Section A-Research paper

ISOLATION, PURIFICATION AND ASSESSMENT OF ACTIVE COMPOUND MOLECULES AS POTENTIAL DRUGS TO CURE TYPE II DIABETES MELLITUS

Prabas Nandan Rout[1], Dr. Prasanna Purohit[2]

DR.A.P.J. ABDUL KALAM UNIVERSITY INDORE M.P.

ABSTRACT

Since the conventional treatments for diabetes mellitus have their own limitations, people are looking for alternatives from medicinal plants with antihyperglycemic activity to meet the demand. Both traditional Ayurvedic medicine and modern Western research have praised Tinospora cordifolia for its ability to reduce blood sugar levels. Its extracts (such as immune-modulatory, anti-hyperglycemic, antioxidant, adaptogenic, hepatoprotective, hormone-regulating, etc.) and isolated phytoconstituents (such as tinosporin, berberine, jatrorrhizine, etc.) have been shown to have preventative and therapeutic antidiabetic properties. The supposed anti-diabetic effects of tinospora have received conflicting reports. This research accomplishes two goals: (i) it evaluates the plant's claimed antidiabetic qualities in light of previous experimental and clinical studies, and (ii) it sheds light on how the plant could work to address the many underlying causes of diabetes.

Keywords : ethanol extract, inhibitory activity, in vitro anti-diabetic activity, Tinospora cordifolia's

1. INTRODUCTION

Chronically high blood sugar levels are a hallmark of a diverse group of metabolic diseases collectively known as "diabetes mellitus" (DM). The most prevalent reasons are changes in either insulin secretion or insulin action, or both. Most people with diabetes have either Type 1 or Type 2 diabetes. Type 1 diabetics (T1Ds) have no secretion of insulin at all. People with type 1 diabetes have an autoimmune or idiopathic destruction of their pancreatic beta-cells. Non-insulin-dependent diabetes, of which 90% to 95% of all cases are attributable to type 2 diabetes mellitus (T2DM). Type 2 diabetes is characterized by insulin resistance and inadequate insulin secretion compensatory. [1] People with T2DM may have high or normal insulin levels. However, inadequate insulin production leads to insulin resistance in those with higher blood glucose levels. There are several potential contributors to type 2 diabetes. There are, first and foremost, aspects that are passed down from one's parents. Higher rates of concordance between sets of identical twins and between blood relatives provide support to

the idea that T2DM is inherited, as do susceptibility areas revealed via genome-wide association studies.

The gut microbiota has a role in type 2 diabetes as well. The bacteria in our intestines play a crucial role in determining how we react to and engage with the world. The presence of a more hostile gut environment and a moderate amount of microbial dysbiosis are both linked to the development of type 2 diabetes mellitus (T2DM). Both genetic and environmental factors have a role in the development of type 2 diabetes. Obesity, a sedentary lifestyle, physical inactivity, a high-glycemic, low-fiber diet, a deficiency in vitamins, smoking, and alcohol use are all risk factors for type 2 diabetes. Numerous complications have been linked to type 2 diabetes mellitus. The life-threatening effects of chronic hyperglycemia are a hallmark of type 2 diabetes. High blood glucose levels in the circulation over an extended period of time might damage blood vessels. Microvascular complications include retinal damage, kidney damage, and nerve damage. [3] Macrovascular problems include heart disease like myocardial infarction and brain disease like strokes. Diabetic gastroenteropathy is common in people who have had diabetes for a long time. Side effects include heartburn, stomach discomfort, nausea, vomiting, bowel problems (such as constipation, diarrhea, and faecal incontinence), and more. Complications from diabetes raise serious issues, including decreased quality of life, poor prognosis, and increased mortality.

