WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 8.084
Volume 12, Issue 5, 1455-1465. Research Article ISSN 2277– 7105

IN VITRO ANTIDIABETIC ACTIVITY OF ETHANOLIC LEAF EXTRACT OF *HELIOTROPIUM INDICUM* BY ALPHA AMYLASE ENZYME INHIBITION METHOD

***Kathiravan M., Prathap B., Mohamed Halith S., Saran Kumar R., Sameeha Banu B., Selvamani S., Sathishkumar P. and Senthamilvalavan P.**

Department of Pharmaceutical Chemistry, Dhanalakshmi Srinivasan College of Pharmacy, Perambalur-621212.

Article Received on
09 February 2023,

Revised on 02 March 2023,
Accepted on 23 March 2023

DOI: 10.20959/wjpr20235-27600

***Corresponding Author**
Kathiravan M.
Department of Pharmaceutical Chemistry, Dhanalakshmi Srinivasan College of Pharmacy, Perambalur-621212.

ABSTRACT

Medicinal Plants play a key role cure many diseases from immemorial. The usage of plants in traditional medicinal system is the vital process of india.[1] Diabetes mellitus (DM) is a group of metabolic disorders characterized by a chronic hyperglycemic condition resulting from defects in insulin secretion, insulin action or both. According to a report from World Health Organization, about 220 million people have type 2 DM. Its incidence is increasing rapidly, and it is expected to increase to more than 365 million by 2030. From this work carried out by the *in-vitro* antidiabetic activity of ethanolic leaf extract of *Heliotropium indicum* and Acarbose act as standard by inhibition of Alpha amylase enzyme method. It has promoting antidiabetic activity by inhibition of Alpha amylase enzyme method. It has to be further evaluvated to produce a safe herbal formulation for the treatment of diabetes mellitus.

KEYWORDS: Diabetes mellitus, Ethanolic extract, Anti-diabetic activity, *Heliotropium indicum.*

1. INTRODUCTION

Diabetes mellitus is caused by the abnormality of carbohydrate metabolism which is linked to low blood insulin level or insensitivity of target organs to insulin. Despite considerable progress in the treatment of diabetes by oral hypoglycemic agents, search for newer drugs continues because the existing synthetic drugs have several limitations. The herbal drugs with anti-diabetic activity are yet to be commercially formulated as modern medicines, even

though they have been acclaimed for their therapeutic properties in the traditional systems of medicine.

Type 2 Diabetes usually occurs in obese individuals and is associated with hypertension and dyslipidemia. Thus the treatment aims to reduce insulin resistance and to stimulate insulin secretion. Traditional medicine (herbal) is used for treatment of diabetes in developing countries where the cost of conventional medicines is a burden to the population. Despite the introduction of hypoglycemic agents from natural and synthetic sources, diabetes and its secondary complications continue to be a major medical problem. Many indigenous Indian medicinal plants have been found to be useful to successfully manage diabetes. One of the great advantages of medicinal plants is that these are readily available and have very low side effects. Plants have always been an exemplary source of drugs and many of the currently available drugs have been derived directly or indirectly from them.[2]

It has an adverse effect on carbohydrate, lipid and protein metabolism resulting in chronic hyperglycemia and abnormality of lipid profile. These lead to series of secondary complications including polyurea, polyphagia, ketosis, retinopathy as well as cardiovascular disorder. The management of diabetes is a global problem until now and successful treatment is not yet discovered. In the last few years, there has been a growing interest in the herbal medicine in care and management of diabetes both in developing and developed countries, due to their natural origin.[3]

Herbal medicines are generally considered to be safe and effective agents. Therefore, people every year turn to herbal medicine because they believe plant remedies are free from undesirable side effects. Approximately 8% of all hospital admissions in the United State are due to adverse reactions to synthetic drugs. At least 100,000 people a year die from these toxicities.[4]

Etiology

1. Autoimmune reaction leads to destruction of beta cells.
2. Heredity.
3. Viral infection affecting the pancreas.
4. Secondary to drugs and stress.
5. Obesity causes insulin resistance.

