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Possible Hypoglycemic Attributes of *Morus indica* l. and *Costus speciosus:* An *in vitro* Study

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ABSTRACT

Introduction: Medicinal plants have been reported to play an important role in modulating glycemic responses; they are also known to have preventive and therapeutic implications in disorders of carbohydrate and lipid metabolism. This study reports the possible hypoglycemic effects of *Morus indica* (Mulberry) and *Costus speciosus* (Insulin plant) in an i*n vitro* system. **Methods:** Glucose adsorption, diffusion and starch hydrolysis of Mulberry leaf powder (MLP) and Insulin plant powder (IPP) were studied using *in vitro* techniques that simulated gastrointestinal conditions and compared with commercial dietary fibre sources such as wheat bran (WB), acarbose (ACB) and guar gum (GG) at three different levels (2, 4, and 6 %). **Results:** The glucose binding capacity of both *Morus indica.*L (MLP) and *Costus speciosus* (IPP)increased with increased levels and was significantly high compared to wheat bran and acarbose. At higher levels (4 and 6 %), the diffusion rate of glucose was lower compared to wheat bran, acarbose and guar gum. The α-amylase inhibitory effect was significantly high in MLP (51%) and IPP (18%) compared to WB (8%). The effect of samples on glucose diffusion was also studied in a system comprising of starch-α-amylase sample. The glucose diffusion rate was significantly low in the systems where MLP (6%) and IPP (6%) were used compared to the positive control and to commercial sources of fibre (ACB and GG). **Conclusion:** The data reveals that the samples may lower the rate of glucose absorption and as a result, decrease postprandial hyperglycemia by these mechanisms.

Keywords: α-amylase inhibition, diffusion, *Costus speciosus,* glucose adsorption, *in vitro* hypoglycemic effect, *Morus indica.* L

INTRODUCTION

Traditional medicinal plants have been used since ancient times to treat a great variety of human diseases such as diabetes, coronary heart disease and cancer (Tiwari & Rao, 2002) Clinical examinations have demonstrated hypoglycemic activity in extracts from many plants (Kelkar *et al.*, 1996, Ragavan & Krishnakumari 2006; Kumar, Shetty & Salimath, 2005; Vasanthamani & Savitha, 2001; Chandrashekar, Mukherjee & Mukherjee, 1989). Nevertheless, the mechanisms of action and potential toxicity of plant tissues and extracts that can influence blood glucose levels are generally unknown.

Many Indian medicinal plants have been found to be useful in successfully managing diabetes, significant among them are *Gymnema sylvestre, Pterocarus marupium,*

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Eugenia jambolana, Swertia chiraita, Syzigium cumini, Momordica charantia, Fenugreek, *T. arjuna, Nigella sativa* L. The active principles have been isolated from some of them (Kaleem *et al.*, 2006; Shukla *et al.*, 2000). The extracts of *Morus indica* L, have been reported to possess medicinal properties including hypoglycemic, hypotensive and diuretic activities (Andallu *et al.*, 2001).

The main interest of studies into medicinal plants with hypoglycemic effect is to understand the physiological effects that they may have in the human gastrointestinal tract. Among the chemical constituents of plant foods, dietary fibre components are well known for their different physiological effects, both direct and indirect (Brigenti *et al.*, 1995). High fibre foods have been suggested to play an important role in modulating the glycemic response, on the basis of studies with purified fibres (Jenkins *et al.*, 1978) and with fibre rich foods, particularly, legumes (Jenkins *et al.*, 1980).

The objectives of the present study were to determine the functional properties of two medicinal plants viz., *Morus indica*.L, and *Costus speciosus* and to evaluate their potential hypoglycemic effects by using several *in vitro* methods. The influence of these samples on the diffusion of glucose and the enzymatic degradation of starch was determined and compared with commercial fibres viz., acarbose and wheat bran.

METHODS

Fresh leaves of *Morus indica* L. (MLP, mulberry) were obtained from the Department of Studies in Sericulture, University of Mysore, India; *Costus speciosus* (IPP, common name- insulin plant) from a local horticultural farm and commercial sources of dietary fibre-guar gum (GG), acarbose (ACB) and wheat bran (WB) were purchased from a local store.

