Research Article

Effect of various Ayurvedic formulations and medicinal plants on carbohydrate hydrolyzing enzymes and glucose uptake by yeast cells-an *in vitro* study

Faiyaz Ahmed*, Sudha Sairam and Asna Urooj

*Department of Studies in Food Science and Nutrition, University of Mysore, Mysore, India.

For correspondence: Dr. Faiyaz Ahmed, Department of Studies in Food Science and Nutrition, University of Mysore, Mysore, India. Telefax: +91-821-2419632.

E-mail: fayaz_ahmed09@yahoo.co.in

ABSTRACT

The present study evaluated the effect of three Ayurvedic formulations and five medicinal plants commonly used in the treatment of diabetes on α-amylase, α-glucosidase, sucrase and glucose uptake by yeast cells *in vitro*. All the Ayurvedic formulations inhibited the enzymes to varying degrees. Although all the medicinal plants studied inhibited α-amylase to an extent of 16-74%, only *Eugenia jambulana* and *Ficus racemosa* inhibited all the three enzymes while, *Tinospora cardifolia and Gymnema sylvestrae* activated α-glucosidase and sucrase. *Aegle marmelos* inhibited sucrase but increased the activity of α-glucosidase. All the samples except *Aegle marmelos* significantly increased (p=0.01) glucose uptake in yeast cells. The enhancement of glucose uptake was dependent on both the sample and glucose concentration. It is proposed that, the mechanism of hypoglycemic action varies greatly from one medicinal plant to other and hence, there is a need for in depth research to establish the mode of action of each medicinal plant for their effective utilization as therapeutic agents.

Key words : Medicinal plants, α-amylase, α-glucosidase, glucose uptake, sucrase, diabetes

INTRODUCTION

Diabetes mellitus is a metabolic disorder of multiple aetiology characterized by chronic hyperglycemia with disturbance of carbohydrate, fat and protein metabolism resulting from defects in insulin secretion, insulin action or both[1]. Recent studies suggest that postprandial hyperglycemia could induce non-enzymatic glycosylation of various proteins, resulting in the development of chronic complications such as micro and macro vascular diseases and has been proposed as an independent risk factor for cardiovascular diseases[2]. Therefore, control of postprandial plasma glucose levels is critical in the early treatment of Diabetes Mellitus and in reducing chronic vascular complications[3]. The aim of antidiabetic therapy is to reduce hyperglycemia, which is the result of decreased insulin sensitivity or decreased insulin secretion from pancreatic β-cells, which can further inhibit insulin secretion from pancreas and diminish insulin mediated glucose uptake in peripheral tissues[4]. Medicinal plants continue to play an important role in the treatment of diabetes, particularly in developing countries where most people have limited resources and do not have an access to modern treatment[5]. A recent review states that more than 1123 plant species have been used ethnopharmacologically or experimentally to treat symptoms of diabetes[6]. There are more than 200 pure compounds from plant sources that have been reported to show blood glucose lowering activity[7].

The wide variety of chemicals classes indicates that a variety of mechanisms of action are likely to be involved in lowering blood glucose levels and several mechanisms have been proposed for the hypoglycemic effect of phytochemicals, such as inhibition of carbohydrate metabolizing enzymes, manipulation of glucose transporters, β-cell regeneration and enhancing insulin releasing activity[8]. With this background, the present study was planned to evaluate the effect of three Ayurvedic formulations and five medicinal plants commonly used in the treatment of diabetes on carbohydrate hydrolyzing enzymes and glucose uptake by yeast cells.

MATERIALS AND METHODS

Plant materials

Mehari churna (MC), *Madhumehari churna* (MMC), *Madhumardan churna* (MMD), *Gymnema sylvestrae* leaf powder (GS), *Tinospora cardifolia* leaf powder (TC), *Eugenia jambulana* seed powder (JB), *Ficus racemosa* bark powder (FR) and *Aegle marmelos* leaf powder (AM) were purchased from a local Ayurvedic dispensary.