Pathophysiology

Type 1: diabetes is characterized by destruction of the pancreatic beta cells mostlikely cause of these conditions is combined genetic, immunological and possibly environmental (e.g. viral) factors contribute to cell destruction. This is normal response of the body in which the bodies are direct against the normal tissue as if they were foreign and eventually can damage islet of Langerhans, specific area of the pancreas that produce insulin, reducing the production of insulin or totally no production of insulin.

Type 2: diabetes mellitus is a onset, and non-insulin depended .there are two main problems related to insulin in type 2, first one is insulin resistance and repaired (insulin secreting glands release irregular amount of insulin).

Complications

- Skin infections
- Arthrosclerosis
- Diabetic retinopathy
- Heart damage
- Permanent kidney damage
- Fatty liver
- Osteoporosis

Causes

- Obesity/overweight (especially excess visceral adiposity.
- Excess glucocorticoids (cushing's syndrome or steroid therapy)
- Excess growth hormone (acromegaly)
- Pregnancy, gestational diabetes
- Polycystic ovary disease
- Lipodystrophy (acquired or genetic, associated with lipid accumulation in liver)
- Autoantibodies to the insulin receptor
- Mutations of insulin receptor
- Mutations of the peroxisome proliferators' activator receptor γ (PPAR γ)
- Mutations that cause genetic obesity (e.g., melanocortin receptor mutations)
- Hemochromatosis (a hereditary disease that causes tissue iron accumulation)

Symptoms

Type 1

- Polydipsia
- Polyurea
- Hyperphagia
- Fatigue
- Blurred vision

Type 2

- Weight gain or Weight loss
- Poor wound healing
- Fatigue
- Blurred vision
- Frequent infections

Diagnosis

Early screening and diagnosis allow for the identification of at-risk persons (so that preventive measures, primarily life- style changes, may be undertaken) and those with early disease (so that treatment can be initiated). The diagnostic cutoff point for diabetes is a fasting plasma glucose level of 126 mg per deciliter (7.0 mmol per liter) or more or a glycated hemoglobin level of 6.5% or more; the diagnosis requires confirmation by the same or the other test. A fasting glucose level of 100 to 125 mg per deciliter (5.6 to 6.9 mmol per liter) is consistent with prediabetes; the range of glycated hemoglobin levels that are diagnostic of prediabetes is controversial, but the American Diabetes Association recommends a range of 5.7 to 6.4%.

Laboratory Examination

- Fasting blood glucose (FBG)
- Random blood glucose
- Glucose tolerance test (OGTT)
- Glycosylated hemoglobin (HbA1C)
- Glycosuria
- Ketonuria

Management

The Major Components of The Treatment of Diabetes Are

A. Diet and exercise

B. Oral hypo glycemic therapy

C. Insulin therapy

Diet

Diet is a basic part of management of every case. Treatment cannot be effective unless adequate attention is given to ensuring appropriate nutrition. Dietary treatment should aim at.

- Ensuring the control
- Provide nutritional requirements
- Allowing good glycemic control with blood glucose levels asclose to normal as possible
- Correcting any associated blood lipid abnormalities

Exercise

Physical activity promotes weight reduction and improves insulin sensitivity, thus lowering blood glucose levels, people should be educated about the potential risk of hypoglycemia and how to avoid it.

TREATMENT

Medication

- Insulin
- Non insulin

2. MATERIALS AND METHODS

Fig 1: Enitre plant of *Heliotropium indicum.*

Fig 2: Leaf of *Heliotropium indicum.*

Plant Taxonomy

Domain : Eukarytoa
Kingdom : Plantae
Phylum : Spermatophyta
Subphylum : Angiospermae
Class : Dicotylendonae
Order : Boraginales
Family : Boraginaceae
Genus : *Heliotropium*
Species : *Heliotropium indicum* L.[5]

Collection

The plant Heliotropium indicum was collected from Viruddhachalam, Tamil Nadu, India during the month of November - December.

Authentication

The collected plant was authenticated by Dr. S. Mutheeswaran M Sc., M.phil.M, Ph.D, Xavier Research Foundation St Xavier's college, Palayamkottai, Tamil Nadu and herbarium vouche specimen was produced in our department of pharmacognosy, Dhanalakshmi Srinivasan College Of Pharmacy, Permabalur for future reference.