Processing treatment

Fresh leaves of mulberry and insulin plant were cleaned, oven dried (55-60°C), ground, passed through a sieve and stored in an airtight container at 4° C until further use.

Water and oil holding capacity

Water holding capacity of the samples was determined by centrifuge method (Sosulki, 1962). Samples amounting to 5 g were taken in 50 ml centrifuge tubes, Distilled water (30ml) was added to each tube and the contents were mixed well (30sec) using a glass rod. The tubes were allowed to stand for 10 min and mixed a further seven times at 10-min intervals. Adhering particles were washed into the sample with 10 ml of distilled water. The suspensions were centrifuged at 2,300 rpm for 25 min, the supernatant was decanted, the tubes were drained and dried in the oven at 50° C for 25 min and then cooled in a dessicator and weighed. The oil holding capacity of the samples was also determined following the above procedure. The results are expressed as g/g water and oil bound by the samples.

Glucose-adsorption capacity

MLP and IPP at three different levels viz, 2, 4 and 6%, and wheat bran (2%) each were added to 25 ml of glucose solution (5 - 50 mmol/L); the mixture was stirred well, held in a water bath at 37° C for 6 h, and then centrifuged at 4000rpm for 20min. The glucose content in the supernatant was determined. The bound glucose was calculated as follows. Bound glucose = (glucose concentration of original solution - glucose concentration after 6 h) x volume of solution divided by wt of sample (Ou *et al.*, 2000).

Guar gum (0.8%) and acarbose (0.2%) were added to 25ml of glucose solution (20 mmol/L). The solution mixtures were dialysed against 200 ml of distilled water at 37°C. The glucose content in the dialysate was determined after 6 h. Bound glucose

was calculated as follows:
Bound glucose=[glucose concentration in retentate before start of diffusion x (volume of retentate)-(glucose concentration in dialysate after 6 h) x (total volume of retentate and dialysate)] divided by wt of sample.

Glucose Dialysis Retardation Index (GDRI)

The glucose-dietary fibre system comprised 20 mmol/L of glucose and one of the following dietary fibres: MLP and IPP at three different levels (2, 4 and 6%), guar gum (0.8%), wheat bran (2%) and acarbose (0.2%). A total of 37 samples (2 replicates of each fibre and a blank), 25 ml each, were dialysed in dialysis bags with a cut-off molecular weight of 12,000 (Hi media laboratories Pvt. Ltd Mumbai, India) against 200 ml of distilled water at 37°C. The glucose content in the dialysate was determined after 30, 60, 120 and 180 min (Ou *et al.*, 2000). It was calculated using the following equation:
GDRI= 100 – [(glucose content with the addition of fibre)/ (glucose content of the control)] x 100.

α-amylase inhibitory activity (%)

The effect of selected samples on α -amylase activity was studied using enzyme-starch system. A total of 24 samples viz. two replicates of control, positive control, MLP (2%), IPP (2%), WB (0.8%) and acarbose (0.2%) were studied. Each of the samples were mixed by stirring 25 ml of potato starch solution (4%) in a beaker, 100 mg of α–amylase (from porcine pancreas 1300 u/ mg, from Hi media Laboratories Limited, Mumbai, India.), stirred vigorously and incubated at 37°C for 60 min. After a period of incubation, 0.1mol/L NaOH was added to terminate α-amylase activity. The mixture was centrifuged for 15 min (3000 rpm) and glucose content in the supernatant was determined. A control test was also run without the addition of fibre. The amylase inhibitory activity (%) is defined as the percent decrease in the glucose production rate over the control.

Determination of amylolysis kinetics

Forty grams of potato starch was added to » 900 ml of 0.05M phosphate buffer (pH 6.5). The solution, after stirring at 65°C for 30 min, was made up to a final volume of 1000 ml to give a 4 % (w/v) starch solution. The starch-α-amylase-dietary fibre system comprised the above starch solution, α-amylase (0.4%), and one of the following dietary fibres: MLP and IPP at three different levels (2, 4 and 6%), guar gum (0.8%), wheat bran (2%) and acarbose (0.2%). A total of 37 samples (2 replicates of each fibre and a blank), 25 ml each, were dialysed in dialysis bags with a cutoff molecular weight of 12,000 against 200ml of distilled water at 37°C (pH 7.0). The glucose content in the dialysate was determined after 60, 120, 180 and 240 min. A control test was carried out without the addition of samples/fibre. The glucose content in the above experiments was estimated using GOP-POD enzymatic kit (Span Diagnostics, Surat, India).

