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In vitro α-glucosidase and α-amylase enzyme inhibitory effects of *Andrographis paniculata* extract and andrographolide

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There has been an enormous interest in the development of alternative medicines for type 2 diabetes, specifically screening for phytochemicals with the ability to delay or prevent glucose absorption. The goal of the present study was to provide *in vitro* evidence for potential inhibition of α-glucosidase and α-amylase enzymes, followed by a confirmatory *in vivo* study on rats to generate a stronger biochemical rationale for further studies on the ethanolic extract of *Andrographis paniculata* and andrographolide. The extract showed appreciable α-glucosidase inhibitory effect in a concentration-dependent manner (IC_{50} = 17.2 ± 0.15 mg/ml) and a weak α-amylase inhibitory activity (IC_{50} = 50.9 ± 0.17 mg/ml). Andrographolide demonstrated a similar (IC_{50} = 11.0 ± 0.28 mg/ml) α-glucosidase and α-amylase inhibitory activity (IC_{50} = 11.3 ± 0.29 mg/ml). The positive *in vitro* enzyme inhibition tests paved way for confirmatory *in vivo* studies. The *in vivo* studies demonstrated that *A. paniculata* extract significantly ($P < 0.05$) reduced peak blood glucose and area under curve in diabetic rats when challenged with oral administration of starch and sucrose. Further, andrographolide also caused a significant ($P < 0.05$) reduction in peak blood glucose and area under the curve in diabetic rats. Hence α-glucosidase inhibition may possibly be one of the mechanisms for the *A. paniculata* extract to exert antidiabetic activity and indicates that AP extract can be considered as a potential candidate for the management of type 2 diabetes mellitus.

Keywords: α-glucosidase, α-amylase, *Andrographis paniculata*, andrographolide, peak blood glucose, post prandial hyperglycaemia.

INTRODUCTION

Andrographis paniculata (Burm.f.) Nees (Acanthaceae) (Creat in English) is a traditional medicinal plant common in South East Asia and found from India to Indo-China. It is commonly known as king of bitter, kariyat, kalmegh, hempedu bumi and pokok cerita. It is an annual, erect and branched plant with lanceolate green leaves and attains heights of 60–70 cm. The leaves and aerial parts of the plant have been used to cure various kinds of ailments. *A. paniculata* contains andrographolide as the major active principle and also others like 14-deoxy-11,12-didehydroandrographolide, 14-deoxyandrographolide etc. The plant was also reported for its hypotensive action by Zhang and Tan (1996). Further studies by Zhang and Tan (1997), Zhang *et al.* (1998), and Tan and Zhang (1998) showed the hypotensive effect to be strongly correlated with 14-deoxy-11,12-didehydroandrographolide and 14-deoxyandrographolide. Amroyan *et al.* (1999) reported on the antithrombotic effect of *A. paniculata*. Anticancer activity of dichloromethane fraction of methanolic extract from *A. paniculata* was reported by Kumar (2004) and the active principles responsible were found to be andrographolide, 14-deoxyandrographolide and 14-deoxy-11,12-didehydroandrographolide. The ameliorative activity of andrographolide and arabinogalactan

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Abbreviations: Acar, acarbose; AG, andrographolide; AP, *Andrographis paniculata* extract; AUC, area under curve; DC, diabetic control; NC, normal control; NYDMM, non-insulin-dependent diabetes mellitus; PGB, peak blood glucose.

proteins against alcohol-induced hepatic and renal toxicity was reported by Singha *et al.* (2007). Antiangiogenic activity of ethanolic *A. paniculata* extract and andrographolide on mice was reported by Sheeja *et al.* (2007). Zhang and Tan (2000b) evaluated the antihyperglycemic effect and antioxidant effects with 14-day treatment with ethanolic extract of the aerial parts of *A. paniculata* (100, 200, and 400 mg/kg) in normal and streptozotocin-induced type 1 diabetic rats. Also, Yu *et al.* (2003) studied the antihyperglycemic action of andrographolide, in streptozotocin-induced diabetic rats and reported that andrographolide treatment at a dose of 1.5 mg/kg decreased the plasma glucose concentration. *A. paniculata* standardized dried extract was evaluated for toxicity in male rats. No toxicity was found with the treatment of 20, 200 and 1000 mg/kg (Burgos *et al.,* 1997). It was concluded that *A. paniculata* dried extract did not produce sub chronic toxicity in male rats. The LD_{50} of andrographolide in male mice was established at 11.46 g/kg i.p. (Handa & Sharma, 1990).

