

ORIGINAL ARTICLE

OPEN LABEL CLINICAL TRIAL TO STUDY ADVERSE EFFECTS AND TOLERANCE TO DRY POWDER OF THE AERIAL PART OF *ANDROGRAPHIS PANICULATA* IN PATIENTS TYPE 2 WITH DIABETES MELLITUS

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Adverse effects and tolerance to dry powder of aerial part of *Andrographis paniculata* (Burm.f.) Nees were studied in 20 patients with type 2 diabetes mellitus for a period of 12 weeks. Patients were given powdered *A. paniculata* starting with 600 mg daily, gradually increasing to a maximum of 1.8 gm daily. Parameters monitored included body weight, blood pressure, liver function tests, renal function tests, cardiac enzymes, haemogram, serum electrolytes, fasting blood glucose, HbA1c, blood cholesterol, serum triglycerides and blood hormone levels (tri-iodotyronine, thyroxine, thyrotropin, insulin, fasting cortisol). None of the above mentioned parameters showed significant change during the study period except for a fall in HbA1c by 5.46% ($p < 0.01$) and fasting S. insulin by 20.93% ($p < 0.003$). In conclusion, *A. paniculata* powder did not induced significant adverse events based on parameters observed in our study but significantly lowered HbA1c and fasting serum insulin in patients with type 2 diabetes.

Key words : *Andrographis paniculata*, diabetes mellitus type 2

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Introduction

Traditional medicines in South East Asia are sourced from plants, animals and minerals. People often turn to traditional medicine when modern

western medicines fail or to treat chronic medical illnesses such as diabetes mellitus. *Andrographis paniculata* (Acanthaceae) is among many local herbs in South East Asia, which are claimed to have antidiabetic properties. The known active chemical

Figure 1 : Chemical structure of andrographolide

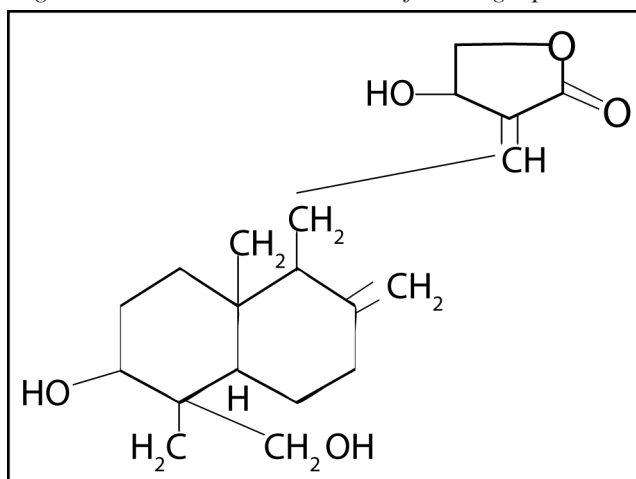
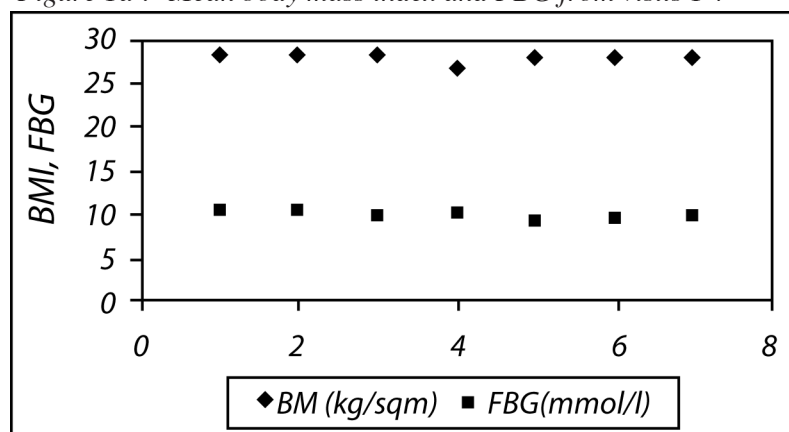


Figure 1a : Mean body mass index and FBG from visits 1-7



of this herb is a diterpene lactone, andrographolide (Fig. 1). Other chemicals include 14-deoxyandrographolide, 14-deoxy-11-oxo - andrographolide, 14 - deoxy-11, 12 - didehydroandrographolide and neoandrographolide.