Statistical analysis

All determinations, which were replicated twice were subjected to one way ANOVA and Tukey's multiple comparison test using 11.0 version SPSS computer software.

RESULTS

Water and oil holding capacity

The water holding capacity was higher in MLP (4.45g/g)compared to WB (2.55g/g), IPP (1.94 g/g) and ACB (1.20 g/g). The oil holding capacities of MLP, IPP and WB were 1.63, 1.41 and 1.82 g/g respectively.

Glucose adsorption capacity of the selected samples

Table 1 shows the glucose adsorption capacities of the selected samples over time. The glucose binding capacity increased

Table 1. Glucose bound by the samples at different glucose concentrations

Sample	*Glucose adsorbed*			
	50 mmol	*20 mmol*	*10 mmol*	*5 mmol*
WB (2%)	17.29 ± 0.10^{al}	5.56 ± 0.07^{am}	3.16 ± 0.10^{an}	1.25 ± 0.04^{ao}
ACB (0.02%)	18.54 ± 0.06^{bl}	6.97 ± 0.10^{bm}	3.21 ± 0.03^{an}	3.56 ± 0.13^{bo}
GG (0.8%)	32.11 ± 0.05^{cl}	15.28 ± 0.03^{cm}	5.96 ± 0.03^{bn}	2.02 ± 0.06^{co}
MLP (2%)	17.91 ± 0.13^{dl}	7.60 ± 0.06^{dm}	2.44 ± 0.10^{cn}	1.87 ± 0.05^{do}
MLP (4%)	26.35 ± 0.10^{el}	10.66 ± 0.10^{em}	3.50 ± 0.06^{dn}	2.81 ± 0.05^{eo}
MLP (6%)	29.83 ± 0.14^{fl}	13.79 ± 0.11^{fm}	4.75 ± 0.10^{en}	3.51 ± 0.13^{ao}
IPP (2%)	16.76 ± 0.15^{gl}	6.64 ± 0.20^{am}	0.94 ± 0.10^{fn}	0.50 ± 0.08^{fo}
IPP (4%)	24.64 ± 0.15^{hl}	7.30 ± 0.12^{gm}	2.51 ± 0.10^{gn}	1.00 ± 0.11^{go}
IPP (6%)	26.18 ± 0.10^{il}	8.09 ± 0.10^{hm}	3.77 ± 0.11^{hn}	1.53 ± 0.10^{ho}

Notes: Mean values carrying different superscripts a, b, c….i, in columns differ significantly. Mean values carrying different superscripts l, m, n…..o, in rows differ significantly. WB – Wheat Bran; ACB– Acarbose; GG – Guar Gum; MLP – Mulberry Leaf Powder; IPP– Insulin Plant Powder.

with increased levels of the samples. All the five samples could bind glucose effectively, and the amounts of bound glucose was dependent on the concentration of the sample and glucose. The samples were effective in adsorbing glucose at both low and higher concentrations of glucose used in the study (5 and 50 mmol/L). At different concentrations (50, 20, and 10 mmol) of glucose solution, the adsorption capacity of the samples studied was significantly (p<0.05) higher compared to commercial fibres (ACB and WB) but lower compared to GG. At a lower concentration (5mmol), there was no significant difference between ACB and MLP (6%). From the data, it was observed that as the concentration of the glucose decreased from 50 to 5 mmol, the adsorption capacity of all the samples also decreased significantly. It was interesting to observe that the adsorption capacity of the samples increased with increased concentration of glucose while the trend was inverse in commercial fibres.