Inhibition of α-glucosidase (EC 3.2.1.20) and α-amylase (EC 3.2.1.1), enzymes involved in the digestion of carbohydrates, can significantly decrease the postprandial increase of blood glucose after a mixed carbohydrate diet and therefore can be an important strategy in the management of postprandial blood glucose level in type 2 diabetic patients and borderline patients (Ali *et al.,* 2006). Currently there is renewed interest in functional foods and plant-based medicines modulating physiological effects in the prevention and cure of diabetes and obesity. Hence the attractive targets like *in vitro* inhibition of α-glucosidase and α-amylase enzymes are currently in vogue. Previously, several *in vitro* studies have been performed yielding potential α-glucosidase inhibitors from various food components and plants like cranberry (Apostolidis *et al.,* 2006), *Cuscuta reflexa* (Anis *et al.*, 2002), pepper (Pullela *et al.*, 2006), soy bean extracts, etc. (Georgetti *et al.*, 2006), and α-amylase inhibitors from cheese (Apostolidis *et al.*, 2007), oregano (McCue *et al.*, 2004), cranberry extract (Apostolidis *et al.,* 2006), Fenugreek and Balanite (Gad *et al.*, 2006), etc. Therefore, natural α-glucosidase and α-amylase inhibitors from plant sources offer an attractive strategy for the control of postprandial hyperglycemia.

Plant extracts have long been used for the ethnomedical treatment of diabetes in various systems of medicine and are currently accepted as an alternative for diabetic therapy. However, for many plant extracts, there is no clear understanding of the mechanism of action. The *in vitro* α-glucosidase inhibitory activity (Shen & Chen, 2002) may not always correlate with the *in vivo* one (Ye *et al.*, 2002). So, it is necessary to confirm the *in vivo* action after oral administration to live animals, which is an important step in screening plant extracts for physiological and pharmacological effects.

However, *in vitro* data is also useful, particularly when a large number of compounds are to be tested, or when compounds are synthesised with minor modifications in functional groups or different percentages of extract/fractions etc.; then a simple *in vitro* test can be performed to rule out inactive compounds and hence save considerable time and money.

There are no previous reports of any *in vitro* α-glucosidase and α-amylase inhibitory activity of *A. peniculata* extract and andrographolide. Hence *in vitro* α-glucosidase and α-amylase enzyme inhibition studies together with *in vivo* studies to confirm the activity in live animals were carried out to evaluate the possible antihyperglycemic potential of ethanolic extract of *Andrographis paniculata* (AP) and the principal active constituent andrographolide (AG) (Akowuah *et al.*, 2006).

MATERIALS AND METHODS

Plant material and preparation of extracts. Dried leaves and aerial parts of *Andrographis paniculata* were obtained from cultivated nurseries of the Malaysian Agriculture Development Institute (MARDI, Kelantan, Malaysia). The dried leaves were powdered using a milling machine and were extracted with 20% (v/v) ethanol (R & M Chemicals, Essex, UK) using the technique of cold maceration. Solvent was replenished every 24 h for 7 days. Extracts from previous days were pooled and filtered through a porous plug of absorbable cotton. The filtrate was then concentrated at 60°C using a rotary evaporator (Buchi Labortechnik, Flawil, Switzerland). Finally the concentrated extract was freeze dried (Labconco Corporation, Kansas City, MO, USA) to yield a dry powder. The yield of the extract was around 7.7%.