In early type 2 diabetes, the pancreas may produce insulin despite high blood sugar. Insulin production is insufficient due to body resistance. Insulin production declines as the condition advances. Childhood type 2 diabetes is becoming common. It begins in people over 30 and becomes more common with time. 25% of 65-year-olds have type 2 diabetes. African American, Asian American, American Indian, and Spanish or Latino Americans are twice as likely to acquire type 2 diabetes. Type 2 diabetes is inherited like type 1. [2]

Older Indians are far more likely to get diabetes. Asian Indians have the highest insulin levels for a particular BMI. Indians' higher body fat may cause insulin resistance. Asian Indians are predisposed to hyperinsulinemia and type 2 diabetes due to excess body fat, a particular abdominal deposition pattern, poor muscle mass, and racial susceptibility. Hyperglycemia is the main symptom of diabetes. Untreated diabetes may cause retinopathy, neuropathy, and mortality.[4]

2. MATERIALS AND METHODS

2.1 Plant Material

In the Western Ghats of Kamataka, the leaves, stems, and roots of T. cordifolia were extracted, detected, and analysed qualitatively.

2.2 Procurement and extraction of raw materials.

Various parts of the T. cordifolia plant, including the leaves, stem, and roots, were gathered from the Western Ghats of Kamataka, near the Kuvempu University campus. Kuvempu University herbaria's herbarium voucher specimen was used to verify the authenticity of this plant. It was shade dried, manually pulverised (sieve No. 10/44), and stored in airtight containers for both species' plant materials.

2.3 Prepare crude extracts

Both facilities' powdered components went through several rounds of solvent extraction. Each batch of 500 g of powdered T. cordifolia leaves, stems, and roots was put into its own one-liter capacity Soxhlet apparatus thimble and then heated for 48 hours in a mixture of Hexane (B.P.69°C), Ethanol (B.P.79°C), and Petroleum ether (B.P.60-80°C), followed by Chloroform (B.P.60-62°C). Before using a new solvent to extract the marc, all of the previous solvent had to be removed. It took 30 minutes to boil 1 kg of T. cordifolia pulverised stem, leaf, and roots in distilled water, followed by 3 days of intermittent shaking, before the aqueous extract was filtered out. All of the aqueous extracts were filtered and vacuum-concentrated with a rotary flash evaporator before being analysed for biological activity. The remaining solvent was thoroughly removed using a water bath, and then the desiccator was used to dry it all out. It was necessary to label, weigh, and record the yield % of the crude extracts obtained from each of the solvents.

2.4 Petroleum Ether Extracts of T. Cordifolia Leaf and Stem

In 10 ml of petroleum ether, we dissolved 5.0 grammes of petroleum ether extract from the leaves and adsorbed it to silica gel (2g). In an 8:2 ratio, petroleum ether and chloroform were used to chromatograph the petroleum ether extract on a silica gel (60-120, Merck, Darmstadt, Germany) column. To monitor TLC (Silica Gel G, visualisation; Iodine chamber or UV chamber or vanillin-sulphuric acid, heated at llOC), the eluted fractions were collected at 5-ml intervals. Using petroleum ether to crystallise compound I, the elute was collected, concentrated, and then re-crystallized to produce compound II. lOOmg of silica gel was added to the stem extract residual (3,0g), which was then dissolved in 10ml of pefroleum ether. On a 75 x 2.5 cm silica gel 60 packed column, the extract was separated (70-230 mesh, Merck, Darmstadt, Germany). Eluted fractions were collected and profiled by TLC (Silica Gel G, visualisation; Iodine chamber or UV chamber or vaniUin-sulphuric acid heated to llOC). Fraction samples were obtained in 5-ml portions. We blended, condensed, and labelled Fractions With Similar Characteristics.

2.5 T. Cordifolia Leaves and Stem Extract by Methanolic Technique

Leaves were soaked in methanol for five grammes and then rinsed with acetone before the methanol settled. Using acetone as a solvent, the insoluble fraction was resuspended and washed three times with new acetone before being separated. This process took about two

hours to complete. Using a silica gel column and chloroform/acetone (6:4) as solvents, the acetone soluble fraction was chromatographed. Five-milliliter samples of the eluted fractions were collected and thin-layer chromatography was used to keep track of the results. A white substance was synthesised via acetone recrystallization of the higher concentration fraction. Chromatography on Silica gel with methanol/water separated the dark brown residue (lOg) from the stem methanol extract into a main portion (9.5:0.5). TLC profiling of the elution revealed a single spot. After then, HPLC analysis of the sample revealed only one peak. Cold methanol was used to wash and filter the obtained chemical. This chemical had a label on it.