Effect of selected samples on GDRI

Glucose diffusion in the model system was affected by the selected samples (Table 2). The diffusion of glucose was significantly (p<0.05) low in all the systems compared to the control at each time interval. The glucose diffusion rates for MLP (2%) and ACB were found to be similar at all time intervals. Diffused glucose was significantly low (P<0.05) for both MLP and IPP at 4 and 6% over 3 h. Among all samples, IPP (6%) could significantly decrease the diffusion of glucose at all time intervals. On the basis of the retardation in glucose diffusion, GDRI for different fibre sources could be calculated (Table 2). The GDRI values for all the samples were found to be significantly high (p< 0.05) at 30-min time intervals, with the GDRI decreasing at 60 and 120 min but again increasing significantly at 180 min in all the systems. IPP (6%) had higher GDRI value at all time intervals.

α-amylase inhibitory effect of the selected samples

The effect of selected samples on the inhibitory effect of α -amylase was studied (Figure 1). The α-amylase inhibitory effect was found to be significantly high in MLP (51%) and IPP (18%) compared to WB (8%), whereas it was significantly low compared to ACB (80%). The order of α-amylase inhibitory effect of the selected samples was as follows: positive control >WB>IPP> MLP >ACB.

Table 2. Effect of selected samples on glucose diffusion in glucose dietary fibre system and GDRI

Sample	*Glucose content in the dialysate (mmol)*			
	30 min	*60 min*	*120 min*	*180 min*
Control	0.75 (34.78)^{b}	1.36 (0.0)^{a}	1.53 (0.0)^{a}	1.90 (0.0)^{a}
WB (2%)	0.75 (34.78)^{b}	1.18 (13.24)^{b}	1.60 (2.00)^{b}	1.78 (10.50)^{b}
ACB (0.02%)	0.63 (45.22)^{c}	0.63 (45.22)^{e}	1.48 (3.30)^{c}	1.65 (21.43)^{c}
GG (0.8%)	0.55 (52.20)^{de}	1.08 (21.00)^{c}	1.29 (15.70)^{d}	1.48 (29.52)^{d}
MLP (2%)	0.60 (48.00)^{cd}	1.12 (18.00)^{bc}	1.43 (6.54)^{c}	1.68 (20.0)^{c}
MLP (4%)	0.48 (59.32)^{ef}	0.91 (33.10)^{d}	1.16 (24.20)^{e}	1.35 (35.71)^{e}
MLP (6%)	0.44 (61.74)^{f}	0.80 (41.20)^{g}	0.98 (35.94)^{f}	1.16 (44.76)^{f}
IPP (2%)	0.55 (52.20)^{de}	0.98 (41.20)^{d}	1.36 (35.94)^{g}	1.36 (35.94)^{g}
IPP (4%)	1.36 (35.94)^{g}	0.82 (40.00)^{g}	1.09 (28.76)^{h}	1.25 (40.50)^{g}
IPP (6%)	0.33 (71.30)^{g}	0.68 (50.00)^{f}	0.89 (42.00)^{i}	1.05 (50.00)^{h}

Notes: Values given in parenthesis indicate Glucose Dialysis Retardation Index (GDRI). Mean values carrying different superscripts a,b, c....g, in columns differ significantly ($p<0.05$).

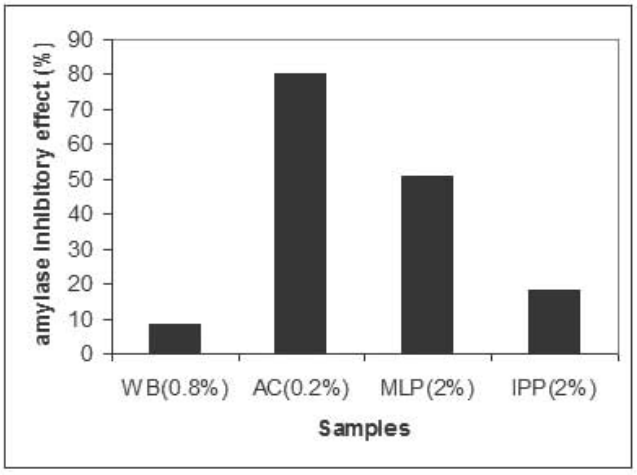

Figure 1. Amylase inhibitory activity of the samples,
Note: WB – Wheat Bran; ACB – Acarbose; MLP – Mulberry Leaf Powder; IPP – Insulin Plant Powder.