***In vitro* α-glucosidase inhibition study** (Kim *et al.*, 2000). Yeast α-glucosidase (Cat. No. G 5003, Sigma Aldrich Chemical Co, USA) was dissolved at a concentration of 0.1 U/ml in 100 mM phosphate buffer, pH 7.0, containing bovine serum albumin 2000 mg/ml (Sigma Aldrich Chemical Co, USA), and sodium azide 200 mg/ml (Sigma Aldrich Chemical Co, USA) which was used as enzyme source; *para*-nitrophenyl-α-D-glucopyranoside (Cat No: N 1377, Sigma Aldrich Chemical Co, USA) was used as substrate. *A. paniculata* extract (20%, v/v) was weighed and serial dilutions of 62.5, 31.25, 15.6, 7.8, 3.9, and 1.95 mg/ml were made up with equal volumes of dimethylsulfoxide and distilled water. Ten microliters of AP extract dilutions was incubated for 5 min with 50 µl enzyme source. After the incubation, 50 µl of substrate was added and further incubated for

5 min at room temp. The pre substrate and post substrate addition absorbance was measured at 405 nm on a microplate reader (Power Wave X 340™, Biotek Instruments Inc, USA). The increase in absorbance on substrate addition was obtained. Percent α-glucosidase inhibition was calculated as follows: (1–B/A) × 100, where A is the absorbance of control and B is the absorbance of samples containing extracts. The inhibitory concentration of the extract required to inhibit the activity of the enzyme by 50% (IC_{50}) was calculated by regression analysis. Experiments were performed in duplicate. Pure andrographolide (98% purity, Cat. No. 36,564-5, Sigma Aldrich Chemical Co, USA) was dissolved in methanol and serial dilutions of 10, 5, 2.5 and 1.25 mg/ml were prepared. Acarbose (Bayer Pharmaceuticals, Leverkusen, Germany) was dissolved in distilled water and serial dilutions of 10, 5, 2.5 and 1.25 mg/ml were made and used as positive control.

***In vitro* α-amylase inhibition study** (Apostolidis, 2007). Twenty-five microliters of 20% (v/v) AP extract and 25 µl of 20 mM phosphate buffer pH 6.9, containing porcine α-amylase (Cat. No. 10080, Sigma Aldrich Chemical Co, Steinheim, Germany) at a concentration of 0.5 mg/ml were incubated at 25°C for 10 min. After pre incubation, 25 µl of 0.5% starch (R & M Chemicals, Essex, UK) solution in 20 mM phosphate buffer, pH 6.9, was added. The reaction mixtures were then incubated at 25°C for 10 min. The reaction was stopped with 50 µl of 96 mM 3,5-dinitrosalicylic acid (DNS) (Cat. No. D 0550, Sigma Aldrich Chemical Co, USA) color reagent. The microplate was then incubated in a boiling water bath for 5 min and cooled to room temp. Absorbance (A) was measured at 540 nm. Percent inhibition was calculated as follows:

$$\% \text{ Inhibition} = \frac{A_{540}\,\text{control} - A_{540}\,\text{exp.}}{A_{540}\,\text{control}} \times 100$$

Control incubations represent 100% enzyme activity and were conducted in a similar way by replacing extracts with vehicle (25 µl dimethylsulfoxide and distilled water). For blank incubation (to allow for absorbance produced by the extract), enzyme solution was replaced by buffer solution and absorbance recorded. Separate incubation carried out for reaction t = 0 was performed by adding samples to DNS solution immediately after addition of the enzyme. The concentration of the extract required to inhibit the activity of the enzyme by 50% (IC_{50}) was calculated by regression analysis. Experiments were performed in duplicate.

Confirmatory *in vivo* studies

Experimental animals. Female Sprague-Dawley rats weighing 200–250 g were obtained from the Central Animal House, Universitiy Sains Malaysia (Penang, Malaysia), and housed in the Animal Transit Room, School of Pharmaceutical Sciences, University Sains Malaysia (Penang, Malaysia), 2–3 days before the start of experiment. All the animals used were cleared by the Animal Ethics Committee, University Sains Malaysia, and maintained according to international and national ethical guidelines. The animals had access to food and water *ad libitum*. Diabetes was induced in the animals by a single intraperitoneal injection of 45 mg/kg body mas streptozotocin (Sigma Aldrich Chemical Co., USA) in ice cold citrate buffer, pH 4.5 (Cyberscan® 500 pH meter, Eutech Cybernetics, Singapore). Blood glucose levels were constantly monitored using Accu-Chek® Advantage-II Glucose meter (Roche Diagnostics, Manheim, Germany) and rats showing blood glucose level around 10–15 m.mol/lit (180–270 mg/dl) were included in the study. Acarbose was used as a positive control at a dose of 10 mg/kg body mass. Andrographolide was used at a dose of 10 mg/kg.