Preparation of samples and aqueous extracts

An emulsion containing 10 mg of sample/ml was prepared by triturating 100 mg of sample with 2 drops of Tween 20 in maleate buffer (pH 6.0, 0.1M) and the volume was made upto 10 ml using a volumetric flask. Aqueous extracts were prepared by extracting powdered materials with hot water (70°C) in a mechanical shaker (24 h), filtered and freeze dried and redissolved in distilled water to obtain the final concentrations of 10 mg/ml.

ISSN: 0974-6943

Faiyaz Ahmed et al., Effect of various Ayurvedic formulations and medicinal plants on carbohydrate hydrolyzing enzymes and glucose uptake by yeast cells-an *in vitro* study

Preparation of enzyme solution (**a** -glucosidase & sucrase) from rat small intestinal brush border

The crude enzyme solution was prepared by the method of Dahlqvist[9]. Wistar rats weighing 140-160 grams were fasted overnight, sacrificed by cervical dislocation and the small intestines were immediately excised. The intestines were washed with ice cold maleate buffer (pH 6.0, 0.1M); the brush border was carefully removed and homogenized with maleate buffer (1:5 w/v) in cold condition. The homogenate was then centrifuged for 15 min (10,000 g, 4°C) and the supernatant was used as crude enzyme source of α-glucosidase and sucrase. All animal procedures have been approved by the Animal Ethical Committee of University of Mysore in accordance with animal experimentation and care.

Preparation of yeast cells

Yeast cells were prepared according to the method of Cirillo[10]. Commercial baker's yeast was washed by repeated centrifugation (3000 g, 5 min) in distilled water until the supernatant fluids were clear and a 10% (v/v) suspension was prepared in distilled water.

a -amylase activity

The effect of selected samples on α-amylase activity was studied using enzyme- starch system[11]. Each of the samples (1%) were mixed by stirring with 25ml of potato starch (4%) in a beaker, 100 mg of α-amylase was added to the starch solution, stirred vigorously and incubated at 37°C for 60min. After the incubation period 0.1mol/L NaOH was added to terminate α− amylase activity. The mixture was centrifuged for 15min (3000 g) and glucose content in the supernatant was determined by glucose oxidase peroxidase diagnostic kit. A control test was also run without the addition of test sample.

a -glucosidase activity

The effect of various samples on α-glucosidase activity was assayed according to the procedure of Honda and Hara[12]. The enzyme solution (10 µl) and the sample emulsion (10 µl) were incubated together with 200 µl maleate buffer (pH 6.0) for 10 min at 37°C. The enzyme reaction was started by adding 200 µl of p-nitrophenyl-α-D-glucopyranoside (NPG) solution (2 mM) and incubated at 37°C. After 30 min, the reaction was terminated by treating the mixture in a boiling water bath for 5 min. After the addition of 1.0 ml of disodium hydrogenphosphate solution (0.1 M), the absorbance of the liberated p-nitrophenol was read at 400 nm. An untreated enzyme solution was used as the control.

Sucrase activity

The effect of various samples on sucrose activity was assayed according to the procedure of Honda and Hara[12]. The enzyme solution (10 µl) and the sample emulsion (10 µl) were incubated together with 180 µl maleate buffer (pH 6.0) for 10 min at 37°C. The enzyme reaction was started by adding 100 µl sucrose solution (60 mM). After 30 min, the reaction was terminated by adding 200 µl of 3,5- dinitrosalysilic acid reagent and treating the mixture in a boiling water bath for 5 min. The absorbance of the solution was read at 540 nm. An untreated enzyme solution was used as the control.

The percent inhibitory activities of α-amylase, α-glucosidase and sucrase were calculated using the following formula.

$$\% \text{ inhibition} = \frac{Abs \; control - Abs \; sample}{Abs \; control} \times 100$$

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.

Glucose uptake by yeast cells

Various concentrations of the aqueous extracts (1-5 mg) were added to 1 ml of glucose solution (5-25 mM) and incubated together for 10 min at 37°C. Reaction was started by adding 100 µl of yeast suspension, vortex and further incubated at 37°C for 60 min. After 60 min, the tubes were centrifuged (2500 g, 5 min) and glucose was estimated in the supernatant. The percent increase in glucose uptake was calculated using the following formula.

$$\% \text{ increase in glucose uptake} = \frac{Abs \; control - Abs \; sample}{Abs \; control} \times 100$$

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.

