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EFFECTS OF *SYZYGIUM AROMATICUM*-DERIVED OLEANOLIC ACID ADMINISTRATION ON POSTPRANDIAL GLUCOSE CONCENTRATION AND KEY INTESTINAL CARBOHYDRATE HYDROLYZING ENZYMES IN STREPTOZOTOCIN-INDUCED DIABETIC RATS.

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INTRODUCTION

The magnitude and duration of postprandial hyperglycaemia due to hydrolysis of carbohydrates in the small intestine are major risk factors of macro- and microvascular complications in diabetes [1]. Indeed, diabetes management strategies may involve several α-glucosidase inhibitors (acarbose, voglibose, miglitol) that suppress postprandial glucose peaks [2].
Reports from our laboratory indicate that *Syzygium aromaticum*-derived oleanolic acid (OA) inhibits the absorption of glucose across the small intestine [3].
The influence of this triterpene on postprandial blood glucose concentrations is not yet established.

OBJECTIVES

The objectives of this study were to:

i. evaluate the influence of OA on postprandial hyperglycaemia after mono-, di- and polysaccharide loading in normal and STZ-induced diabetic rats

ii. examine the effects of OA on the hydrolysis of di- and polysaccharides *in vitro*.

MATERIALS AND METHODS

Isolation of oleanolic acid
OA was isolated from *Syzygium aromaticum* [(Linnaeus) Merrill & Perry] [Myrtaceae] (cloves) flower buds using a standard protocol that has been validated in our laboratory [3].
S. aromaticum cloves were sequentially extracted twice at 24 h intervals with 1 L on each occasion of dichloromethane (DCM), and ethyl acetate yielding dichloromethane solubles (DCMS) and ethyl acetate solubles (EAS). Recrystallization of EAS with ethanol yielded pure OA whose structure was confirmed by spectroscopic analysis using 1D and 2D, ^{1}H and ^{13}C NMR techniques.

EXPERIMENTAL DESIGN

The effects of OA on postprandial blood glucose changes were assessed in non-diabetic and STZ-induced diabetic male Sprague Dawley rats while the effects on intestinal carbohydrate-hydrolyzing enzymes were investigated *in vitro*.

IN VIVO STUDIES

Oral glucose tolerance (OGT) responses
OGT responses were monitored in non-diabetic and STZ-induced diabetic rats loaded with monosaccharide (glucose; 0.86 g/kg, p.o.), disaccharide (sucrose; 1.72 g/kg, p.o.) and polysaccharide (starch; 0.086 g/kg, p.o.) after an 18 h fast (n = 6 in each group). Rats treated with deionized water (3 ml/kg, p.o.), or acarbose (100 mg/kg, p.o.) acted as negative and positive controls, respectively. Blood glucose was monitored at 15 min intervals for the first 60 minutes and once at 120 minutes.

IN VITRO STUDIES

Effects of OA on carbohydrate hydrolyzing enzymes
Various concentrations of OA (20-100 µg/ml) were used to determine the IC50 value for OA on sucrase, α-amylase and α-glucosidase with sucrose (2 mg/ml) and starch (2 mg/ml) serving as substrates.

STATISTICAL ANALYSIS

All data are expressed as means ± S.E.M. The $AUC_{0.5-2h}$ values were calculated using blood glucose concentrations following the 2-hour loading with mono, di- or polysaccharide. The IC_{50} values of OA against carbohydrate hydrolyzing enzymes were calculated using the inhibitory activity of OA at various concentrations. Overall statistical comparisons between the control means and experimental groups were performed with GraphPad InStat Software (version 5.00, GraphPad Software, San Diego, California, USA), using one-way analysis of variance (ANOVA), followed by Tukey-Kramer multiple comparison test. A value of $p < 0.05$ was considered significant.