Oral carbohydrate challenge tests (Ye *et al.*, 2002). The oral carbohydrate tolerance tests were carried out using starch, sucrose and glucose separately both in normal and diabetic groups of rats and were equally divided into various treatment groups.

Oral starch tolerance test. Rats were divided into six groups consisting of six rats (n = 6) in each group. The rats were fasted overnight for 18 h but had free access to water. Treatment group 1 rats were treated orally with 250 mg/kg body mass of 20% (v/v) AP extract (D1), treatment group 2 rats was treated orally with 500 mg/kg body mass of 20% (v/v) AP extract (D2), treatment group 3 rats received orally 1000 mg/kg body mass of 20% (v/v) AP extract (D3), treatment group 4 rats were treated orally with distilled water 4 ml/kg (normal control, NC). Treatment group 5 rats were treated orally with positive control acarbose (Acar) 10 mg/kg body mass and group 6 rats treated with andrographolide (AG) 10 mg/kg. After 10 min, all rats were given starch 3 g/kg body mass orally and the tail was snipped for blood glucose estimation before (0 min) and at 30, 60, and 120 min after starch administration. Blood glucose concentrations were recorded and peak blood glucose (PBG) and area under the curve (AUC) determined. The maximum blood glucose concentration found during blood glucose determination was taken as the PBG. The formula for AUC determination is as follows:

$$\text{AUC}\,(\text{m}\cdot\text{mol}/(\text{l}\cdot\text{h}) = \frac{BG_0 + BG_{30}}{2} \times 0.5 + \frac{BG_{30} + BG_{60}}{2} \times 0.5 + \frac{BG_{60} + BG_{120}}{2} \times 1$$

For diabetic controls (DC), rats were administered distilled water 4 ml/kg.

Oral sucrose tolerance test. The oral sucrose tolerance test was carried out with 20% AP extract, Acar, and AG in the same way as above, but in this test sucrose (R & M Chemicals) at a dose of 4 g/kg body mass was used.

Oral glucose tolerance test. The oral glucose tolerance test was carried out with 20% AP extract, Acar, and AG in the same way as above, but glucose was used here (R & M Chemicals) at a dose of 2 g/kg body mass.

Statistical analysis. The values are expressed as mean ± S.E.M. Statistical difference in PBG and AUC between control and treatment groups was determined using Statistical Package for Social Sciences (SPSS) one-way analysis of variance (ANOVA) followed by Tukey's test for post hoc analysis. $P < 0.05$ was considered as significant.

RESULTS

In vitro α-glucosidase inhibition study

The *in vitro* α-glucosidase inhibitory studies demonstrated that both AP extract and AG had α-glucosidase inhibitory activity. The percentage inhibition at 62.5, 31.25, 15.6, 7.8, 3.9, 1.95 mg/ml concentrations of AP extract showed a concentration-dependent reduction in percentage inhibition. Thus the highest concentration of 62.5 mg/ml tested showed a maximum inhibition of nearly 89%. The percentage inhibition varied from 89–3.2% from the highest concentration to the lowest concentration of 1.95 mg/ml. AG showed a strong inhibitory potential with percentage inhibitions ranging from 53.7–3.5% for concentrations ranging from 10–1.25 mg/ml. Figures 1, 2 and 3 illustrate the inhibitory activity of AP extract, AG and Acar against yeast α-glucosidase. AP extract seems to be less potent in α-glucosidase inhibitory potential compared to Acar and AG. It may be that α-glucosidase is more sensitive towards Acar with the concentration required for 50% inhibition (IC_{50}) found to be 6.2 mg/ml. The inhibitory concentrations of AP extract and AG were found to be 17.2, and 11 mg/ml respectively. The IC_{50} values are summarised in Table 1.

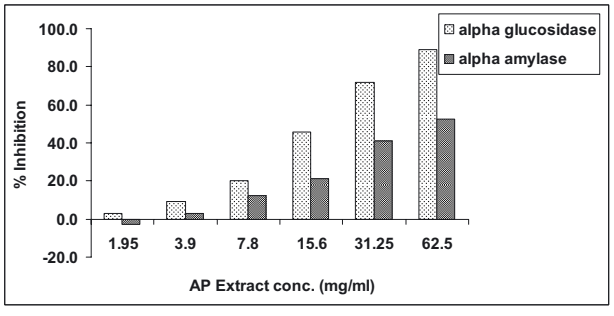

Figure 1. Inhibitory activity of AP extract against yeast α-glucosidase (0.1 U/ml) and porcine α-amylase (0.5 mg/ml). Experiments were run as detailed in Materials and Methods, result represented as mean ± S.D. of percent enzyme activity.