Statistical analysis

All determinations were carried out in triplicates and the data was subjected to one way ANOVA followed by Tukey's multiple comparisons test and the correlations were calculated by Pearson correlation using SPSS 14.0 software and the graphs were plotted using Origin 6.1 software. The values were considered significant at p=0.05 and p=0.01.

RESULTS

The effect of selected samples on the activities of α-amylase α-glucosidase and sucrase is presented in Table 1. Among various samples studied MMD exhibited significantly higher a-amylase inhibitory activity (81%) followed by MMC>JB>MC while, AM & FR showed a moderate inhibition of 47% and 39% respectively. However, GS and TC
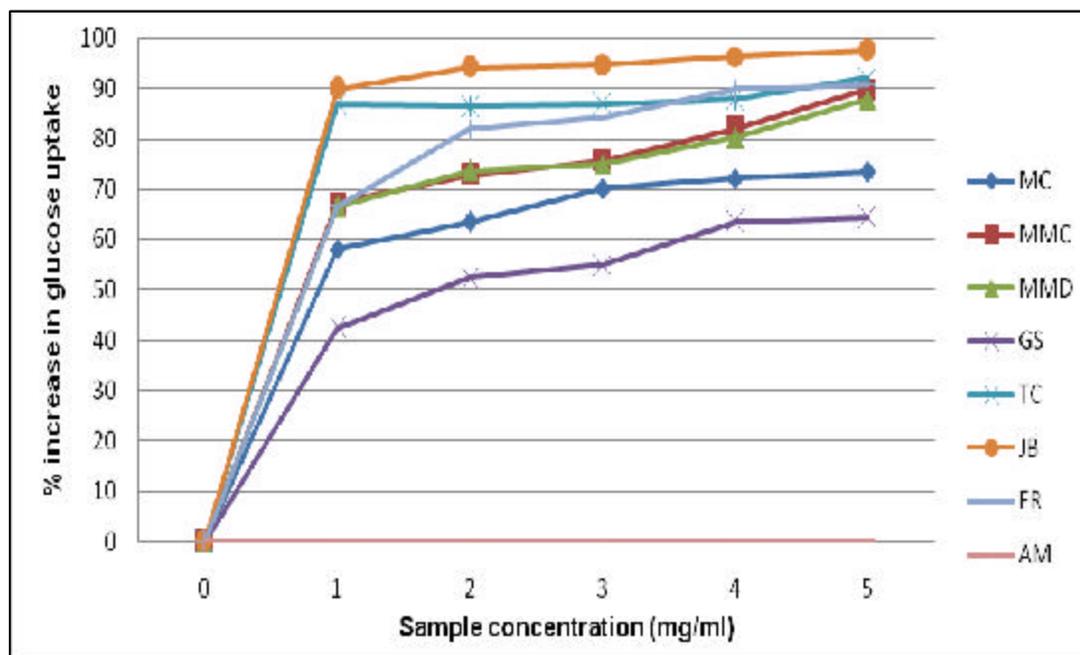
ISSN: 0974-6943

Faiyaz Ahmed et al., Effect of various Ayurvedic formulations and medicinal plants on carbohydrate hydrolyzing enzymes and glucose uptake by yeast cells-an *in vitro* study

Table 1. Effect of the selected samples on carbohydrate hydrolyzing enzymes

Sample	α-amylase	α-glucosidase	Sucrase
MC	77.30 ±3.59 [a b]	22.8 ± 0.86[d]	14.9 ± 0.88[d]
MMC	68.62 ±3.53[c]	5.3 ± 1.32[c]	2.6 ± 1.18[c]
MMD	81.30 ±5.11[a]	5.9 ± 1.56[c]	12.7 ± 1.52[d]
GS	15.95 ±1.10[f]	-14.0 ± 1.73[b]	-8.3 ± 1.37[a]
TC	17.25 ±1.88[f]	-11.6 ± 3.76[b]	-16.2 ± 3.00[b]
JB	73.95 ±2.78[b]	54.7 ± 3.92[e]	22.9 ± 0.57[e]
FR	39.95 ±4.36[e]	17.5 ± 2.19[d]	7.6 ± 1.80[c]
AM	47.92 ±1.88[d]	-11.8 ± 5.25 [b]	16.4 ± 1.75[d]