Preparation of extract

The plant was collected from Viruddhachalam, India in the month of November. The collected the plant material were dried in the shade for one month. Then, the shade-dried plant material was powdered to get a coarse powder and was subjected to hot continuous percolation with the help Soxhlet apparatus by using different solvent, according to the polarity.

About 500gm of powder was extracted with 2 liters of solvent (Chloroform, Ether and Ethanol 70% v/v) by continuous hot percolation method successively at 60°C- 80°C.Aqueous extract was prepared by cold maceration method (0.25% v/v of CHCl3 in H2O).

Each plant extract was concentrated by distilling off the solvent and then evaporated to dryness on a boiling water bath. The extract obtained with each solvent was weighed and its

percentage was calculated. The color and consistency of the extract was noted and stored in adesiccators.

Preliminary Phytochemical Screening

The therapeutic potentials of plant and animal origin are being used from the ancient times in the form of crude drugs or galenicals prepared from them without the isolation of the pure compounds. The pharmacological action of crude drug is determined by the nature of its phytoconstituents. To obtain these pharmacological effects, the plant materials are used as such in their crude form or they were extracted with suitable solvents to take out the desired components and the resulting principles employed as therapeutic agents. All the extracts were subjected to preliminary phytochemical.

Plant material and Extraction process

The mature and succulent leaves of Heliotropium indicum were collected. The collected leaves were brought to the laboratory, washed with distilled water to remove dust and other contaminants. The clean leaves were air dried for 5-6 days at room temperature (28 ± 2ºC) until all the moisture content was evaporated and dried leaves were pulverized using domestic grinder. The powder of Heliotropium indicum was extracted with Ethanol for 24 h by soxhlet procedure**,** which was repeated for 3 times to ensure the complete extraction of chemical constituents from the leaves. The extract was filtered through Whatman (No. 1) filter paper and concentrated by a rotatory evaporator under low pressure. Dark-green residue obtained was stored in glass vials and kept in a refrigerator (4ºC) until use.[6]

Determination of Antidiabetic Activity

Procedure

The α- amylase inhibitory activity was assessed by the method described by Dong et al., 2012, with suitable modification. Briefly, (500, 250, 100, 50 and 10 µg/ml) of the eucalyptus test sample (A) was remixed with 200 µl of α- amylase solution (1.0 U/ml in phosphate buffer pH 6.9), and incubated at 25oC for 30 min. After pre-incubation, 400 µl of 0.25 % starch solution in the phosphate buffer (pH 6.9) was added to each tube to start the reaction. The reaction was carried out at 37oc for 5 min and terminated by the addition of 1.0 ml of the DNS reagent (1%3,5-dinitrosalicylic acid and 12% sodium potassium tartrate in 0.4 M NaOH). The test tubes were then kept over a boiling water bath for 10 min and cooled to room temperature. The reaction mixture was then diluted by making up the volume to 10 ml of distilled water and absorbent (A) was measured at 540 NM. Control incubations

representing 100% enzyme activity were conducted in a similar way by replacing extracts with buffers. For blank incubation (to allow for absorbance produced by the extracts), enzyme solution was replaced by buffers solution and absorbance recorded. The α- amylase inhibitory activity was expressed as percent inhibition and was calculated as follows.

$$\% \text{ Inhibition} = \frac{\text{A control} - (\text{A test} - \text{A background})}{\text{Control}} \times 100$$

Where a control, a test, a background represented the absorbance of 100% enzyme activity, test sample with the enzyme and test sample without the enzyme, respectively.[6]

3. RESULTS AND DISCUSSION

Preliminary phytochemical screening.

Table 1: Preliminary phytochemical screening of heliotropium indicum.