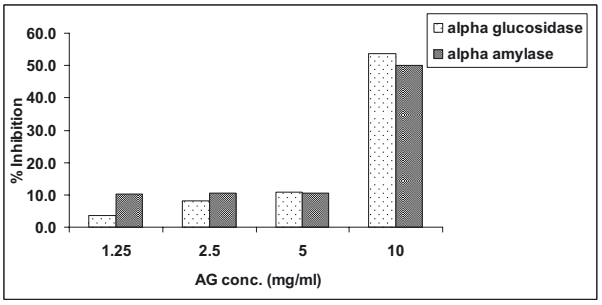

Figure 2. Inhibitory activity of AG against yeast α-glucosidase (0.1 U/ml) and porcine α-amylase (0.5 mg/ml). Experiments were run as detailed in Materials and Methods, result represented as mean ± S.D. of percent enzyme activity.

Table 1. IC_{50} values for *in vitro* yeast α-glucosidase and α-amylase inhibition by AP extract, Acar and AG.

Analyte	Inhibitory concentration	(IC_{50})
α-glucosidase	α-amylase	
AP extract	17.2 ± 0.15	50.9 ± 0.17
Acarbose	6.2 ± 0.33	14.9 ± 0.23
Andrographolide	11.0 ± 0.28	11.3 ± 0.29

All the analyses were done in duplicate. At least four serially diluted solutions of each analyte were taken for calculation of the IC_{50} values.

In vitro α-amylase inhibition study

The AP extract produced a weak α-amylase enzyme inhibition. The maximum inhibition was 52.5% at a concentration of 62.5 mg/ml. The percent-

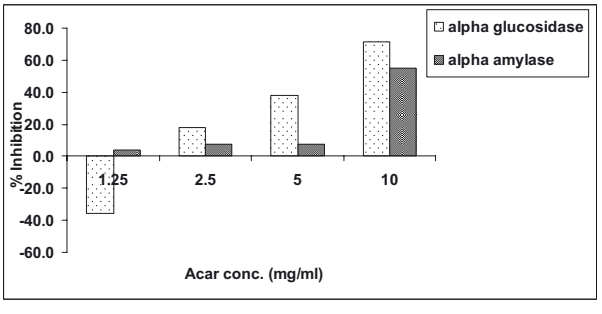

Figure 3. Inhibitory activity Acar against yeast α-glucosidase (0.1 U/ml) and porcine α-amylase (0.5 mg/ml). Experiments were run as detailed in Materials and Methods, result represented as mean ± S.D. of percent enzyme activity.

Table 2. Effect of AP extract, Acar, and AG on AUC after starch or sucrose loading in normal and diabetic rats

	AUC (m.mol/lit)	
Group	Starch	Sucrose
Normal rats		
D1	12.2 ± 0.44	12.8 ± 0.57
D2	11.6 ± 0.46	11.6 ± 0.59*
D3	11.7 ± 0.46	11.3 ± 0.54*
NC	12.9 ± 0.54	13.8 ± 0.48
AG	11.6 ± 0.47	11.3 ± 0.42*
Acar	8.8 ± 0.26*	9.9 ± 0.45*
Diabetic rats		
D1	25.7 ± 1.15*	26.3 ± 0.47
D2	22.1 ± 0.51*	23.8 ± 0.90*
D3	20.7 ± 0.51*	22.3 ± 0.76*
DC	29.8 ± 0.77	30.0 ± 1.12
AG	22.2 ± 0.78*	24.7 ± 0.82*
Acar	19.9 ± 0.35*	21.9 ± 0.97*

D1: 250 mg/kg AP extract; D2: 500 mg/kg AP extract; D3: 1000 mg/kg AP extract; NC: normal control administered 4 ml/kg distilled water; DC: Diabetic control administered 4 ml/kg distilled water; AG: andrographolide 10 mg/kg, and Acar: acarbose 10 mg/kg. Starch was used at 3 gm/kg and sucrose was used at 4 gm/kg. Values are the mean ± S.E.M. (n = 6). *$P < 0.05$ compared with the control. One way ANOVA followed by Tukey's test for post hoc analysis.