For centuries leaves of this tropical plant are used by local people either as complementary treatment or the only medication to treat diabetes. Antidiabetic properties of *Andrographis paniculata* are substantiated by a number of animal experiments conducted in Malaysia (1) and elsewhere (2). Moreover it has not been associated with any significant side effects in animal and human studies (3,4). *Toxicity of Andrographis paniculata*, has been studied by a number of researchers in rats (5) and rabbits (6). A few animal studies suggest antifertility (7) action when the herb was given in very high doses (2gm / kg body weight for 6 weeks), but these results are controversial (8). The aim of the current study is to assess the tolerance and adverse effects to dry powder of aerial part of *Andrographis paniculata* in patients with type 2 diabetes mellitus.

Materials and Method

Andrographis paniculata dry powder capsules

The plant *Andrographis paniculata* (AP) was cultivated at Universiti Sains Malaysia herbal garden according to physiological and agronomical guidelines. The species is identified by the Division of Medicinal Plants, Forest Research Institute of Malaysia. Voucher specimen deposited to Forest Research Institute of Malaysia [PPSP/HB/1/(2002)]. Total amount of raw material (the aerial part of the plant) required for this 12-week study was collected in one batch to avoid changes in the concentration of active chemical (batch to batch variation). After thorough cleaning of aerial part of the plant it was subjected to drying at 40°C for 72 hours in electric oven. The dried plant extract was then made into powder. Capsules of this powder were then prepared at Pharmacology Laboratory, School of Medical Sciences, Universiti Sains Malaysia. Each capsule contained 300 mg of dry herb.

Figure 2 : Blood pressure and pulse rate from visits 1-7

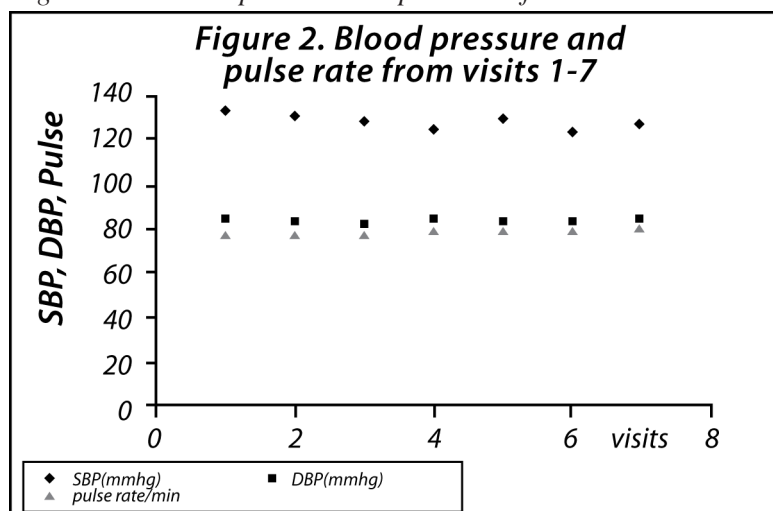
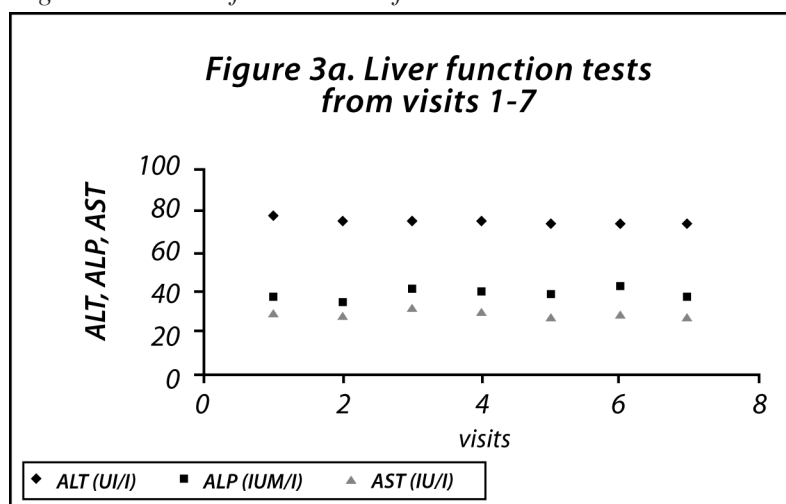