Phytoconstituents	Ethanolic extract
Alkoloids	+
Anthraquinones	-
Steroids	-
Cardiac glycosides	-
Saponins	+
Tanins	+
Flavonoids	+
Protein	-
Amino acids	-
Carbohydrates	+
Terpinoids	-
Quinones	-
Saponins	+
Glycosides	+

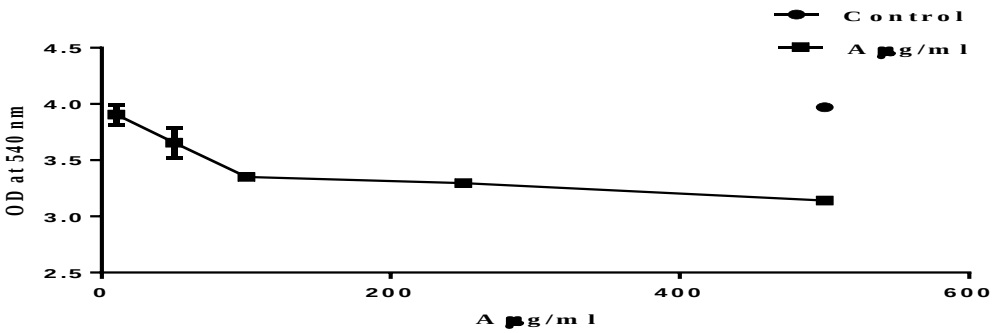
(+) = Presence of phytoconstituents

(-) = Absence of phytoconstituents

A) OD Value at 540 nm

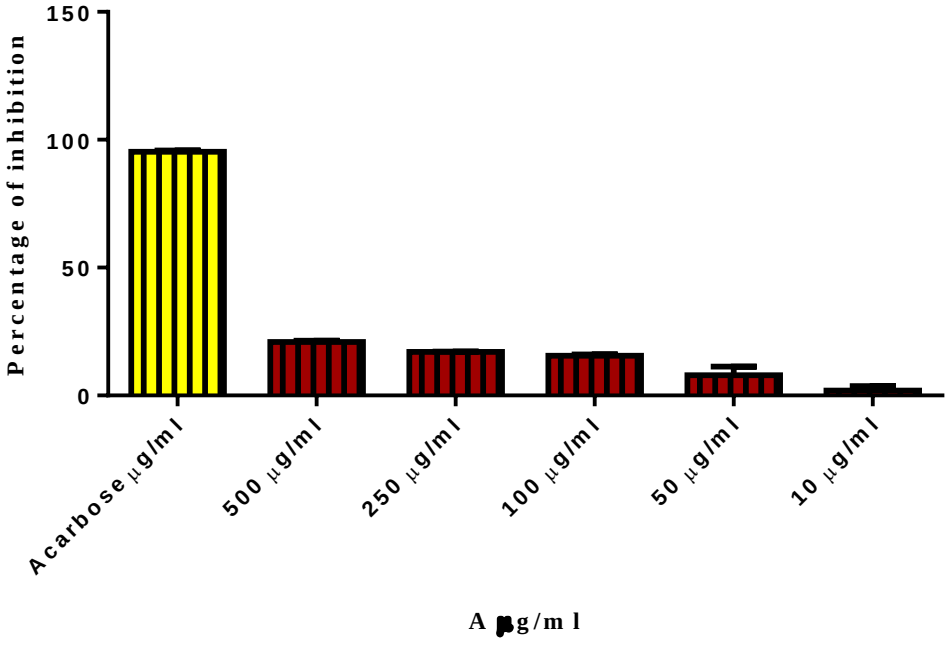
S.No	Tested sample concentration (µg/ml)	OD Value at 540 nm		
1.	Control	3.991	3.981	3.941
2.	500 µg/ml	3.135	3.126	3.163
3.	250 µg/ml	3.294	3.289	3.304
4.	100 µg/ml	3.366	3.333	3.353
5.	50 µg/ml	3.577	3.580	3.810
6.	10 µg/ml	3.827	3.889	4.000
7.	Acarbose	0.165	0.187	0.210

OD value:3.971



B) Percentage of inhibition

S. No	Tested sample concentration (µg/ml)	Percentage of inhibition (in triplicates)			Mean value (%)
1.	Acarbose	95.84488	95.29086	94.71166	95.28246
2.	500 µg/ml	21.05263	21.27927	20.34752	20.89314
3.	250 µg/ml	17.0486	17.17452	16.79678	17.00663
4.	100 µg/ml	15.23546	16.06648	15.56283	15.62159
5.	50 µg/ml	9.921934	9.846386	4.054394	7.940905
6.	10 µg/ml	3.626291	2.064971	0	1.897087



