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INVITRO ANTI-DIABETIC ACTIVITY OF ETHANOLIC EXTRACT IN *ANDROGRAPHIS PANICULATA* LEAVES

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ABSTRACT

Plant research has been intensified worldwide in the recent times. A large number of medicinal plants and their purified constituents have immense therapeutic potentials and have been reported to exhibit anti-diabetic activity of ethanolic extract *Andrographis paniculata. In vitro* anti-diabetic was investigated by using alpha amylase, alpha glucosidase, tyrosinase, glucose uptake in yeast cells, heamoglobin glycosylation, extracts on heamoglobin glycosylation, extracts at physiological glucose concentration. The preliminary phytochemical analysis is also carried out. The results of the present study clearly depicted that ethanolic extract of *Andrographis paniculata* has potent antidiabetic activity.

KEYWORDS: α-amylase, α-glucosidase, glucose uptake in yeast cells, glycated heamoglobin, tyrosinase.

INTRODUCTION

Diabetes mellitus (DM) is a metabolic disorder resulting from a defect in insulin secretion, insulin action or both. Insulin deficiency in turn leads to chronic hyperglycemia with disturbances of carbohydrate, fat and protein metabolism. As the disease progresses tissue or vascular damage ensues leading to severe diabetic complications such as retinopathy, nephropathy, cardiovascular complications and microbial complications. Thus, diabetes covers a wide range of heterogenous diseases (Bastaki, 2005). The classification and diagnostic criterion of diabetes was first put forward by the WHO in 1965. The latest recommendations have been published by the American diabetes association expert

committee (Expert committee on the diagonosis and classification of Diabetes mellitus, 1997) and by the WHO consultation (World Health Organisation consultation, 1999). The vast majority of diabetic patients are classified into one of two broad categories: type 1 diabetes (insulin dependent diabetes mellitus), which is caused by an absolute deficiency of insulin, and type 2 diabetes (non-insulin dependent diabetes mellitus), which is characterized by the presence of insulin resistance with an inadequate compensatory increase in insulin secretion. In addition, women who develop diabetes during their pregnancy are classified as having gestational diabetes. Finally, there are a variety of uncommon and diverse types of diabetes which are caused by infections, drugs, endocrinopathies, pancreatic destruction and genetic defects. These unrelated forms of diabetes are classified separately.

PREVALENCE OF DIABETES MELLITUS

The world prevalence of diabetes among adults (aged 20-79 years) was estimated to be 6.4%, affecting 285 million adults, in 2010, and will increase to 7.7% and 439 million adults by 2030 (Sharma *et al*., 1995). Globally, diabetes prevalence is similar in men and women but it is slightly higher in men <60 years of age and in women at older ages (International Diabetes Federation, 1995). According to Sharma *et al*. (2010), India had 50.8 million diabetic subjects in the year 2010 and this number would increase to 87 million by the year 2030.

Diagnostic criteria for diabetes mellitus

There are three ways to diagnose diabetes mellitus and each, in the absence of unequivocal hyperglycemia, on a subsequent day, by anyone of the three methods given below (Expert committee on the diagnosis and classification of diabetes mellitus, 1997). The use of glycated hemoglobin for the diagnosis of diabetes mellitus is not recommended at this time.

- Symptoms of diabetes plus causal plasma glucose concentration ≥ 200 mg/dl (11. 1 mmol/l). Causal is defined as any time of the day without regard to time since last meal. The classic symptoms of diabetes mellitus include polyuria, polydipsia and unexplained weight loss.
- Fasting plasma glucose (FPG) ≥ 126 mg/dl (7.0 mmol/l). Fasting is defined as no caloric intake for at least 8h.Post load glucose (2 h) ≥ 200 mg/dl (11.1 mmol/l) during an oral glucose tolerance test (OGTT).

COMPLICATIONS OF DIABETES MELLITUS

Diabetes mellitus is a chronic condition that can lead to complications over a period of time (Fowler, 2008). These complications include.