*MC: *Mehari Churna*, **MMC:** *Madhumehari Churna*, **MMD:** *Madhumardan churna*, **GS:** *Gymnema sylvestrae*, **TC:** *Tinospora cardifolia*, **JB:** *Eugenia jambulana*, **FR:** *Ficus racemosa*, **AM:** *Aegle marmaloes.*****Mean values carrying different superscript letters a, b, c.., in columns differ significantly (*p*=0.05).$^{\$+}$ve values indicate % inhibition and ⁻ve values indicates % activation of the enzyme.**

Figure 1: Effect of various Ayurvedic formulations and medicinal plants on glucose uptake by yeast cells at low concentration of glucose (5 mM)

*MC: *Mehari Churna*, **MMC:** *Madhumehari Churna*, **MMD:** *Madhumardan churna*, **GS:** *Gymnema sylvestrae*, **TC:** *Tinospora cardifolia*, **JB:** *Eugenia jambulana*, **FR:** *Ficus racemosa*, **AM:** *Aegle marmelos*.

Although the some samples inhibited a-amylase and α-glucosidase, none of them were found to be potent inhibitors of sucrase.However, JB exhibited significantly higher (*p*=0.05) inhibitory effect on sucrase (23%) followed by AM (16%), MMC (15%), MMD (13%) and FR (8%). GS and TC were found to increase the activity of sucrase by 8% and 16% respectively. JB and MMC which were found to be potent inhibitors of á-glucosidase also resulted in significant inhibition of sucrase. While, AM which had activated á-glucosidase caused considerable inhibition of sucrase (Table 1). However, a significant correlation (r = 0.690, *p*=0.01) was observed between α-glucosidase and sucrase inhibitory activities of the samples.

The effect of the samples on glucose transport across

exhibited least inhibition of 15% & 17% respectively. Among the samples studied five samples inhibited á-glucosidase to an extent of 5 to 54%, where as the other three samples increased the activity of the enzyme to an extent of 11.6 to 14%. Among the inhibitors, *Eugenia jambulana* exhibited significantly higher (*p*=0.05) activity (55%) followed by MMC (23%) and FR (14%). MC and MMD showed a marginal inhibition of around 5%. Among activators, GS significantly increased (*p*=0.05) the activity ofa-glucosidase followed by TC and AM.

yeast cell membrane was studied in an *in vitro* system comprising of yeast cells suspended in glucose solution of varying concentration (5-25mM) in the presence/absence of the extracts (Figure 1-3). The amount of glucose remaining in the medium after a specific time serves as an indicator of the glucose uptake by the yeast cells.

All the Ayurvedic formulation increased the glucose uptake in yeast cells (20-90%) while; medicinal plants increased the glucose uptake by 10-91% except AM which did not cause any changes in the glucose transport across the yeast cell

ISSN: 0974-6943

Faiyaz Ahmed et al., Effect of various Ayurvedic formulations and medicinal plants on carbohydrate hydrolyzing enzymes and glucose uptake by yeast cells-an *in vitro* study