2.6 T. Ethanolic Cordifolia Leaf and Root Extract

T. H. cordifolia and Indicus dried leaves and roots were blended into a smooth paste in ethanol.We used cotton fabric to filter the ethanol extract, then spun it at 10,000 g for 30 minutes. After evaporation, the concentrated supernatant was washed with hot water to remove any remaining contaminants. The ethylacetate fraction was concentrated by evaporation after the hot water fraction was partitioned with ethyl acetate. Ethanol was added to the dried ethylacetate fraction and the supernatant was collected to create a new ethylacetate fraction. Column chromatography was used to further separate the ethylacetate component. It took some time, but eventually a precipitate formed in the ethanol's ethylacetate fraction. After several washings and dissolves in DMSO, the precipitate reformed. Characterization necessitates identifying the molecule's molecular framework, determining the type of functional group it contains, locating it within the skeleton, and establishing any possible stereochemical relationships. Compound characterisation has been transformed thanks to the increasing use of spectroscopic techniques of all kinds. These have been widely used to confirm the structure of the anticipated compounds in the preparative section. To condense the structure of newly isolated molecules, this method was used extensively in the pre-processing portion.

2.7 Experimental Diabetes

The experiments were carried out on rats weighing between 150 and 200 grammes. Three groups of six rats each were formed from the rats. One group was kept as a healthy control, while the others received intraperitoneal administration of streptozotocin at a dose of 100 milligrammes per kilogramme of body weight. Rats were fasted overnight before induction (streptozotocin was prepared freshly in 0.5 mL of sterile saline just prior to injection).

Medicine

The powder was dissolved in 100 ml of distilled water and heated for an hour with 5 gramme of it in there. 70 cc of the filtrate was collected after the solution had been filtered. The collected supernatant was kept at a temperature of 4°C. Every week, it was made from scratch. Tinospora cordifolia stem extract was administered to the test animals twice daily, in the morning and evening, at a dose of 0.5 ml. One hundred milligrammes of dry stem powder per rat per day is about the same as this dosage. TC treated rats are the name given to these particular rodents. An OGTT was performed as were measures of liver glycogen and

histology as well as blood glucose and urine glucose. A pathology lab used glycosylated haemoglobin and mean blood glucose levels to make their findings.

Glucose uptake research

During a 24-hour fast, rats were slaughtered, dissected, and their diaphragms were quickly removed and placed in a cold tyrode solution for use in the study. The diaphragms were carefully dissected out without causing any damage to the surrounding tissue. The diaphragm's fat tissues were removed during surgery. The assay made use of a diaphragm as soon as practicable. To obtain a hemi diaphragm, cut the tissue in half. The weight of the hemi diaphragm was tallied and employed in the subsequent process. Three milligrammes of glucose and three millilitres of tyrode were added to the reaction mixture, then a known weight of diaphragm was added. The reaction mixture in question here is the one being used as a check.

Tyrode solution was used to make up to 3 ml of a solution containing the chemicals of interest in a known quantity for the test. After a 30-minute incubation at 37° C, the glucose concentration was determined. The glucose concentration absorption by the tissue is determined by the difference between the glucose concentrations before and after incubation.

High-density lipoprotein (HDL) cholesterol

The heart of the rat was used to gather blood samples after it had been sacrificed. The sample was left to clot for a period of time, and then serum was drawn. HDL-cholesterol was measured with 200 pL of serum and total cholesterol with 25 pL. With the Qualigens kit, we were able to determine HDL-C and total cholesterol levels. There were no significant differences between the cholesterol levels in the two groups when the samples were heated in a boiling water bath for 10 minutes to develop colour and read at 530 nm. The cholesterol levels in the two groups were the same when the samples were heated in boiling water baths for 10 minutes. The precipitating reagent was used to precipitate VLDL and LDL before the sample was subjected to HDL-cholesterol measurement.

Hypoglycemic Action

One millilitre of the medication was administered to overnight-fasted rats. A glucometer was used to monitor blood glucose levels before and after the delivery of a single dose, and the results were plotted against the passage of time.