age inhibition ranged from 52.5 to 12.5%. The 3.9 and 1.95 mg/ml concentrations failed to produce any detectable inhibition. Acar showed a maximum percentage inhibition of 50.1 at 10 mg/ml. The inhibition ranged from 50.1–10.3% and was not concentration dependent. AG produced a maximum inhibition of 54.8% at 10 mg/ml. At the lowest concentration of 1.25 mg/ml, there was about 4.1% inhibition. Figures 1, 2 and 3 show the percentage inhibition values of AP extract, AG and Acar against porcine α-amylase. The IC_{50} values for AP extract, Acar, and AG are

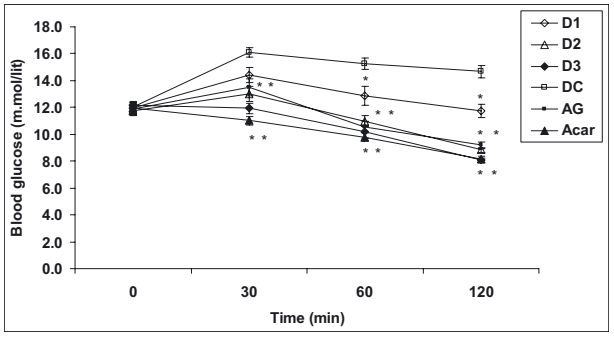

Figure 4. Blood glucose response during oral starch tolerance test in diabetic rats treated with AP extract, Acar or AG.
Starch used at 3 gm/kg. D1: 250 mg/kg AP extract; D2: 500 mg/kg AP extract; D3: 1000 mg/kg AP extract; DC: Diabetic control administered distilled water 4 ml/kg; AG: andrographolide 10 mg/kg; Acar: acarbose 10 mg/kg. Values are the mean ± S.E.M. (n = 6). *$P < 0.05$ compared with the control. One way ANOVA followed by Tukey's test for post hoc analysis.

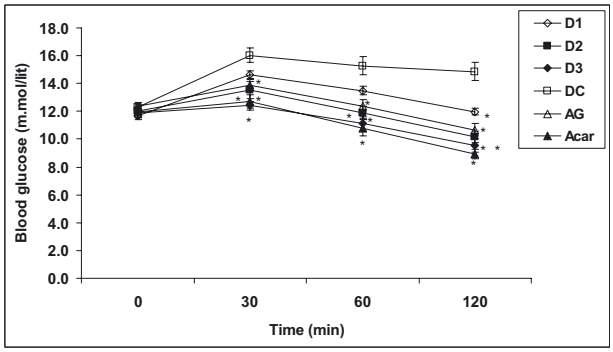

Figure 5. Blood glucose response during oral sucrose tolerance test in diabetic rats treated with AP extract, Acar and AG.
Sucrose used at 4 gm/kg. D1: 250 mg/kg AP extract; D2: 500 mg/kg AP extract; D3: 1000 mg/kg AP extract; DC: Diabetic control administered distilled water 4 ml/kg; AG: andrographolide 10 mg/kg; Acar: acarbose 10 mg/kg. Values are the mean ± S.E.M. (n = 6). *$P < 0.05$ compared with the control. One way ANOVA followed by Tukey's test for post hoc analysis.

50.9 mg/ml, 14.9 mg/ml, and 11.3 mg/ml, respectively. The IC_{50} values are tabulated in Table 1.

Oral starch tolerance test

In normal rats, extract-treated groups D1, D2, and D3 did not show any significant decrease in PBG and AUC levels (Table 2) compared to control rats. AG also failed to demonstrate any significant decreases in PBG and AUC but reduced blood glucose levels significantly ($P < 0.05$) at 120 min. But Acar managed to reduce significantly ($P < 0.05$) PBG and AUC and also the blood glucose levels at 30 min, 60 min and 120 min. But in diabetic rats ex-