Figure 2: Effect of various Ayurvedic formulations and medicinal plants on glucose uptake by yeast cells at medium concentration of glucose (15 mM)
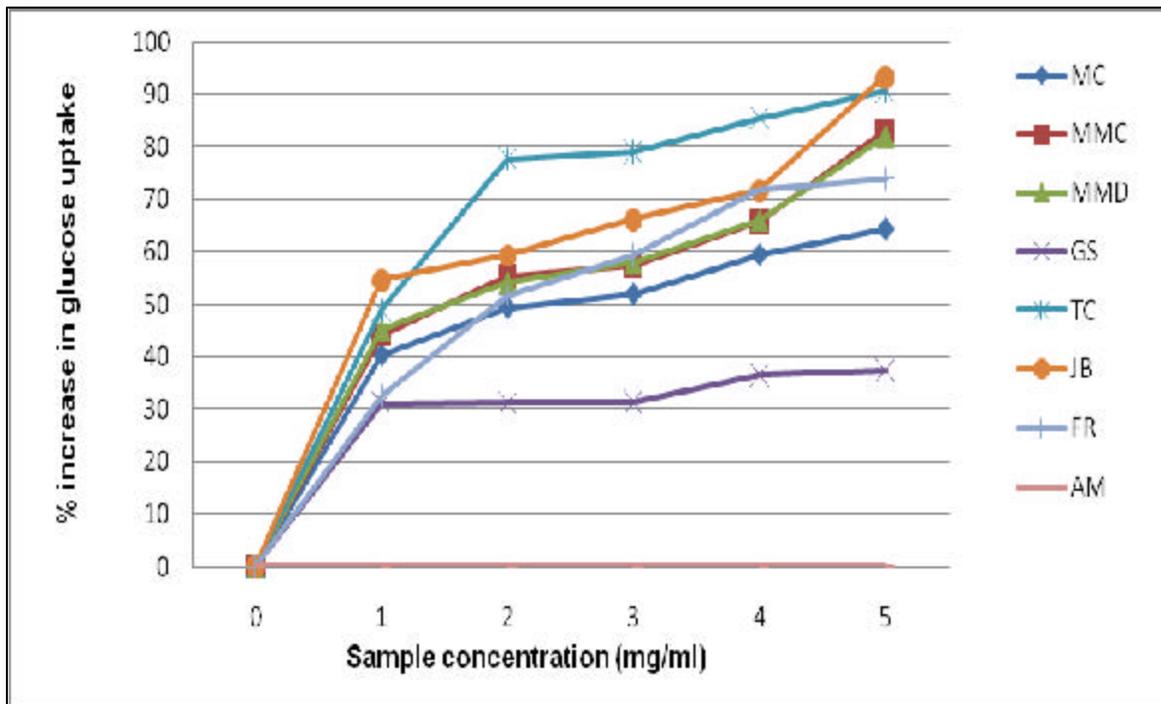
*MC: *Mehari Churna*, MMC: *Madhumehari Churna*, MMD: *Madhumardan churna*, GS: *Gymnema sylvestrae*, TC: *Tinospora cardifolia*, JB: *Eugenia jambulana*, FR: *Ficus racemosa*, AM: *Aegle marmelos.*

Figure 3: Effect of various Ayurvedic formulations and medicinal plants on glucose uptake by yeast cells at high concentration of glucose (25 mM)
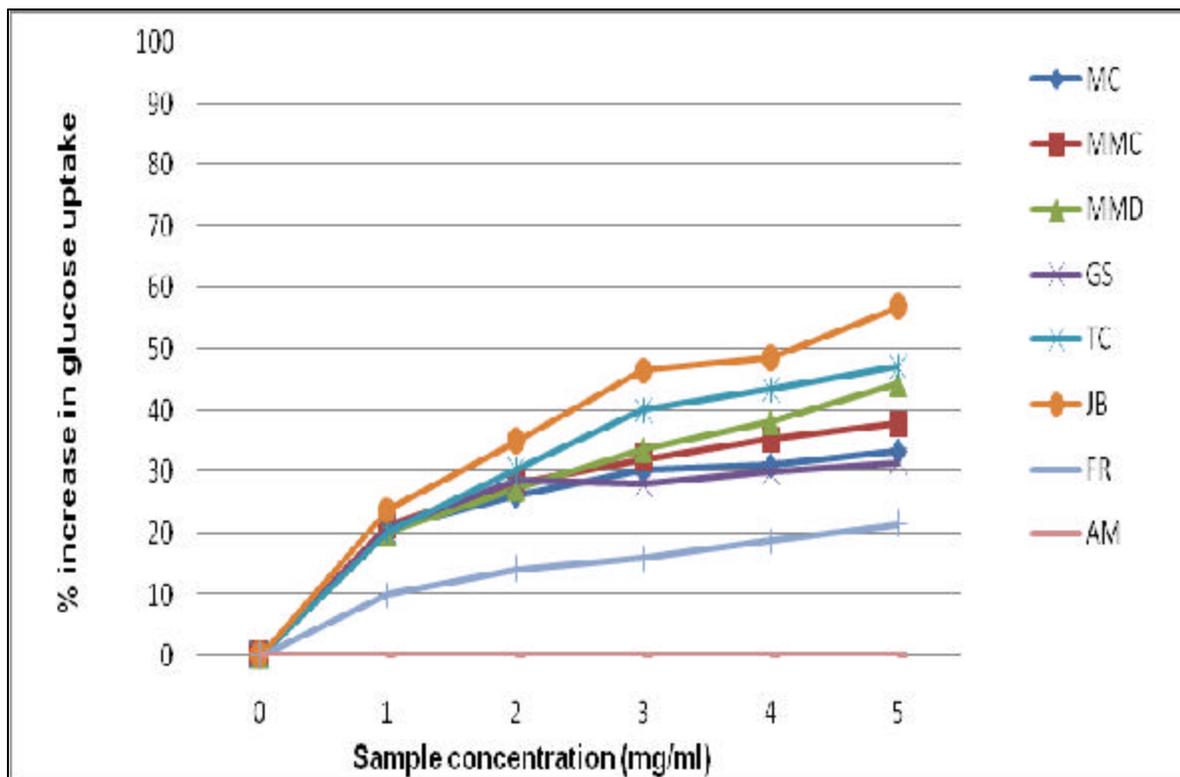
*MC: *Mehari Churna*, MMC: *Madhumehari Churna*, MMD: *Madhumardan churna*, GS: *Gymnema sylvestrae*, TC: *Tinospora cardifolia*, JB: *Eugenia jambulana*, FR: *Ficus racemosa*, AM: *Aegle marmelos.*