Figure 3a : Liver function tests from visits 1-7



HPLC method validation to measure andrographolide contents

For validation of HPLC method Waters™ 610 fluid unit coupled with Waters' 600 controller, Waters' 486 tunable UV absorbance detector(230 nm). Lichrosorb reverse phase (RP-18) analytical column(250×4.6 mm, ID) and 50ul loop was used. The column consisted of particles of the size 5 um, 3390A integrator from Hewlett Packard. The mobile phase was prepared using 30% acetonitril and 70% distilled water. A flow rate of 1.1 ml/min was maintained throughout and injection volume was 50ul each time. By comparing retention times our HPLC assay was found to be free from possible interference from other diterpene compounds present in the dry leaves of *Andrographis paniculata*. A calibration graph was constructed in the concentration range of 1ug/ml to 60ug/ml using 5 concentrations (n=2 each). The linearity of calibration graph was demonstrated by good

determination coefficient ($r^2=0.9983$) obtained for regression line. The precision of the method was evaluated by determining the interday RSD% (5.49-11.58) intraday RSD% (3.24-14.93) of the measured peak areas for different concentrations. The LOQ defined in the present experiment as lowest andrographolide concentration in the calibration curve that can be measured routinely with acceptable precision (RSD<20%) and accuracy (80-120%) was 1ug/ml. Recovery data was determined at three concentration levels as recommended by center for Drug Evaluation and Research (96.20% to 103%). Andrographolide contents of the herb were found to be 5.45% as determined by first extracting the dry powder with methanol and then using above mentioned validated high pressure liquid chromatography method.

Selection of subjects

Twenty patients, already diagnosed with diabetes mellitus type 2 and undergoing treatment