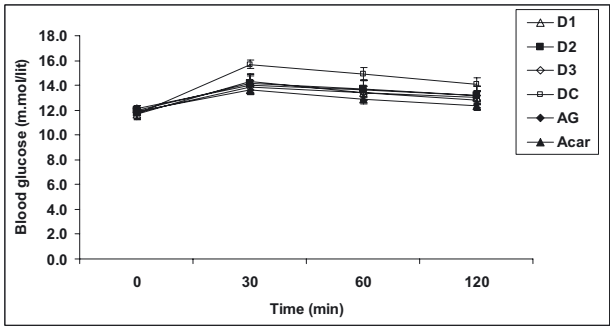

Figure 6. Blood glucose response during oral glucose tolerance test in diabetic rats treated with AP extract, Acar and AG.
Glucose used at 2 gm/kg. D1: 250 mg/kg AP extract; D2: 500 mg/kg AP extract; D3: 1000 mg/kg AP extract; DC: Diabetic control administered distilled water 4 ml/kg; AG: andrographolide 10 mg/kg; Acar: acarbose 10 mg/kg. Values are the mean ± S.E.M. (n = 6). One way ANOVA followed by Tukey's test for post hoc analysis.

tract-treatment groups D2, and D3, produced a significant ($P < 0.05$) drop in PBG (Fig. 4), and D3 lowered AUC (Table 2) levels significantly ($P < 0.05$) in comparison to diabetic control group. At 30 min, D2 and D3 reduced blood glucose levels significantly ($P < 0.05$) while D1, D2, and D3 showed significantly ($P < 0.05$) reduced blood glucose levels at 60 min and 120 min. AG and Acar also significantly ($P < 0.05$) lowered PBG (Fig. 4) and AUC (Table 2). Diabetic rats treated with AG or Acar also showed significant ($P < 0.05$) decreased blood glucose levels at 30 min, 60 min, and 120 min (Fig. 4).

Oral sucrose tolerance test

In normal rats, the extract-treated groups did not show any reduction in PBG levels. But D2 and D3 groups managed to lower AUC significantly ($P < 0.05$), whereas D1 did not demonstrate any significant decrease in PBG and AUC (Table 2). when compared to normal control rats. At 30 min, none of the extract-treated groups showed any significant decrease in blood glucose lowering response, but at 60 min D3 significantly ($P < 0.05$) reduced blood glucose level and at 120 min both D2 and D3 treated groups showed a significant ($P < 0.05$) reduction in blood glucose levels. In normal rats, AG did not show any decrease in PBG but a significant ($P < 0.05$) reduction in AUC (Table 2) was observed. AG also produced a significant ($P < 0.05$) reduction in blood glucose concentration at 60 min and 120 min. Acar demonstrated a significant ($P < 0.05$) decrease in PBG and AUC in comparison to normal control group. Acar in normal rats also produced a significant ($P < 0.05$) blood glucose lowering response at 30 min, 60 min and 120 min. In diabetic rats, D2 and D3 showed a significant ($P < 0.05$) reduction in PBG (Fig. 5) and AUC (Table 2) in comparison to diabetic control group, whereas D1 showed no tendency in PBG and AUC reduction. At 30 min and 60 min, D2 and D3 produced a significant ($P < 0.05$) decrease in blood glucose (Fig. 5). But at 120 min, D1, D2, and D3 groups demonstrated a significant ($P < 0.05$) blood glucose lowering. The higher doses of the ethanolic extract of *A. paniculata* shifted and delayed the blood glucose concentrations from 30 to 60 min concurrently suppressing PBG and reducing AUC. AG and Acar demonstrated a significant ($P < 0.05$) reduction in PBG, and AUC levels. There was a significant ($P < 0.05$) blood glucose lowering at 30 min, 60 min, and 120 min (Fig. 5) on treatment with AG and Acar.

Oral glucose tolerance test

In normal rats, none of the extract-treated groups managed to reduce PBG and AUC significantly in comparison to normal control group. But D3 showed a significant ($P < 0.05$) decrease in blood glucose at 60 min. D1 and D2 did not produce any significant decrease in blood glucose lowering at the stipulated times. Both AG and Acar failed to significantly suppress the PBG, AUC and also lower blood glucose levels at stipulated times after glucose loading. No significant lowering of blood glucose was observed at 30 min, 60 min, and 120 min on Acar treatment. These results reveal that ethanolic extracts of *A. paniculata* and AG did not affect absorption of glucose in the small intestine. In diabetic rats also none of the administered doses of the extract was capable of producing significant reductions in PBG and AUC compared to diabetic control group. No significant reductions in blood glucose levels was observed at 30 min, 60 min and 120 min (Fig. 6) with the extracts. AG and Acar also did not produce any significant reductions in PBG and AUC. No significant reductions in blood glucose response were seen at the stipulated times (Fig. 6).