3. RESULTS AND ANALYSIS

Table 1. Phytochemical evaluation of bioactive compounds of T. cordifolia Leaf

Test	T.cordifolia Leaf		
	Pet.Ether extract	**Ethanol extract**	**Aqueous extract**
Steroids	+	+	+

Alkaloids	+	+	+
Flavonoids	+	+	+
Glycosides	+	+	+
Reducing sugars	+	+	+

'+' – Present '-' - Absent

Table 2 Phytochemical evaluation of bioactive compounds of T. cordifolia stem

Test	**T.cordifolia stem**		
	Pet. Ether extract	**Ethanol extract**	**Aqueous extract**
Steroids	+	+	+
Alkaloids	+	+	+
Flavonoids	+	+	+
Glycosides	+	+	+
Reducing sugars	+	+	-

'+' – Present '-' - Absent

Table 3 Phytochemical evaluation of bioactive compounds of T. cordifolia Root

Test	**T.cordifolia Root**		
	Pet.Ether extract	**Ethanol extract**	**Aqueous extract**
Steroids	-	+	-
Alkaloids	+	+	+
Flavonoids	+	+	+
Glycosides	+	+	+
Reducing sugars	-	+	+

'+' – Present, '-' - Absent

Table 4 Qualitative analysis of T. cordifolia Leaf

Test	T. cordifolia Leaf		
	Pet.Ether extract	**Ethanol extract**	**Aqueous extract**
Carbohydrates	-	+	+
Proteins	-	+	+
Tannins	-	+	+
Saponins	+	+	+
Triterpenoids	+	+	+
Flavonoids	+	+	+
Quinones	-	-	-
Sterols	+	-	-
Glycosides	+	+	+
Alkaloids	+	+	+

'+' – Present '-' - Absent

Table 5 Qualitative analysis of T. cordifolia stem

Test	T. cordifolia stem		
	Pet. Ether extract	**Ethanol extract**	**Aqueous extract**
Carbohydrates	-	+	+
Proteins	-	+	+
Tannins	-	+	+
Saponins	+	+	+
Triterpenoids	+	+	-
Flavonoids	+	+	+
Quinones	-	-	-
Sterols	+	-	-
Glycosides	+	+	+
Alkaloids	+	+	+

'+' – Present '-' - Absent

Table 6 Qualitative analysis of T. cordifolia Root

Test	T. cordifolia Root		
	Pet. Ether extract	**Ethanol extract**	**Aqueous extract**
Carbohydrates	-	+	+
Proteins	-	+	+
Tannins	-	+	+
Saponins	+	+	+
Triterpenoids	+	+	-
Flavonoids	+	+	+
Quinones	-	-	-
Sterols	+	-	-
Glycosides	+	+	+
Alkaloids	+	+	+

'+' – Present '-' - Absent

Table 7 Blood glucose level of induction of diabetes and treated TC experimental animals mg/dl

S.No	Normal blood glucose	Diabetic blood glucose	Medicine treated blood glucose
1	80	410	95
2	80	415	80
3	60	420	85
4	60	420	90
5	100	426	120
6	85	423	101

4. DISCUSSIONS

The prevalence of diabetes mellitus is rising as people's eating habits and lifestyles change. The condition is still incurable despite some claims to the contrary, according to the available evidence. Medication, a well-controlled diet, and regular exercise can help manage the condition well. Mismanagement of the latter two components, on the other hand, is what's been driving up diabetes rates. Current treatments have a variety of negative effects, and one of the most significant is the necessity to gradually raise dosage over time due to Type II

diabetes's worsening insulin resistance. As a result, new medicines must be developed that have fewer adverse effects and are more effective. Even though the formulation includes active principles to treat disease, it also includes components with redundant, synergetic, or even antagonist qualities that play a function in safeguarding the active principles. When compared to traditional medical practises, modern medicine is driven by a single, effective principle that must be demonstrated at the molecular level in order to be effective.

5. CONCLUSION

Since skeletal muscle insulin-mediated glucose absorption is impaired, glucose is diverted to the liver." Liver lipids limit insulin's ability to regulate glucose and glycogen synthesis. Increased dietary glucose and unaltered lipogenesis cause NAFLD. Impaired insulin action in adipose tissue increases lipolysis, which re-esters lipids in other tissues including the liver and worsens insulin resistance. Due to low insulin levels, insulin resistance may lead to pancreatic-cell failure and hyperglycemia. Thus, treating type 2 diabetes requires insulin action and secretion.

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