Figure 3b : Liver function tests from visits 2-7

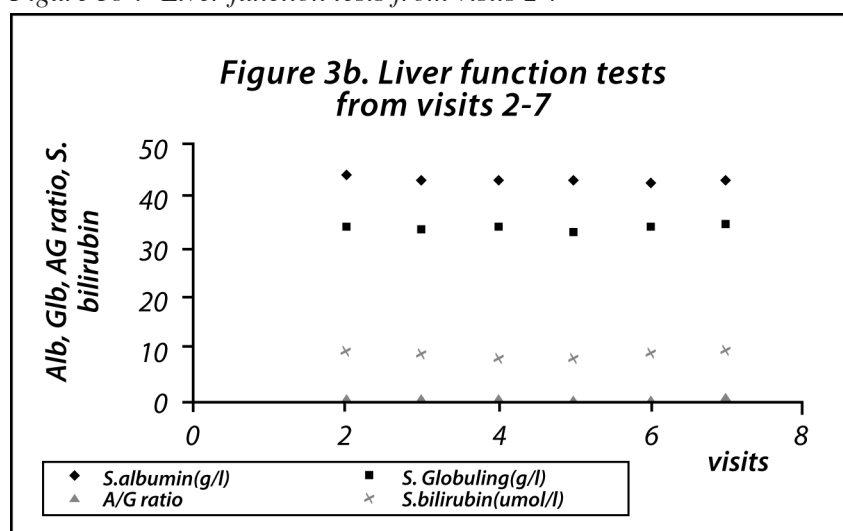
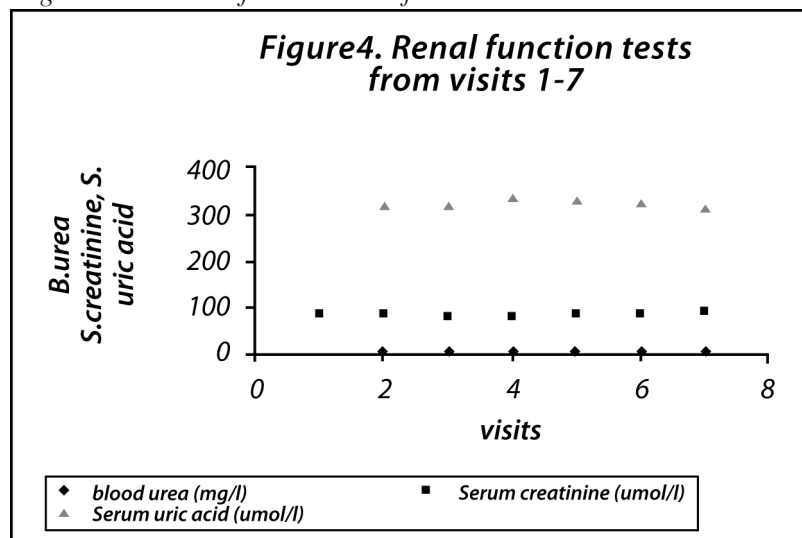


Figure 4 : Renal function tests from visits 1-7



in diabetes clinic, Hospital Universiti Sains Malaysia (USM) were recruited for this study. These included 6 males and 14 females. For subjects to be included into the trial, they were required to fulfill inclusion and exclusion criteria. Subjects of both sexes between the age of 35 and 70 years, body mass index between 25 and 40 kg/m², glycosylated haemoglobin (HbA1c) between 7% and 10% and fasting plasma glucose (FPG) between 7mmol and 15mmol were included in the trial if they fulfilled all the exclusion criteria. Exclusion criteria were, impaired hepatic function (ALT or ALP >2x ULN), impaired renal function (S. creatinine >150 μ mol/l), history of cardiac problem (IHD, cardiac failure), uncontrolled blood pressure (SBP >160mmHg DBP >100mmHg), history of treatment with insulin, proteinuria (2+ and above) and pregnant/breast feeding mothers). All subjects were required to give written informed consent.

Clinical evaluation

At first screening visit, subjects were evaluated for eligibility criteria. After physical examination, blood was collected to determine eligibility for the study. On 2nd visit (2 weeks after 1st visit) all subjects came after overnight fasting and blood results were reviewed for eligibility. For eligible subjects, general physical examination was done, blood was collected for baseline investigations, which included FBG, HbA1c, complete haemogram, LFT, RFT, electrolytes, CK, LDH, cholesterol, triglycerides, insulin, T4, TSH and cortisol.

Subjects were then instructed to take 2 capsules of AP (600 mg) daily with breakfast as an add on treatment to their ongoing medication. Subsequently subjects were asked to attend Clinical Trial Unit at 2 weekly intervals after overnight fasting for a total of 7 visits. During visits 3, 4, 5 and 6 general physical examination was repeated and blood was collected for FBG, ALT, ALP, and