- Nephropathy (disease of the kidney), which can lead to kidney failure and the need for dialysis.
- Retinopathy (disease of the eye), which can lead to blindness.
- Neuropathy (disease of the nerves), which can lead to, among other things, ulceration of the foot requiring amputation.
- Coronary heart disease, which can lead to heart attack.
- Cerebrovascular disease, which can lead to strok.
- Microbial complications.

TREATMENT OF DIABETES MELLITUS

The main goals of the treatment of hyperglycemia are: 1) Alleviating symptoms, 2) Minimizing acute complications, 3) Increasing the sense of well being and quality of life and 4) Eliminating or minimizing chronic complications. Current therapeutic agents available for type 2 diabetes include sulfonyl ureas and relatedcompounds, biguanides, α-glucosidase inhibitors and thiazolidinediones. A rational approach would be to begin with the agents particularly suited to the stage and nature of the disease, progressing, if necessary, to combination therapy. The treatment of type 1 diabetes includes the use of exogenous insulin.

USE OF MEDICINAL PLANTS FOR THE TREATMENT OF DIABETES MELLITUS

Management of diabetes without any side effects is still a challenge in the medical field, as presently available drugs for diabetes have one or more adverse effects (Bohannon, 2002). Since the existing drugs for the treatment of DM do not satisfy our need completely, the search for new drugs continues. In recent years, herbal remedies for the unsolved medical problems have been gaining importance in the research field. Apart from the currently available therapeutic options, many herbal medicines have been recommended for the treatment of diabetes. Herbal drugs are prescribed widely because of their effectiveness, less side effects and relatively low cost (Venkatesh *et al*., 2003; Devaki *et al*., 2011).

MATERIALS AND METHODS

The fresh plant leaves was collected from Madukkur, Pattukkottai,Thanjavur district. The fresh leaves were shade dried and the leaves were powdered and sieved and used for the analysis of preliminary phytochemical analysis, *in vitro* anti diabetic activities.

Preliminary phytochemical screening

Qualitative phytochemical examinations were carried out of all the extract as per the standard methods.

ENZYME INHIBITORY ASSAYS OF PLANT FRACTIONS

Determination of *α- amylase* inhibitory activity

The assay mixture containing 200 µl of 0.02M sodium phosphate buffer, 20 µl of alpha amylase enzyme and the plant fractions (20-100 µg/ml) were incubated for 10 minutes at room temperature followed by addition of 200 µl of starch. The reaction was terminated with the addition of 400 µl DNS reagent and placed in boiling water bath for 5 minutes, cooled and diluted with 15 ml of distilled water and absorbance was measured at 540 nm.

The control samples were prepared without any plant fractions. The % inhibition was calculated as follows.

$$\text{Inhibition (\%)} = \frac{\textbf{Absorbance 540 (control) – Absorbance 540 (extract)}}{\textbf{Absorbance 540 (control)}} \times 100$$

Metformin was used as the reference alpha amylase inhibitor. All tests were performed in triplicate.

DETERMINATION OF *α - GLUCOSIDASE* INHIBITORY ACTIVITY

P-Nitrophenyl-α-D-glucopyranoside, Baker's Yeast alpha glucosidase were purchased from Sigma (USA).The yeast alpha glucosidase was dissolved in 100 mm phosphate buffer pH 6.8 and was used as the enzyme extract. P-Nitrophenyl-α-D-glucopyranoside was used as the substrate. Plant fractions were used in the concentration ranging from 20-100 µg/ml. Different concentrations of plant fractions were mixed with 320 µl of 100 mm phosphate buffer pH 6.8 at 30 °C for 5 minutes. 3 ml of 50 mm sodium hydroxide was added to the mixture and the absorbance was read at 410 nm. The control samples were prepared without any plant fractions. The % inhibition was calculated according to the formula.

$$\text{Inhibition (\%)} = \frac{\textbf{Absorbance 410 (control)} - \textbf{Absorbance 410 (extract)}}{\text{Absorbance } \textbf{410} \text{ (control)}} \times 100$$

Metformin was used as the reference alpha glucosidase inhibitor. All tests were performed in triplicate.