C) IC50 Value of tested sample: 69.73 µg/ml

log(inhibitor) vs. normalized response -- Variable slope		
Best-fit values		
LogIC50		1.843
HillSlope		-1.767
IC50		69.73
Std. Error		
LogIC50		0.04577
HillSlope		0.3541
95% Confidence Intervals		
LogIC50		1.745 to 1.942
HillSlope		-2.532 to -1.002
IC50		55.53 to 87.55
Goodness of Fit		
Degrees of Freedom		13
R square		0.9268
Absolute Sum of Squares		1483
Sy.x		10.68
Number of points		
Analyzed	3	15

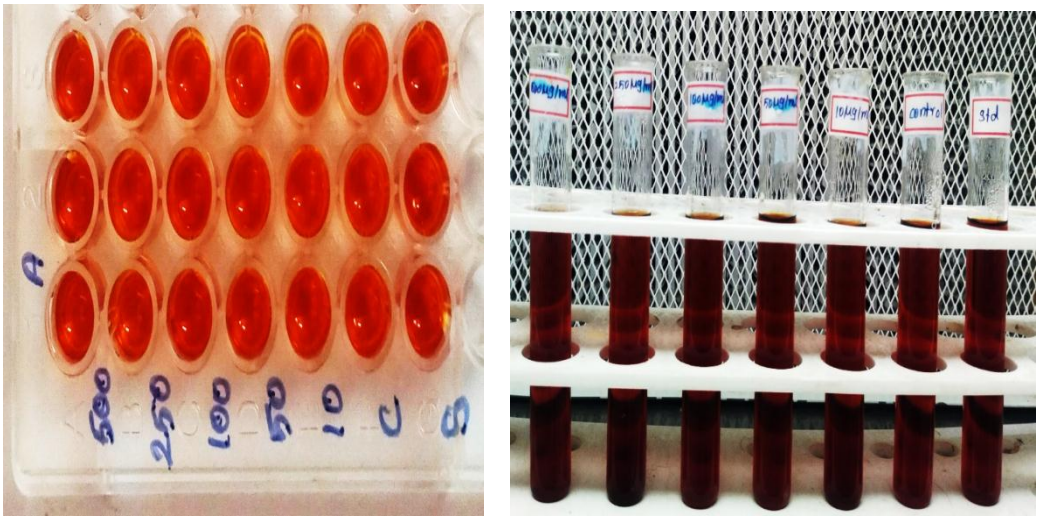


FIG 3: Samples.

4. CONCLUSION

The present investication posses the *invitro* anti-diabetic activity of ethanolic leaf extract and determined IC50 Values and percentage of enzyme inhibition using various concentration of ethanolic leaf extract of *Heleiotriopium indicum* and as well as Acarbose. It can be concluded that the isolated leaves of *Heleiotriopium indicum* has promoting anti-diabetic activity inhibit alpha amylase enzyme. It has further evaluated to produce a safe herbal formulation for treatment of diabetis mellitus.

5. REFERENCES

1. DN Sangeetha and Rajamani, Invitro anti-diabetic activity of methanolic extract of Indianthus virgatus By Glucose uptake method. Pharmacognosy journal, 2019; 14: 674-677.
2. G ARUMUGAM et al. journal of acute disease of diabetes mellitus, 2013; 196-200.
3. ANM mamunr or Rasid et al, journal of pharmacognosy and phytochemistry, 2014; 149-159.
4. Hamid nasri et all, journal of herbmed pharmacology Toxicity and safety of medicine Pranabesh Ghosh et all, Morphological characteristics and phyto-pharmacological detailing of hatishur (Heliotropium indicum Linn.)
5. Muthusamy et all, influence of incubation temperatures on total phenolic, and free radical scaverging activity of callus from heliotropium indicum L.
6. Pranabesh Ghosh et all, Morphological characteristics and phyto- pharmacological detailing of hatishur (Heliotropium indicum Linn).