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Comparative assessment of *in vitro* antidiabetic activity of *Tinospora cordifolia* stem, leaves and its callus extracts

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Abstract

Callus can be a viable alternative to obtain important phytochemicals and analyze crude extract for pharmacological activities rather than going the cumbersome way of collecting and destroying possibly endangered plants. In this study, callus was produced using stem and leaf explants of *Tinospora cordifolia*, and methanol extract of stem leaf, stem callus and leaf callus were evaluated for their antidiabetic potential. Inhibition of glycosylation of haemoglobin and α- amylase inhibition was in a dose dependent manner and glucose transport differs with the sample and glucose concentration. The results of the work indicate that the both native plant extracts and callus extracts possessed considerable *in vitro* anti diabetic activity and can be applied as alternative in the treatment of diabetes and diabetic induced complication.

Keywords: *Tinospora cordifolia,* stem, leaf, callus, *in vitro* antidiabetic.

Introduction

Diabetes mellitus is a metabolic disorder characterized by a loss of glucose homeostasis with disturbances of carbohydrate, fat and protein metabolism resulting from defects in insulin secretion, insulin action, or both (Rajiv Gandhi and Sasikumar, 2012). Over several years diabetes mellitus has become a major health problem worldwide; reaching epidemic proportions (Modak *et al.,* 2007)**.** Nowadays many new drugs are discovered as treatment to diabetes. But, some risks are involved in the usage of these therapeutic agents. Glucosidase and lipase inhibitors, which have been used as medicines give rise to certain side effects. For instance, acarbose has a low efficacy in decreasing the glycemic levels. Lipase inhibitor produces a weight loss in patients and some others cause hepatotoxicity, abdominal pain, flatulence, diarrhea and hypoglycemia (Ramirez *et al*., 2012). Thus searching for a new class of compounds is essential to overcome diabetic problems. There is continuous search for alternative drugs**.**

Herbal preparations are used to treat diabetes, as an alternative therapy but their reported hypoglycemic effects are multifarious. Folk medicinal and other types of traditional medicinal practitioners use medicinal plants intheir formulations but most often disregard the conservation status of the plants. Moreover, if roots, tubers or rhizomes of a medicinal plant are necessary in traditional medicinal formulations, the whole plant is uprooted thus destroying the plant. This is done with scant regard to the plant's re-cultivation, thus making such plants becoming rapidly endangered. One way out of this impasse is to conduct pharmacological studies on calluses produced from nodal explants of the plant. If calluses can be seen to give the desired pharmacological effect or have the requisite phytochemical(s), they can serve the purpose of various plant parts including underground parts, and such calluses can be obtained within a relatively small space in the laboratory or industry and so negating the uprooting of plants and as a result endangering them.

Traditional practitioners can substitute whole plants for calluses. Callus culture and concomitant pharmacological studies are rapidly gaining attention of scientists (Islam *et al.,*2015)

The herb *Tinospora cordifolia* (Menispermaceae) is commonly known as Guduchi in India. A variety of chemical constituents such as alkaloids, diterpenoid lactones, steroids, glycosides aliphatic compounds, polysaccharides have been reported from different parts of *Tinospora cordifolia*. It has a long history of use in Ayurvedic medicine (the traditional medicine of India). Evidence hints that *Tinospora* may have anti-cancer (Singh *et al*., 2005; Singh *et al*., 2004), immune stimulating (Rawa l*et al*., 2004), anti-diabetic (Stanely *et al*., 2003; Rathi *et al*., 2002), cholesterol-lowering (Stanely *et al*., 2003) and liver-protective (Bishayi *et al*., 2002) actions. *T. cordifolia* has also shown some promising speed in healing the diabetic foot ulcers (Purandare *et al*., 2007). The objective of the present study was to determine the *invitro* antidiabetic potential of methanol extract of stem, leaf and their callus of *T. cordifolia.*

Materials and Methods

Tinospora cordifolia callus cultures were initiated from stem and leaf explants. Explants were collected from a 10-year old plant, sterilized, and then cultured on a Murashige and Skoog supplemented with 2,4-dichlorophenoxy acetic acid (1.5 mg/l) and benzyl aminopurine (0.3 mg/l). The cultures were maintained at 25^0C under 16 hrs lights per day photoperiods (3000 lux) for multiple callus induction.

The fresh stem leaves, stem callus and leaf callus of *T. cordifolia* were shade dried and powdered mechanically and stored in an air tight container. The extraction was carried out by hot percolation method using Soxhlet apparatus. The solvent used was ethanol. About 40 gm of powder was extracted with 200 ml of ethanol. The extract was concentrated to dryness under controlled temperature 40-50°C. The extract was preserved in refrigerator till further use.

Non-enzymatic glycosylation of haemoglobin method - (Acharya *et al.,* 1980)

Antidiabetic activity of plant and callus extracts of *T. cordifolia* were investigated by estimating degree of non-enzymatic haemoglobin glycosylation, measured colorimetrically at 520nm. Glucose (2%), haemoglobin (0.06%) and Gentamycin (0.02%) solutions were prepared in phosphate buffer 0.01 M, pH 7.4. 1 ml each of above solution was mixed. 25, 50 and 100µg/ml of native plant and callus extracts concentrations were added to above mixture. Mixture was incubated in dark at room temperature for 72 hrs. The degree of glycosylation of haemoglobin was measured colorimetrically at 520nm. Metformin was used as a standard drug for assay. % inhibition was calculated

$$\% \text{ inhibition} = \frac{As - Ac}{As} \times 100$$

Glucose uptake in Yeast cells method- (Cirillo, 1962)

The commercial baker's yeast was washed by repeated centrifugation (3,000×g; 5 min) in distilled water until the supernatant fluids were clear and a 10% (v/v) suspension was prepared in distilled water. Various concentrations of extracts (25, 50 and 100µg/ml) were added to 1ml of glucose solution (5, 10 and 25 mM) and incubated together for 10 min at 37 °C. Reaction was started by adding 100µl of yeast suspension, vortex and further incubated at 37°C for 60 min. After 60 min, the tubes were centrifuged (2,500 × g, 5 min) and glucose was estimated in the supernatant. GLINOSE was taken as standard drug.