DETERMINATION OF *TYROSINASE* INHIBITORY ACTIVITY

Test reaction mixtures were prepared by adding 10 µl tyrosinase to10 µl plant fractions and then adding 20 µl 1.5 mm L-tyrosine and 110 µl of 0.1 M sodium phosphate buffer (pH 6.5). The resulting mixture (150 µl) was incubated for 10 minutes at 37°C and absorption at 490 nm was measured. The percent inhibition of *tyrosinase* activity was calculated as follows.

$$\text{Inhibition \%} = \frac{\textbf{Absorbance 490 (control)} - \textbf{Absorbance 490 (extract)}}{\text{Absorbance } \textbf{490} \text{ (control)}} \times 100$$

Metformin was used as the reference tyrosinase inhibitor. All tests were performed in triplicate.

EVALUATION OF HAEMOGLOBIN GLYCOSYLATION: (CIRILLO, 1962)

GLUCOSE UPTAKE IN YEAST CELLS

The commercial baker's yeast was washed by repeated centrifugation (3,000×g; 5 minutes) in double distilled water until the supernatant fluids were clear and a 10% (v/v) suspension was prepared in distilled water. Various concentrations of JFF, BFF and PFF (50 - 2000 µg/ml) were added to 1mL of glucose solution (5, 10 and 25 mm) and incubated together for 10 minutes at 37 °C. Reaction was started by adding 100 µl of yeast suspension, vortex and further incubated at 37 °C for 60 minutes.

The tubes were then centrifuged (2,500 × g, 5 minutes) and amount of glucose was estimated in the supernatant was estimated by DNS method.

The percent increase in glucose uptake by yeast cells was calculated using the following formula.

$$\text{Increase in glucose uptake (\%)} = \frac{\textbf{Absorbance (sample)} - \textbf{Absorbance (control)}}{\textbf{Absorbance}_{\textbf{(sample)}}} \text{ X } 100$$

L-Acarbouse was used as the reference tyrosinase inhibitor. All tests were performed in triplicate. 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 triplicated.

EVALUATION OF NON-ENZYMATIC GLYCOSYLATION OF HAEMOGLOBIN

PREPARATION OF HAEMOGLOBIN

The blood was collected from a healthy human volunteer and transferred into a blood bottle containing Ethylene diamine tetraacetic acid as anticoagulant. Hemolysate was prepared based on the principle of hypotonic lysis. The red blood collected were washed thrice with 0.14 M sodium chloride solution and 1 volume of red blood cells suspension was lysed with 2 volume of 0.01 M phosphate buffer, pH 7.4 and 0.5 volume of carbon tetrachloride. The haemolysate was then freed from the debris by centrifugation at 2300 g for 15 minutes at room temperature3. The haemoglobin rich fraction (upper layer) was separated and dispensed into sample bottle for storage in the refrigerator until required for use.

ESTIMATION OF HAEMOGLOBIN GLYCOSYLATION: (Adisa *et al.*, 2004)

1ml each of haemoglobin fraction was transferred in to three test tubes each containing 1 ml solution of different concentration (2, 6, and 10 mg/ ml) of glucose in 0.01M phosphate buffer pH 7.4, 1 ml each of haemoglobin fraction was transferred. The contents were incubated at room temperature for 72 hours. A blank solution in which the addition of glucose solution was omitted was used as control. The amounts of hydroxymethylfurfural in nanomole released were estimated at different incubation periods of 0, 24, 48 and 72 hours which correspond to the degree of glycosylation.

RESULTS AND DISCUSSION

Diabetes is a chronic disease that occurs when the pancreas does not produce enough insulin (a hormone that regulates blood sugar) or alternatively, when the body cannot effectively use the insulin it produces. The overall risk of dying among people with diabetes is at least double the he risk of their peers without diabetes. Diabetes and its complications impose significant economic consequences on individuals, families, health systems and countries. In this regard, our present study is concentrated on antidiabetic activity.