Effect of selected samples on amylolysis kinetics and GDRI

The selected samples affected the diffusion rate of glucose in the starch-α-amylase-dietary fibre system (Table 3). Compared to positive control, the diffusion rate of glucose in the systems containing samples/ fibres, were significantly low at each time interval. The diffusion rate of glucose decreased in the systems with increased levels of the sample. The glucose diffusion rate was significantly low in the systems where MLP (6%) and IPP (6%) were used, compared to the positive control and to commercial sources of fibre (ACB and GG). It was interesting to observe that there was

Table 3. Effect of selected samples on amylolysis kinetics and GDRI

Samples	*Glucose content in the dialysate (mmol)*			
	60 min	*120 min*	*180 min*	*240 min*
Control	0.12 (0.0)^{ab}	0.28 (0.0)^{a}	0.50 (0.0)^{a}	0.75 (0.0)^{a}
WB (2%)	0.09 (25.00)^{cd}	0.15 (46.42)^{b}	0.20 (60.00)^{b}	0.24 (57.33)^{b}
ACB (0.02%)	ND	ND	ND	ND
GG (0.8%)	0.01 (91.66)^{f}	0.10 (35.71)^{c}	0.20 (60.00)^{c}	0.25 (66.66)^{c}
MLP (2%)	0.10 (16.66)^{bc}	0.22 (40.00)^{d}	0.30 (40.00)^{d}	0.37 (50.66)^{d}
MLP (4%)	0.09 (25.00)^{d}	0.17 (39.30)^{e}	0.25 (50.00)^{c}	0.30 (60.00)^{c}
MLP (6%)	0.04 (66.66)^{e}	0.07 (75.00)^{f}	0.14 (72.00)^{e}	0.16 (78.66)^{f}
IPP (2%)	0.14 (8.33)^{a}	0.26 (7.14)^{g}	0.40 (20.00)^{f}	0.47 (37.33)^{g}
IPP (4%)	0.11 (8.33)^{c}	0.16 (43.00)^{d}	0.28 (44.00)^{d}	0.35 (53.33)^{d}
IPP (6%)	0.04 (66.00)^{e}	0.07 (25.00)^{f}	0.11 (78.00)^{e}	0.12 (76.00)^{h}

Notes: Mean values carrying different superscripts a,b, c....g, in columns differ significantly. Values given in parenthesis indicate Glucose Dialysis Retardation Index (GDRI). WB – Wheat Bran; ACB – Acarbose; GG – Guar Gum; MLP – Mulberry Leaf Powder; IPP– Insulin Plant Powder; ND – No diffusion.

no diffusion of glucose at all time intervals in the system where ACB was used.

The GDRI values for both MLP and IPP increased over time. The GDRI value for MLP at 2, 4 and 6% were found to be 50.66, 60.00 and 78.66 and for IPP at 2, 4 and 6% were 37.33, 53.33 and 76.00 respectively. The maximal GDRI values for GG and WB was reached at 180 and 60 min respectively.

DISCUSSION

The most challenging goal in the management of type 2 diabetes is the achievement of blood glucose level as close to normal as possible with the potential of phytochemicals to combat diabetic disorders being also reported (Tiwari & Rao, 2002). Several mechanisms have been proposed for the hypoglycemic effect of phytochemicals, such as inhibition of carbohydrate metabolism enzymes, manipulation of glucose transporters, β-cell regeneration and enhancing insulin releasing activity (Tiwari & Rao, 2002).

The present study was designed to form the basis of simple *in vitro* methods to measure the potential hypoglycemic effects of two medicinal plants viz., *Morus indica.* L and *Costus speciosus.*

Reduction in postprandial hyperglycemia by dietary fibres is well known with this effect being attributed to the inhibition of amylase activity and a delayed glucose adsorption in the gastro intestinal tract. The medicinal plants were found to be considerably good sources of dietary fibre (MLP-13.4 and IPP-11.2%) (Devi, 2009) which might have exerted an effect on both glucose adsorption and diffusion.