DISCUSSION

AG, the major biomarker and the principal component of the ethanolic extract of aerial parts *A. paniculata*, has been reported to have antidiabetic activity by causing an increase in glucose utilization, simultaneously lowering plasma glucose in diabetic rats (Yu *et al.*, 2003). So, perhaps AP extract could have an effect on glucose absorption from the gut and may prolong absorption process, suppressing the peak blood glucose levels.

Although there are citations of antihyperglycemic (Zhang & Tan, 2000b) and antidiabetic (Zhang & Tan, 2000a) activity of ethanolic extract of *A. paniculata* based on free radical scavenging activity and in part on increased glucose metabolism, there are no previous reports, at least to our knowledge, on the activity of this extract on *in vitro* α-glucosidase activity.

Our *in vitro* studies demonstrated an appreciable α-glucosidase and a weak α-amylase inhibitory activity of 20% (v/v) extract of *A. paniculata*. AG showed similar α-glucosidase and amylase inhibitory effect. The IC_{50} values show that 20% (v/v) ethanolic extract has a lower potency and has a preference for α-glucosidase over α-amylase. AG seems to show equal preference for both α-glucosidase and α-amylase enzymes.

But not always does the *in vitro* inhibitory activity relate to the corresponding *in vivo* activity. Thus proof of concept needs to be demonstrated in preclinical animal studies. For safety and efficacy to be established, it was essential to confirm the *in vivo*

action following oral administration to live animals hence the *in vivo* experiments were performed.

In the *in vivo* experiments, doses 500, 1000 mg/kg of the AP extract and 10 mg/kg AG reduced the blood glucose excursions and decreased the PBG and AUC after starch and sucrose loading in diabetic rats. The lowest dose 125 mg/kg of 20% (v/v) extract showed reduction in AUC and also reduced blood glucose levels caused by starch loading at 60 min and 120 min. D1 also showed blood glucose lowering response on sucrose loading at 120 min in diabetic rats. The above results show a striking similarity to the effects of acarbose. Further, acarbose failed to inhibit glucose-loading induced peak blood glucose at 30 min. Similarly none of the doses of the 20% (v/v) extract could prevent the rise in peak blood glucose in normal or diabetic rats induced by glucose loading. The ethanolic extract of *A. paniculata* and AG seem to delay the quick digestion of starch and sucrose and lengthen the duration of carbohydrate absorption over time, thus reducing the PBG value and AUC. But in the *in vivo* tests the AP extract was observed to be more active as measured by the greater reduction in PBG and AUC than AG, whereas in *in vitro* test AG was found to be more potent than AP extract as measured by the lower IC_{50}. It may be that an unknown compound(s) in the extract may be responsible for this reduction in PBG and AUC in addition to AG exerting the effect. The tendency of the ethanolic extract of *A. paniculata* and AG to suppress the PBG at 30 min in diabetic rats demonstrates α-glucosidase inhibitory activity while preventing the rise in blood glucose levels.

Acarbose-like drugs, drugs that inhibit α-glucosidase present in the epithelium of the small intestine, have been demonstrated to decrease post-prandial hyperglycaemia (Sima & Chakrabarti, 2004) and improve impaired glucose metabolism without promoting insulin secretion in NIDMM patients (Carrascosa *et al.*, 2001). These medications are most useful for people who have just been diagnosed with type 2 diabetes and who have blood glucose levels only slightly above the level considered serious for diabetes. They also are useful for people taking sulfonylurea medication or metformin, who need an additional medication to keep their blood glucose levels within a safe range.

Therefore, the retardation and delay of carbohydrate absorption with a plant-based α-glucosidase inhibitor offers a prospective therapeutic approach for the management of type 2 diabetes mellitus and borderline patients (McCue *et al.*, 2005). Alhough the extract seems to be promising in the treatment of type 2 diabetes mellitus by reducing postprandial hyperglycaemia, it is still early to recommend its use in humans. Only a thorough and full-fledged study can rationalise the use in humans.

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