The following anti-diabetic parameters which are investigated in the current project are enclosed herewith.

Table 1: Preliminary Phytochemical Analysis

S.No.	Name of the compound	Result
1.	Carbohydrates	+
2.	Saponins	+
3.	Phytosterols	+
4.	Tannins	+
5.	Protein and amino acid	+
6.	Chlorogenic acid	+
7.	Steriods	+

+ Presence

Table: 2 Effect of *α – amylase* on *invitro* anti-diabetic activity of ethanolic extract in *Andrographis paniculata.*

S.No.	Concentration	Ethanolic Extract of *Andrographis paniculata*
		%Inhibition of α- amylase
1.	20	11
2.	40	15
3.	60	23
4.	80	26
5.	100	30
6.	60	18

Values are expressed as Mean ± SD

student's 't' test followed and p*** <0.005

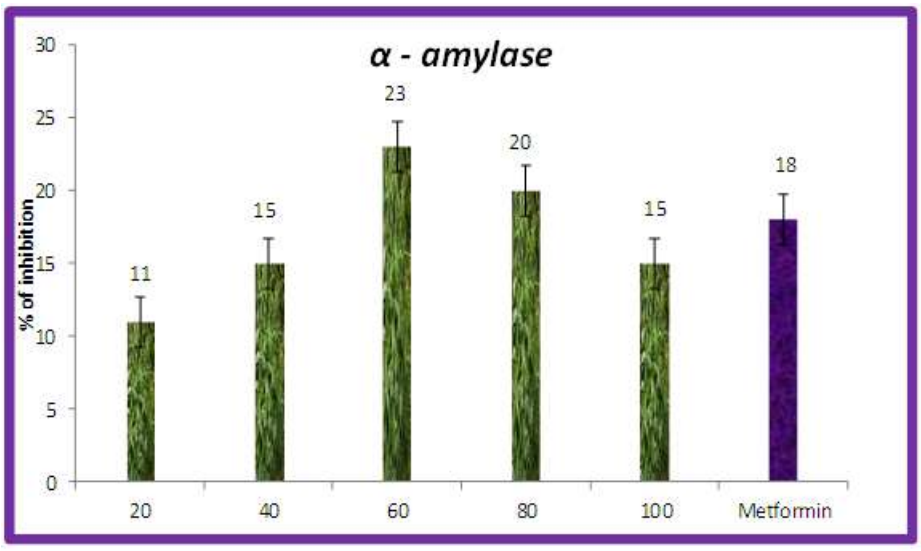

Figure 2: Effect of *α - amylase* on *invitro* antidiabetic activity of ethanolic extract in *Andrographis paniculata.*

Table 3: Effect of *α*- glucosidase on *invitro* anti-diabetic activity of ethanolic extract in *Andrographis paniculata.*

S.No.	Concentration (µg/ml)	Ethanolic Extract of *Andrographis paniculata* in % inhibition of *α-glucosidase*
1.	20	32
2.	40	49
3.	60	64
4.	80	57
5.	100	82
6.	60	63