RESULTS AND DISCUSSION

By comparison with animals pre-loaded with carbohydrates alone, co-administration of OA with glucose, sucrose and starch significantly reduced the peak blood glucose spikes of separate groups of non-diabetic and STZ-induced diabetic rats (Figure 1 and 2) with a concomitant reduction in the C_{max} values (Table 1). The suppression of the postprandial glucose spikes response by OA to carbohydrate loads was associated with the reduction of the area under the blood glucose-time curve ($AUC_{0.5-2h}$) of non-diabetic and diabetic animals (Table 1). The in vitro half-maximal inhibitory concentrations (IC_{50}) of OA on sucrase, α-amylase and α-glucosidase compared with that of acarbose, the standard drug (Table 2).

CONCLUSION

These results suggest that OA suppresses postprandial hyperglycaemia perhaps via the inhibition of the carbohydrate hydrolyzing enzymes in the small intestine.

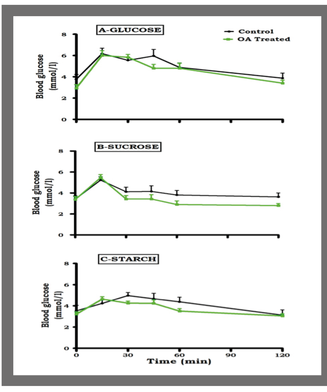

Figure 1: OGT responses to glucose (A), sucrose (B) and starch (C) loading of control and OA treated non-diabetic rats. Values are presented as means, and vertical bars indicate SEM of means (n=6 in each group).
*p<0.05 by comparison with control animals

Table 1: Comparison of the effects of OA on the Cmax and AUC with respective control groups of non-diabetic and STZ-induced diabetic rats following loading with glucose, sucrose and starch. Data are expressed as mean ± SEM (n = 6, in each group).

Group	C_{max} (mmol/l)	AUC (mmol/l/h)
Glucose	7.22±0.46	7.30±0.01
Glucose + OA	4.60±0.28	6.30±0.08*
STZ-induced diabetic glucose	27.9±1.81	45.87±1.37
STZ-induced diabetic glucose + OA	24.70±1.67	36.30±0.86 *
Sucrose	7.22±0.46	7.99±0.05
Sucrose + OA	4.60±0.28	6.30±0.08*
Sucrose + acarbose	3.65±0.31	6.18±0.03*
STZ-induced diabetic sucrose	27.9±1.81	45.87±1.37
STZ-induced diabetic sucrose + OA	24.70±1.67	36.30±0.86 *
STZ-induced diabetic sucrose + acarbose	21.6±2.07	38.79±0.74*
Starch	4.97±0.40	7.94±0.08
Starch + OA	4.83±0.19	7.02±0.08*
Starch + acarbose	3.75±0.22	6.76±0.06*
STZ-induced diabetic starch	24.3±2.03	52.81±1.56
STZ-induced diabetic starch + OA	21.8±1.92	40.95±1.33*
STZ-induced diabetic starch + acarbose	20.1±1.75	38.65±0.92*

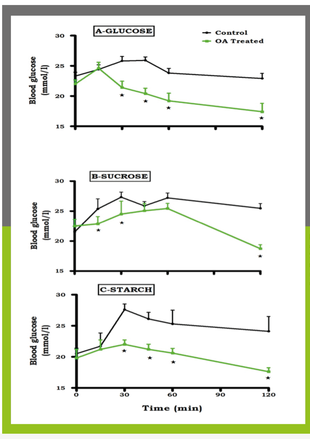

Figure 2: OGT responses to glucose (A), sucrose (B) and starch (C) loading of control and OA treated STZ-induced diabetic rats. Values are presented as means, and vertical bars indicate SEM of means (n=6 in each group). *p<0.05 by comparison with control animals

Table 2: Effects of OA and acarbose on the activity of sucrase, α-amylase and α-glucosidase

	IC_{50}(µg/ml)		
	Sucrase	α-amylase	α-glucosidase
OA	59.74±1.76	62.11±1.80	56.45±1.75
Acarbose	43.65±1.84	80.25±1.90	47.97±1.68

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