Figure 5 : Cardiac enzyme levels from visits 2-7

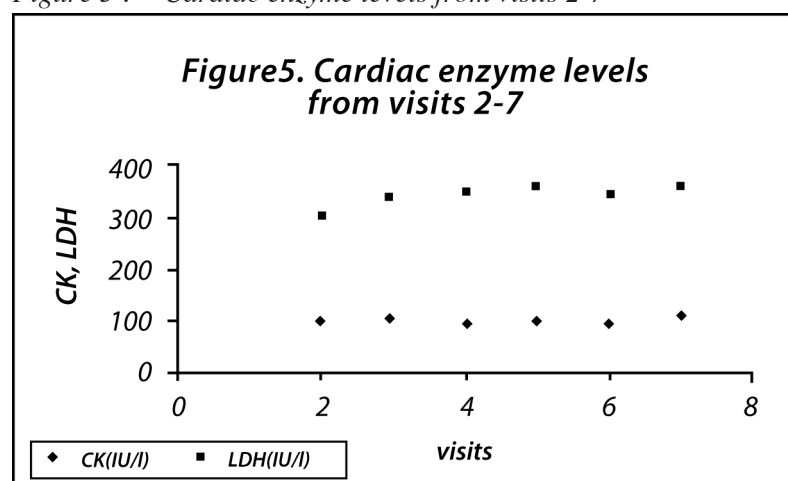
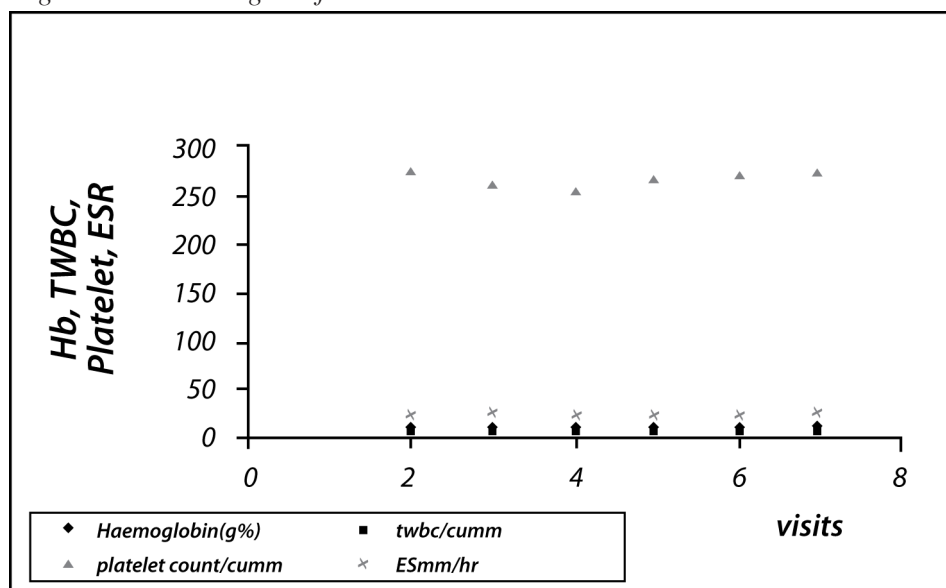


Figure 6 : Haemogram from visits 2-7



creatinine, Urine was also taken for testing of protein and sugar. Those subjects who had FBG above 7 mmol/l, the dose of AP was increased by 1 capsule (300 mg) each time. Maximum doses achieved were 3 capsules (900 mg each) twice a day for 2 weeks. On visit 7 all investigations were repeated as in visit 2. During all visits after starting this herbal treatment subjects were inquired about adverse events experienced since last visit and state of compliance to the medication.

Results

Adverse events

During all visits subjects were asked about occurrence of any adverse event since last visit. During the entire period of trial one patient complained of gastric irritation and nausea after swallowing the capsule. She was prescribed antacid gel and was able to continue with the trial without further experiencing similar symptoms.