ISSN: 0974-6943

Faiyaz Ahmed et al., Effect of various Ayurvedic formulations and medicinal plants on carbohydrate hydrolyzing enzymes and glucose uptake by yeast cells-an *in vitro* study

membrane. Among medicinal plants, JB and TC exhibited significantly higher ($p = 0.05$) activity followed by GS and FR while, among formulations the effect of MMC and MMD were comparable and were significantly higher ($p=0.05$) than that of MC. The glucose uptake enhancement effect of JB and TC were comparable with those of MMC and MMC. The increase in glucose uptake was dose dependent and it was directly proportional to the sample concentration. However, the percent increase in the glucose uptake by the yeast cells was inversely proportional to the glucose concentration and was found to decrease with increase in the molar concentration of the glucose solution.

DISCUSSION

The present study reports the effect of selected Ayurvedic formulations and medicinal plants on carbohydrate hydrolyzing enzymes and the characteristics of glucose transport across yeast cell membrane in their presence/absence. The results reveal that all the Ayurvedic formulations were effective inhibitors of α-amylase. However, among medicinal plants only JB was found to be a good inhibitor of the enzyme whose activity was comparable with that of MMC. The inhibition of a-amylase activity by medicinal plants might be attributed to several possible factors such as fiber concentration, the presence of inhibitors on fibers, encapsulation of starch and enzyme by the fibers present in the sample thereby reducing accessibility of starch to the enzyme and direct adsorption of the enzyme on fibers leading to decreased amylase activity[11]. It was interesting to note that, although MC, MMC and MMD inhibited activities of α-amylase, α-glucosidase and sucrase, JB was the only medicinal plant to exhibit consistent and significant inhibition of enzymes. It is noteworthy here, that JB is a common constituent of all the formulations studied. Hence, the inhibitory effect of various formulations may be attributed to the presence of JB.

Inhibitors of carbohydrate hydrolyzing enzymes delay carbohydrate digestion and prolong overall carbohydrate digestion time, causing a reduction in the rate of glucose absorption and consequently blunting the post prandial plasma glucose rise[13]. The results emphasize that the inhibition of carbohydrate hydrolyzing enzymes is one of the probable mechanism through which many of the medicinal plants/formulations exert their hypoglycemic effect. Several such inhibitors have been recently developed from the natural sources and examples of such inhibitors which are in clinical use are acarbose, miglitol and voglibose[5].