Values are expressed as Mean±SD

student's 't' test followed and p***<0.005

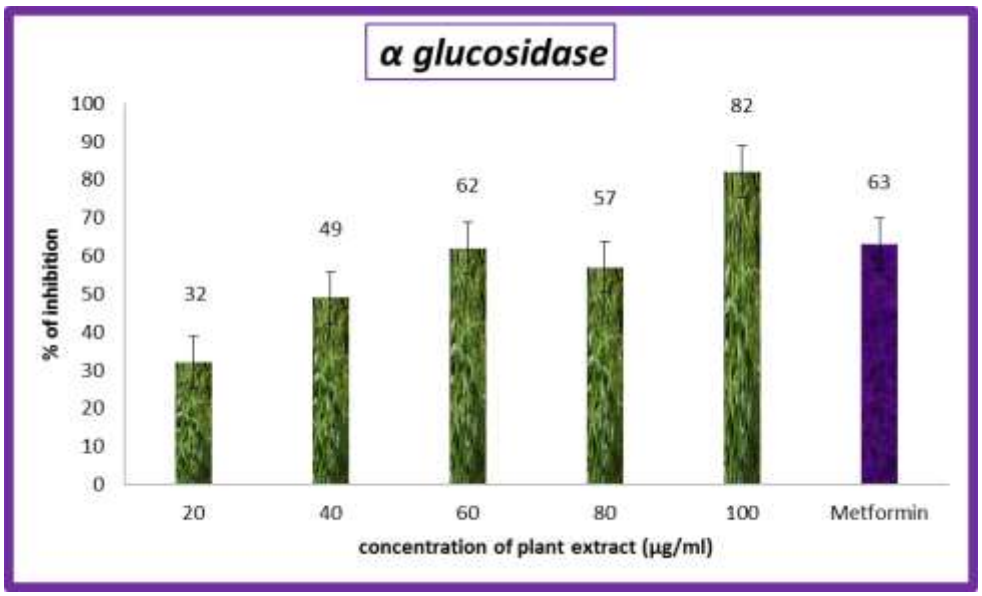

Figure 3: Effect of *α-glucosidase* on *invitro* anti-diabetic activity of ethanolic extract in *Andrographis paniculata.*

Table 4: Effect of *Tyrosinase* on *invitro* anti-diabetic activity.

S.No.	Concentration (µg/ml)	Ethanolic Extract of *Andrographis paniculata* in Inhibition % *Tyrosinase*
1.	20	0.09
2.	40	0.14
3.	60	0.27
4.	80	0.45
5.	100	0.60
6.	60	0.37

Values are expressed as Mean ±SD

Student's 't' test followed and p***<0.005

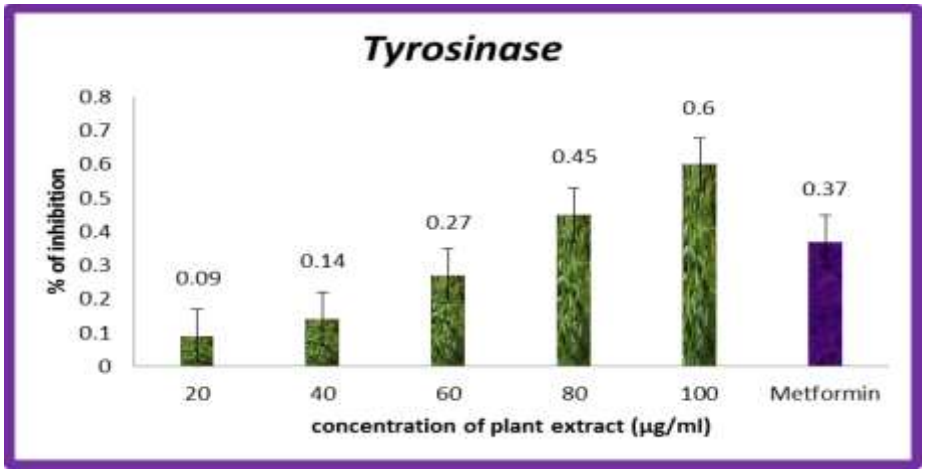

Figure 4: Effect of *Tyrosinase* on *invitro* anti-diabetic activity of ethanolic extract in *Andrographis paniculata.*

Table: 5 Effect of Glucose uptake by yeast cells of ethanolic extract in *Andrographis paniculata.*

5mM of glucose against plant extract		
S.No.	**Concentration (µg/ml)**	**Ethanolic Extract of *Andrographis paniculata***
1.	20	0.14±0.036
2.	40	0.22±0.028
3.	60	0.32±0.101
4.	80	0.38±0.051
5.	100	0.44±0.039
6.	60	0.27±0.105