Clinical and biochemical

Physical examination conducted during all 7 visits showed no significant changes in mean values of body weight (BMI), systolic blood pressure, diastolic blood pressure and pulse rate (Figure 2 and 3). Mean fasting blood glucose was 10.19 (S.E.± 0.53) mmol/L at screening visit and 9.71 (S.E.± 0.76) mmol/L at visit 7 (Figure 2), thus showing no significant change during treatment. However mean HbA1c fell from 8.61 (S.E. ± 0.25)% at screening visit to 8.13 (S.E. ± 0.29)% at visit 7, a fall by 5.46% ($p<0.01$). Liver function tests showed no changes as seen in Figure 4a & 4b. In the same way renal functions were well preserved and comparing results of blood urea, creatinine and uric acid (Figure 5) showed no significant change. Cardiac functions were monitored by measuring CK and LDH enzyme levels from visit 2 to 7. Comparison of these enzyme levels in Figure 6 showed no significant rise or fall. Serum electrolyte values measured from visit 2 to 7 were well within normal limits (serum sodium 139.5 ± 0.97 mmol/L to 139.85 ± 0.54 mmol/L, serum potassium 4.15 ± 0.21 to 4.34 ± 0.09 mmol/L, serum calcium 2.23 ± 0.08 mmol/L to 2.39 ± 0.03 mmol/L).

Table 1 : Changes in FBG, HbA1c and Fasting insulin from Visits 1-7.

	Visit 1	Visit 7	% difference
FBG mmol/l	10.19	9.71	4.71 $p>0.05$
HbA1c%	8.60	8.13	5.46 $p<0.01$
Fasting insulin IU/l	11.18	8.84	20.93 $p<0.003$

L, serum phosphorus 1.77 ± 0.64 mmol/L to 1.23 ± 0.03 mmol/L). Mean hemoglobin, total WBC, platelet counts and ESR also showed no changes (Figure 7). Blood lipid levels (cholesterol 5.7 ± 0.26 mmol/L to 5.85 ± 0.26 mmol/L and triglycerides 1.59 ± 0.12 mmol/L to 1.56 ± 0.15 mmol/L) showed no significant change from visits 2 to 7 and similar results were observed for thyroid hormone (FT4 14.03 ± 0.61 nmol/L to 13.6 ± 0.44 nmol/L, TSH 0.98 ± 0.08 mU/L to 1.06 ± 0.10 mU/L) and fasting cortisol levels (319.63 ± 30.42 nmol/L to 342.16 ± 35.46 nmol/L). Fasting insulin levels however showed a significant fall from 11.18 (S.E. ± 1.46) IU/L at visit 2 to 8.84 (S.E. ± 1.05) IU/L at visit 7, a reduction by 20.93% ($p < 0.003$) (Table 1). Average patient compliance as determined by capsule counting was 90.93%.

Discussion

Many researchers have studied the effects of *Andrographis paniculata* in animals and in human. In various animal experiments it has been found to have hepatoprotective (9, 10), antioxidant, antidiabetic (2), antihypertensive (11) and anti HIV (12) effects. In human clinical trial it was found to be effective against common cold (3, 4) and pharyngotonsillitis (13). Various animal experiments have also been done to study toxicity to *Andrographis paniculata* (5, 6). These experiments have shown *Andrographis* to be a safe herb. The results of our study showed no changes in physical and biochemical parameters of toxicity with doses as high as 900mg twice a day for 2 weeks.

These results support the results of previously conducted studies. However as reported by Zhang et al. (2) the increase in body weight and fall in fasting blood glucose of diabetic rats after treatment with ethanolic extract of AP was not observed in our clinical trial. The fall in HbA1c and fasting insulin level in diabetic patients in our study suggests its efficacy as an antidiabetic agent. These results suggest that the mechanism of action of *Andrographis* is by increasing peripheral utilization of glucose, probably by potentiating insulin action and not by a direct insulin releasing action on islet cells in pancreas. However other possible extrapancreatic effects on glucose metabolism (enzymatic and non enzymatic) in liver or carbohydrate absorption in GIT remain unclear.

In conclusion, dry powder of aerial part of *Andrographis paniculata* caused no significant adverse effects in parameters observed in patients with type 2 diabetes mellitus. Physical and

biochemical parameters of toxicity were not affected during treatment with *Andrographis paniculata* for a period of 12 weeks and at the same time it showed antidiabetic property. *Andrographis paniculata* therefore was found to be a safe herb, which needs to be further evaluated for its long term safety and efficacy as antidiabetic agent.

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