B6 represents standard Withaferin-A,

***In-vitro* Pharmacological Evaluation of Withania *Coagulans* Fruit Extract for its Antidiabetic Activity as a Hypoglycemic Agents:**

***In-vitro* Evaluation of Glucose Uptake by Yeast Cells:** The glucose absorption activity of samples (extract and metformin) by yeast cells is depicted in **Fig. 6**. **Fig. 6** depicts the rate of glucose transport across the cell membrane in yeast cells. The remaining glucose concentration in the medium is determined using this method. The percentage of glucose absorption was seen to increase with an increase in metformin concentration. No dose-dependent glucose uptake was found after the yeast cells were treated with the methanolic extract of *Withania coagulans*. In the presence of 1 mg/ml and 5 mg/ml of *W. coagulans* methanolic extract (WCME), the percentage of glucose uptake by yeast cells increased with increasing glucose concentrations (5, 10, and 25 mM), but not with 2, 3, or 4 mg/ml of WCME, with the exception that in the presence of 25 mM glucose, 62 percent glucose absorption was observed. At all glucose concentrations, the highest concentration of WCME (5 mg/ml WCME) demonstrated maximum activity and showed the greatest increase (81.83 percent) in 25 mM glucose.

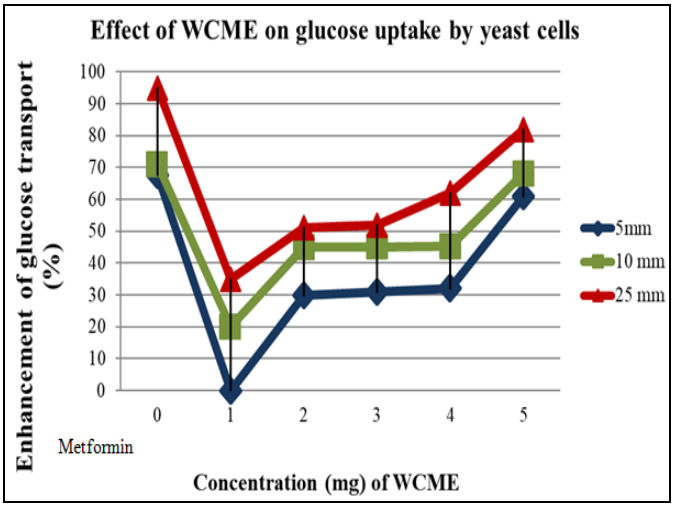

FIG. 6: THE EFFECT OF *WITHANIA COAGULANS* METHANOLIC EXTRACT ON GLUCOSE UPTAKE BY THE YEAST CELL AT DIFFERENT GLUCOSE CONCENTRATIONS

The Percentage of Glucose Uptake by the Yeast Cells was measured in the Presence of Various Concentrations of WCME and Glucose:

WCME: Withania *Coagulans* Methanolic Extract: The current data suggest that the plant extract is capable of effectively enhancing glucose uptake, which implies that it is capable of effectively enhancing effective glucose utilization at 5 mg/ml concentration, thereby controlling blood glucose level, as suggested by other reports. The percentage of glucose uptake increased with increasing metformin concentration. The increased ability of the samples to adsorb glucose in the current study may also be attributed to the dietary fiber (insoluble and soluble fibers) present in the sample. One of mammalian species' most important and tightly regulated processes is the long-term maintenance of plasma glucose concentration under various dietary conditions. The beneficial effects of plant fibres on blood glucose control have been established. *Withania coagulans* has been shown to improve glucose diffusion in neighbouring cells through sodium-dependent glucose absorption.

CONCLUSION: The antidiabetic properties of plants can be evaluated *in-vitro* by several methods such as the study of glucose uptake, effect on glycosylation of the haemoglobin, and inhibition of alpha-glucosidase and alpha-amylase enzymes. The glucose transport mechanism across the yeast cell membrane has been gaining significant importance as an in vitro screening method for evaluating the hypoglycaemic effects of various medicinal plants. The above *in-vitro* studies depict an appreciable increase in the glucose uptake by the yeast cells in combination with the plant extracts. In conclusion, this report suggests that the increase of glucose transport across the cell membrane might be the anti-hyperglycaemic mechanism of action of *Withania coagulans* fruits. The plant extract of *Withania coagulans* under study can serve as a therapeutic agent and can be used as a potential source of novel bioactive compounds for treating type 2 diabetes mellitus. Further *ex-vivo* and *in-vivo* investigations should be done to confirm the antidiabetic activity of these plants. Hence, it can be concluded that this experiment fulfilled the objective.

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CONFLICTS OF INTEREST: Authors have no conflicts of interest to declare.

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