Values are expressed as Mean ±SD

Student's 't' test followed and p***<0.005

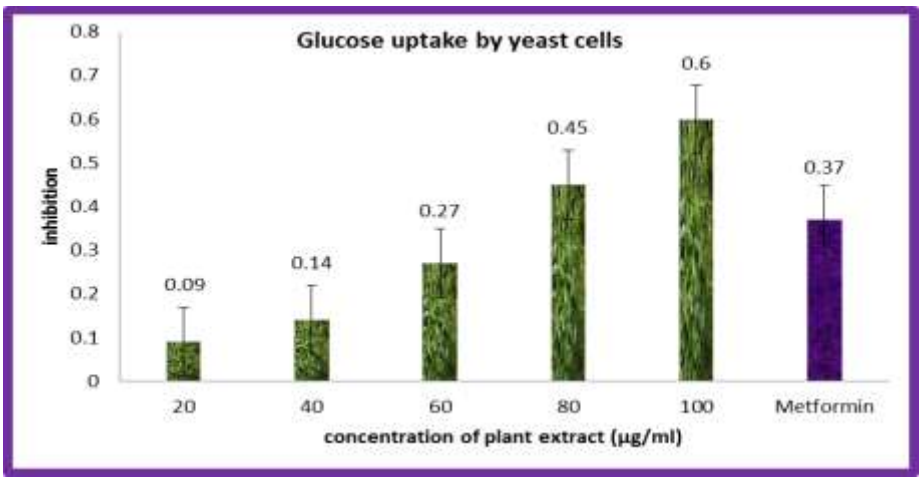

Figure 5: Effect of Glucose uptake by yeast cells on *invitro* anti-diabetic activity of ethanolic extract in *Andrographis paniculata*

Table 5: Effect of Glucose uptake by yeast cell of ethanolic extract in *Andrographis paniculata.*

10mM of glucose against plant extract		
S.No.	**Concentration (µg/ml)**	**Ethanolic Extract of *Andrographis paniculata***
1.	20	0.17±0.036
2.	40	0.25±0.028
3.	60	0.38±0.101
4.	80	0.48±0.051
5.	100	0.60±0.039
6.	60	0.27±0.105

Values are expressed as Mean ±SD

Student's 't' test followed and p***0.005.

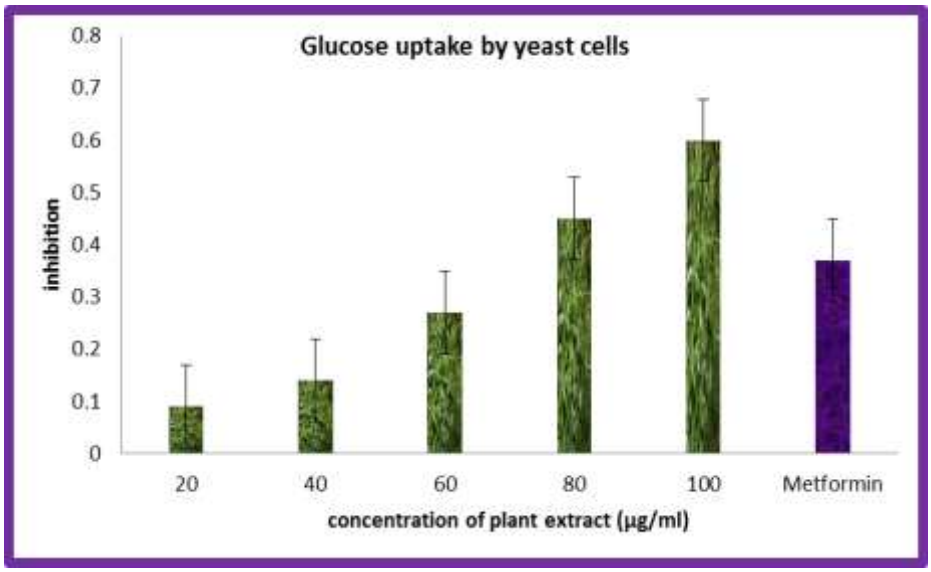

Figure 5: Effect of Glucose uptake by yeast cells on *invitro a*nti-diabetic activity of ethanolic extract in *Andrographis paniculata.*

Table 6: Effect of glycosylated haemoglobin on *invitro a*nti-diabetic activity of ethanolic extract in *Andrographis paniculata.*

S.No.	**Concentration (µg/ml)**	**Ethanolic Extract of *Andrographis paniculata***
1.	20	0.16±0.101
2.	40	0.22±0.253
3.	60	0.39±0.564
4.	80	0.34±0.148
5.	100	0.48±0.596
6.	60	0.48±0.256

Values are expressed as Mean ±SD

Student's 't' test followed and p***<0.005.