α- Amylase Inhibition method – (Nickavar and Yousefiana, 2009).

1ml of substrate-potato starch (1% w/v), 1ml of drug solution (GLINIL drug/methanol extract) of 3 different concentrations such as 25, 50 and 100µg/ml µg/ml. 1ml of α- amylase enzyme (1% w/v) and 2ml of acetate buffer (0.1 M, 7.2pH) was added. The mixture was incubated for 1hr.then 0.1 ml iodine-iodide indicator (635mg iodine and 1gm potassium iodide in 250ml distilled water) was added in the mixture. Absorbance was taken at 565nm in UV-Visible spectroscopy. The percentage increase in glucose uptake by yeast cells and % of α- amylase inhibition were calculated using the following formula-

$$\text{Increase in glucose uptake (\%)} = \frac{\text{Abs sample} - \text{Abs control}}{\text{Abs sample}} \times 100$$

Where, Abs control is the absorbance of the control reaction (containing all reagents except the test sample), and Abs sample is the absorbance of the test sample. All the experiments were carried out in triplicates.

Results and Discussion

Non enzymatic glucosylation of haemoglobin method

Bailey and Day, 1989 reported that the human bodies possess enzymatic and non- enzymatic antioxidative mechanisms which minimize the generation of reactive oxygen species, responsible for many degenerative diseases including diabetes. Increased concentration of glucose in the blood leads to its binding to hemoglobin which may result in the formation of the reactive oxygen species. Plant extracts play an important role the inhibition of the glycosylation end products. An increase in the glycosylation was observed on incubation of

hemoglobin with the increasing concentration of the glucose over a period of 72hrs (Table 1). However, the plant extracts significantly inhibited the haemoglobin glycosylation which is indicated by the presence of increasing concentration of haemoglobin. The leaf callus extracts exhibited higher inhibition of glycosylation (75% in 100µg/ml) as compared with the standard drug 88% in 100µg/ml). The plant extracts also displayed the inhibition of haemoglobin glycosylation at different physiological concentrations of the glucose over the period of 72 hrs, indicating that the plant extracts decreases the formation of the glucose- haemoglobin complex and thus amount of free haemoglobin increases.

Table1:Non enzymatic glucosylation of haemoglobin method

Sample	Concentrations (µg/ml)			
	Abs	**25µg/ml**	**50µg/ml**	**100µg/ml**
Blank	0.076±0.002	**% of inhibition**	**% of inhibition**	**% of inhibition**
Standard (Metformin)		64.8	80.6	88.3
Stem		45.4	62.8	68.2
Leaf		48.7	63.9	70.4
Stem callus		56.4	69.4	74.8
Leaf callus		59.0	71.7	75.6

Glucose uptake in yeast cells

This assay is based on the movement of glucose across the membrane of yeast cells, with the help of the plant extract. The yeast cells were suspended in plant extract and various concentrations of glucose (25µg/ml to 100µg/ml). The plant extract enhances the yeast cells to take in the glucose. The amount of glucose remaining in the solution after incubation was observed. This determines the glucose uptake by the yeast cells (Suhashini *et al*., 2014). From the results, it was found that the percentage increase in glucose uptake by yeast cells at 100µg/ml glucose concentration with methanolic extract ranges from 67 – 76% and minimum uptake of glucose at 25µg/ml glucose concentration (45 – 59%). The result suggests that both callus extract exhibited maximum level inhibition was recorded (76%) than other extracts tested (Table 2).

Table 2: Glucose uptake in yeast cells

Sample	Concentrations (µg/ml)			
	Abs	**25µg/ml**	**50µg/ml**	**100µg/ml**
Blank	0.134±0.016	**% of inhibition**	**% of inhibition**	**% of inhibition**
Standard (Glinose)		80.5	83.7	90.0
Stem		52.8	55.5	67.2
Leaf		58.4	65.2	70.6
Stem callus		64.4	72.7	76.6
Leaf callus		69.4	73.2	76.9

α -Amylase inhibition method

α-amylase is an enzyme that converts starch to glucose in its presence. When α- amylase, glucose, plant extract are taken together as a solution, the plant extract causes the inhibition of enzyme activity (Suhashini *et al*., 2014). The percentage inhibition of α amylase increases from 43 to 72% with increasing concentration of plant extract (25 and 100 µl) (Table 3). The standard drug of Glinil exhibited the rate of glucose inhibition maximum level 84% and minimum level 76%.

In this present study we evaluated *in vitro* Non enzymatic glucosylation of haemoglobin method, Glucose uptake in yeast cells and alpha amylase inhibition of methanol extracts of *T. cordifolia*. However further pharmacological and biochemical investigations will clearly elucidate the mechanism of action and helpful in projecting plant and callus powder as a therapeutic target in diabetes research.

Table 3: α -Amylase Inhibition Method

Sample	Concentrations (µg/ml)			
	Abs	25µg/ml	50µg/ml	100µg/ml
Blank	0.132±0.020	**% of inhibition**	**% of inhibition**	**% of inhibition**
STANDARD (GLINIL)		76.3	82.1	84.2
Stem		43.1	53.2	56.4
Leaf		44.5	52.9	55.0
Stem callus		49.7	58.4	70.2
Leaf callus		63.4	67.2	72.6

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