The behaviour of the hydration properties of dietary fibre is determined by particle structure and their size (Ou *et al.*, 2000; Parrott & Thrall, 1978; Robertson & Eastwood 1981). *In vivo* and *in vitro* studies of glucose absorption have shown that the delay in glucose adsorption in the gastrointestinal tract is determined mainly by the viscosity of soluble polysaccharides (Jenkins *et al.*, 1978, Adiotomre *et al.*,1990). The increased ability of MLP to adsorb glucose may be attributed to the viscosity contributed by the soluble fibre present in the sample. The abilities of both insoluble and soluble fibres from different sources, to adsorb glucose have been reported (Adiotomre *et al.*,1990; Lopez *et al.*, 1996; Ou *et al.*, 2000; Ou, Gao & Li 1999; Chau & Huang 2003).

The increase in WHC may be due to an increase in the amount of water which can be trapped by the samples. Hydration properties are determined by the content of the water soluble dietary components of the food (Sosulki, 1962). The low WHC of WB may be due to the presence of higher amounts of cellulose and starch. Similar observations have been reported by (Sosulki & Cadden, 1982). It is reported that WHC of vegetable fibres is probably due to the vegetable fibre having a greater ability than cereal fibre to trap water within the cell matrix rather than due to its ability to bind water (Ou *et al.*, 1999). OAC is related to the surface and the density or thickness of the particles, so that those particles with the greatest surface area theoretically present a greater capacity to adsorb and bind components of an oily nature (Adiatomre *et al.*, 1990).

It was interesting to note that using MLP and IPP in the systems decreased glucose diffusion significantly compared to commercial sources of fibres. In addition to glucose adsorption, the retardation in glucose diffusion might also be attributed to the physical obstacle presented by insoluble fibre particles toward glucose molecules and the entrapment of glucose within the network formed by fibres (Lopez *et al.*, 1996; Nishimune *et al.*, 1991). From the results obtained, it was observed that the GDRI values of all the samples generally diminished as the time increased in the systems where glucose-samples/ fibre were used. It is also been reported in an earlier study that GDRI values of the fibre samples diminished over time (Chau, Huang & Lee 2003). The effect of insoluble dietary fibre in the inhibition of glucose diffusion in the small intestine is suggested to be due to the adsorption or inclusion of the smaller sugar molecules within the structure of the fibre particles (Nishimune *et al.*, 1991). In addition to the effect of physical properties of the fibre, the higher protein content MLP and IPP (24.6 and 18.7%) (Devi, 2009) obtained may have an influence on the higher values of GDRI for samples than for commercial fibers (Adiatomre *et al.*, 1990; Wood *et al.*,1990; Gourgue *et al.*, 1992). Similar observations for insoluble fractions of artichoke and dietary fibre have been reported (Lopez *et al.*, 1996). In general, MLP and IPP affected the diffusion rates of glucose at all time intervals compared to commercial fibres. However, the effect was more pronounced with higher levels of MLP and IPP.

The retardation of α-amylase activity by the samples might be attributed to several possible factors such as fibre concentration, the presence of inhibitors on fibre capsulation of starch and enzyme by the fibres present in the sample, reduced accessibility of the enzyme to starch and the direct adsorption of the enzyme on fibres leading to a decrease in amylase activity (Ou *et al.*, 2000).

Acarbose was used as it is α-glucosidase inhibitor and is currently the most commonly used oral agent to attenuate the post-prandial hyperglycemia. Inhibition of glucosidase and amylase results in delayed carbohydrate digestion and glucose absorption to attenuate the postprandial hyperglycemia.

In vitro screening is not a reliable predictor of hypoglycemic *in vivo*; however, the model systems used in this study indicate possible mechanisms by which the samples studied may function *in vivo* in lowering postprandial glucose levels. Further studies are needed to investigate whether the medicinal plants are competitive inhibitors of α-amylase or act only as a barrier between the enzyme and starch.

In summary, the plants investigated are safe, inexpensive medicinal species with potential to be developed into alternatives or adjuncts to current antidiabetic medications. Further investigations into *in vivo* studies of the antidiabetic effects of samples are currently in progress.

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