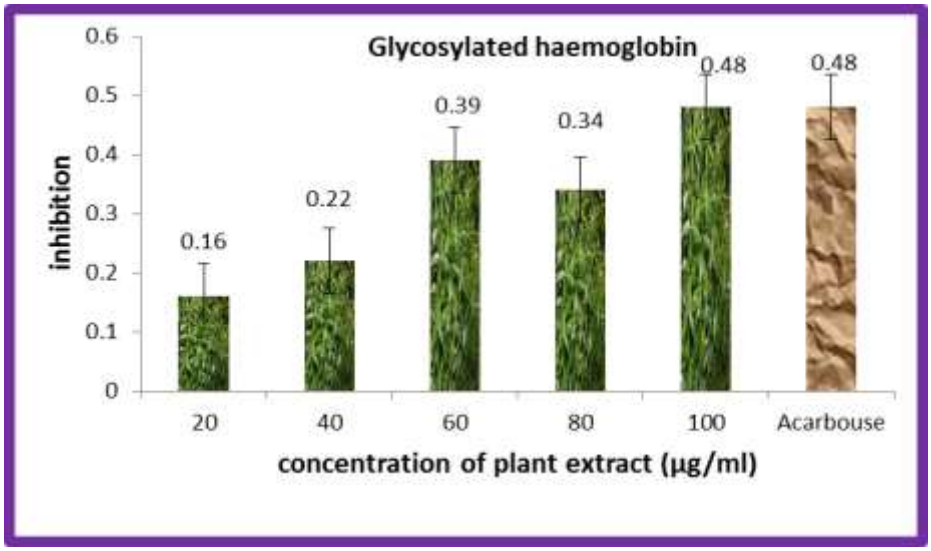

Figure 6: Effect of glycosylated haemoglobin on *invitro* anti-diabetic activity of ethanolic extract in *Andrographis paniculata.*

α-Amylase and *α*-glucosidase

The treatment of diabetes therefore mainly focuses on reducing fluctuations in blood sugar and subsequent complications. The *α*-amylase and *α*-glucosidase inhibitors are currently used for diabetic treatment as oral hypoglycemic agents. starch hydrolysis in the gastrointestinal tract. *α*-Amylase and *α*-glucosidase inhibitory potential of citrus fruits like acarbose would delay the degradation of starch and oligosaccharides, which would in turn cause a decrease in the absorption of glucose and consequently inhibit the increase in postprandial blood glucose. The concentration of 100µg/ml shows the maximum α-amylase of anti-diabetic activity of the plant extract where as 60µg/ml shows the maximum activity in the standard prescribed drug. The concentration of 100µg/ml shows the maximum α-glucosidase of anti-diabetic activity of the plant extract 60µg/ml shows the maximum activity in the standard prescribed drug.

Tyrosinase

Tyrosinase is a polyphenol oxidase with a dinuclear copper active site and is involved in the formation of mammalian melanin pigments, enzymatic browning of fruits and vegetables. Over-activity of this enzyme leads to hyperpigmentation of the skin. Chemical agents that demonstrated tyrosinase inhibitory activity have been used to suppress melanogenesis and can be clinically useful for the treatment of some dermatological disorders associated with melanin hyperpigmentation. Kojic acid is one of the popular chemicals used in whitening cosmetics but chronic, cytotoxic and mutagenic effects have been proved.

The concentration of 100µg/ml shows the maximum Tyrosinase of anti-diabetic activity of the plant extract where as 60µg/ml shows the maximum activity in the standard prescribed drug.