The mechanism of glucose transport across the yeast cell membrane has been receiving attention as an *in vitro* screening method for hypoglycemic effect of various compounds/

medicinal plants. In the present study, it was observed that, the selected samples (except AM) increased glucose uptake by yeast cells in a dose dependent manner and the rate of glucose transport across the yeast cell membrane was a function of external glucose concentration as well as sample concentration. However, this relationship will seize to exist once the yeast cells reach a saturation point (Figure 1-3). It is reported that in yeast cells (*Saccharomyces cerevisiae*) glucose transport is extremely complex and it is generally agreed that glucose is transported in yeast by a facilitated diffusion process. Facilitated carriers are specific carriers that transport solutes down the concentration gradient. This means that effective transport is only attained if there is removal of intracellular glucose[14-15]. We observed that, among 8 samples investigated *Eugenia Jambulana* and *Ficus racemosa* exhibited greater hypoglycemic effect *in vitro* by inhibiting carhohydrate hydrolyzing enzymes and also by increasing glucose uptake by yeast cells. These observations support the use of these medicinal plants in the traditional systems of medicines worldwide for the management of diabetes.

CONCLUSION

It is concluded that, the hypoglycemic action of herbal formulations/medicinal plants varies greatly from one to other and may be mediated by more than one biological effect including inhibition of carbohydrate hydrolyzing enzymes and enhancement of glucose transport across the cell membrane.

ACKNOWLEDGEMENTS

REFERENCES

1. World Health Organisation Consultation., Definition, diagnosis and classification of diabetes mellitus and its complications. Part 1: diagnosis and classification of diabetes mellitus. Report of a WHO Consultation, Geneva., 1999.

2. Baron A.D., Postprandial hyperglycemia and á-glucosidase inhibitors. Diabetes Res Clin Practice., 1998, 40, S51-S55.

3. Shim YJ., Doo HK., Ahn SY., Kim YS., Seong JK., Park IS., Kim BH., Inhibitory effect of aqueous extract from the gall of *Rhus chinensis* on alpha-glucosidase activity and postprandial blood glucose. J Etnhopharmacol., 2003, 85, 283-287.

4. Wolffenbuttel HR., Hoeften WV., Prevention of complications in non-insulin-dependent diabetes mellitus (NIDDM). Drugs., 1995, 50, 263-288.

5. Ali H., Houghton P., Soumyanath A., á-Amylase inhibitory activity of some Malaysian plants used to treat diabetes; with particular reference to *Phyllanthus amarus.* J Ethnopharmacol., 2006, 107, 449-455.

6. Grover J.K., Yadav S., Vats V., Medicinal plants of India with antidiabetic potential. J Ethnopharmacol., 2002, 81, 81-100.

7. Marles R., Farnsworth N., Plants as sources of antidiabetic agents. In: Wagner H, Farnsworth N R, (Eds.), *Economic and Medicinal*

Plant Research, Academic Press Ltd, UK., 1994, 149-187.

8. Tiwari AK., Rao JM., Diabetes mellitus and multiple therapeutic approaches of phytochemicals: Present status and future prospects. Curr Sci., 2002, 23(1), 30-33.

9. Dahlqvist A., Method for assay of intestinal disaccharides. Anal Biochem., 1962, 7, 18-25.

10. Cirillo V.P., Mechanism of Glucose transport across the yeast cell membrane. J Bacteriol., 1962, 84, 485-491.

11. Ou S., Kwok K., Li Y., Fu L., *In vitro* study of possible role of dietary fiber in Lowering postprandial serum glucose. J Agric Food Chem., 2001, 49, 1026-1029.

12. Honda M., Hara Y., Inhibition of rat small intestinal sucrase and á-glucosidase activities by tea polyphenols. Biosci Biotech Biochem., 1993, 57(1), 123-124.

13. Bailey C.J., New approaches to the pharmacotherapy of diabetes. In: Pickup J C, William G, (Eds.), Textbook of Diabetes, 3rd ed. Blackwell Science Ltd., UK., 2003, 73.1-73.21.

14. Illiano G, Cuatrecasas P., Glucose transport in fat cell membranes. J Biol Chem., 1971, 246(8), 2472-2479.

15. Teusink B., Jasper A., Hans DV., Van Dam WK., Walsh MC., Intracellular glucose concentration in derepressed yeast cells consuming glucose is high enough to reduce the glucose transport rate by 50%. J Bacteriol., 1998, 180(3), 556-562.