Glucose uptake in yeast cell

In the glucose uptake in yeast cells method the mechanism of glucose transport across the yeast cell membrane has been receiving attention as in vitro screeing method for hypoglycaemic effect of various compounds/ medicinal plants. Recent studies on the transport of on metabolizable sugars and certain metabolizable glycosides suggest that sugars transport across the yeast cell membrane is mediated by stereospecific membrane carries. It is reported that in yeast cells (Saccharomyces cerevisiae) glucose transport is transported in yeast is by a facilitated diffusion process. Facilitated carries are specific carries that transport solutes down the concentration gradient. This means that effective transport is only attained if there is removal of intracellular glucose. The concentration of 100µg/ml shows the maximum Glycated heamoglobin of anti-diabetic activity of the plant extract 60µg/ml shows the maximum activity in the standard prescribed drug.

Heamoglobin glycosylation

In diabetes mellitus, higher amounts of glycated haemoglobin, indicating poorer control of blood glucose levels, have been associated with cardiovascular disease, nephropathy, and retinopathy. A trial on a group of patients with Type 1 diabetes found that monitoring by caregivers of HbA_{1C} led to changes in diabetes treatment and improvement of metabolic control compared to monitoring only of blood or urine glucose.

The concentration of 100µg/ml shows the maximum Glucose uptake by yeast cells of anti-diabetic activity of the plant extract 60µg/ml shows the maximum activity in the standard prescribed drug.

SUMMARY AND CONCLUSION

Herbs are staging a comeback and herbal ‘renaissance’ is happening all over the globe. The herbal products today symbolise safety in contrast to the synthetics that are regarded as unsafe to human and environment. Although herbs had been priced for their medicinal, flavouring and aromatic qualities for centuries, the synthetic products of the modern age surpassed their importance, for a while. However, the blind dependence on synthetics is over

and people are returning to the naturals with hope of safety and security. Over three-quarters of the world population relies mainly on plants and plant extracts for health care.

In this regard the effect of medicinal plants on diabetes are selected for the present study. In this study, we came to know that the parameters such as α-amylase, β-glucosidase, tyrosinase, glucose uptake by yeast cells, glycosylated haemoglobin were analysed. In our present study, all the parameters on treatment with plant exhibits more restoration of all the above said parameters into normal level than compared to the standard drugs prescribed by the physicians.

REFERENCES

1. Adisa, R. A.; Oke, J.; Olomu, S. A. & Olorunsogo, O. Inhibition of human haemoglobin glycosylation by flavonoid containing leaf extract of *Cnestis ferruginea*. *Journal of the Cameroon Academy of Sciences*, 2004; 4: 351-359.
2. Bastaki, S. Review Diabetes Mellitus and its Treatment. *International Journal of Diabetes and metabolism*, 2005; 13: 111-134.
3. Bohannon NJV. Treating dual defects in diabetes: Insulin resistance and insulin secretion. *American Journal of Health Systems Pharmacy*, 2002; 59: 9-13.
4. Devaki K, Beulah U, Akila G, Narmadha R and Gopalakrishnan VK. Glucose lowering effect of aqueous extract of Bauhinia tomentosa L. on alloxan induced type 2 diabetes mellitus in wistar albino rats. *Journal of Basic and Clinical Pharmacy*, 2011; 2: 167-174.
5. Michael J. Fowler, MD, Microvascular and Macrovascular Complications of Diabetes, *Clinical Diabetes*, 2008 Apr; 26(2): 77-82.
6. Sharma, A. K. I. Ahmed, M. Tadayyon, *International Journal of Diabetes*, 1995; 4: 29-38.
7. Venkatesh S, Reddy GD, Reddy BM, Ramesh M and Rao AVN. Antihyperglycemic activity of Caralluma attenuate. *Fitoterapi*a, 2003; 74: 274-279.
8. Vincent P. Cirillo, Mechanism of Glucose Transport Across The Yeast Cell Membrane *Journal of bacteriology*, April 16, 1962.