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Experimental study on hypoglycemic activity of ethanolic extract of *Andrographis paniculata* Nees. on rats

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The ethanolic extract of the whole plant of *Andrographis paniculata* (aq;cg;NuD;/i&kwfcg;) was tested for its acute hypoglycemic activity in adrenaline-induced hyperglycemic rats. It was examined after single oral administration of tested drug with the dosage of 400 mg/kg and 300 mg/kg body weight, standard drug (Glibenclamide) 0.5 mg/kg and distilled water 4ml/kg on rats which were seven numbers in each group. High dose of tested drug was as effective as standard drug in 4 consecutive hours after drugs administration ($p>0.5$). Low dose of tested drug had no significant difference with standard drug at 2, 3 & 4 hours ($p>0.5$) after the drug administration. The result revealed that *A. paniculata* in high dose was as effective as Glibenclamide whereas low dose of *A. paniculata* had less effectiveness at first hour. This study clearly indicated that an ethanolic extract of a whole plant of *A. paniculata* has a significant acute hypoglycemic activity and gave scientific support in traditional usage of this plant.

INTRODUCTION

The number of people with diabetes is increasing due to population growth, aging, urbanization and increasing prevalence of obesity and physical inactivity. Quantifying the prevalence of diabetes and the number of people affected by diabetes, now and in the future is important to allow rational planning and allowance of resources.

The prevalence of diabetes for all age groups worldwide was estimated to be 2.8% in 2000 and 4.4% in 2030. The total number of people with diabetes is projected to rise from 171 million in 2000 to 366 million in 2030**.** The prevalence of diabetes is higher in men than women. But there are double between 2000 and 2030 .The most important demographic change to diabetes prevalence across the world appears to be the increase in the proportion of people > 65 years of age [1]. In 1997, Amos estimated that 124 million people worldwide have diabetes, 97% NIDDM and that by 2010 the total number with diabetes is projected to reach 221 million [2]. According to Sorensen report, World Health Organization has recorded in 2000 that there was a "global epidemic of obesity" and the prevalence of type 2 diabetes was rising in parallel. In 2001, Boyle estimated the number of Americans with diabetes is projected to increase from prevalence of 4.0% in 2000 to a prevalence of 7.2% in 2050 [2].

In Myanmar, out of 364 subjects 20(5.5%) had diabetes mellitus mentioned in one study which described the patterns of hypertension, obesity, diabetes mellitus and dietary habit of those subjects [3]. A cross-sectional survey among 4613 people aged 20 years and above in Yangon Division in 2003 reported the overall prevalence of diabetes mellitus was 11.9% (95% CI 10.1 to 13.4), urban prevalence was 13.9% and rural prevalence was 7.3%, with a slight female preponderance [4]. Traditional herbal medicines are widely used to treat diabetes mellitus, however, very few have

been clinically evaluated [5]. Diabetes has been treated with plant product medicines. Recent scientific investigation has confirmed the efficacy of many of these preparations, some of which are remarkably effective [5].

Andrographis paniculata (AP) also known commonly as "King of Bitters" is a member of the plant family Acanthaceae, and has been used for centuries in Asia to treat gastrointestinal tract and upper respiratory infections, fever, herpes, sore throat and a variety of other chronic and infectious diseases. It is found in the Indian Pharmacopeia and is prominent in at least 26 Ayurvedic formulas; whereas in Traditional Chinese Medicine (TCM), Andrographis is an important "cold property" herb. Chemically the drug contains flavones and lactones. Among lactones, andrographolide is the main constituent and it is also active principle of the plant [6]. The leaves contain the highest amount of andrographolide (2.39%), while the seeds contain the lowest.

It grows abundantly in southeastern Asia. India (and Sri Lanka), Pakistan and Indonesia but it is cultivated extensively in China and Thailand, the East and West Indies and Mauritius. Normally it grows from seeds in all types of soil. It is, therefore, cultivated quite easily.

The prevalence of diabetes mellitus is increasing worldwide, and patients need lifelong therapy with hypoglycemic drugs. The cost of treatment for lifelong therapy will burden onto the patients and family. On top of that, most of the patients have exhaustion for taking drug and willing to switch on alternative medicine like herbal drugs which are relatively cheap compared to western drugs. However, they are not supported by good scientific studies.

This study made an attempt to fill this gap. Thus the present study was undertaken to evaluate the evidence-based, scientifically proved acute hypoglycemic activity of ethanolic extract of the whole plant of *A. paniculata* Nees. in adrenaline-induced hyperglycemic rat models and to characterize the phytochemical properties of that plant.

MATERIALS AND METHODS

Collection of plant materials and preparation of extracts

The whole plants of *A. paniculata* Nees. were collected from Mandalay Division and shaded air dried until a completely dried product was obtained. The authenticity of the sample was identified by taxonomist from Department of Botany, University of Mandalay.

Before extraction, plant samples (whole plant) were air dried and cut into pieces. Exactly 100 gm of sample was packaged and subjected to solvent extraction by soxhlet assembling. This sample was extracted into two different solvents by chloroform and 96% ethanol successively. The time for extraction was about 6 hours. Then the extract was evaporated to concentrate by means of rotary evaporator. It was followed by drying in air until solid residue was obtained. Such a residue was desiccated and weighed to record respective yield of extracts. All extracts were stored in desiccators.

Phytochemical analysis

Types of compounds present in *A. paniculata* were analysed by doing phytochemical tests [7] and compounds were listed in Table 1.

Chemicals used

Glibenclamide (Gibic) 5mg, Thai, Reg no: 1A 448/38 was purchased from the market. Adrenaline tartrate (adrenaline 0.1% W/V) from Myanmar Pharmaceutical Factory was also obtained.

Animal experiments

Wistar strain rats, both male and female, weighing between 200 to 250 gms produced from Laboratory Animal Services Division, Department of Medical Research (Upper

Myanmar) were used for the study. All the animals were divided into 4 groups of 7 number each and were fed the standard diet and water ad libitum. They were kept in clean and dry cages and maintained in a well-ventilated animal house.

Screening of experimental animals

It was done one week before starting experiment. Animals were made fasting for 18 hours and baseline fasting blood sugar levels were measured by cutting tails about 1 mm length and using Omnitest glucometer. Then they were given subcutaneous injection of adrenaline 0.2 ml/kg body weight and 1 hour, 2 hour post injection fasting sugar levels were measured. Fasting blood sugar levels equal and above 8 mmol/l were selected for the study and those who did not respond the induction were rejected.

Study on acute hypoglycemic activity

Animals were fasted for 18 hours prior to drug administration allowing access only to water. Blood samples were collected by cutting 1 mm length of tail and baseline fasting blood sugar levels were measured with Omnitest glucometer in all four groups. After that following procedures were done.

- Group I & II were given a single oral administration of ethanolic extract of whole plant of *A. paniculata* (suspended in distilled water) at doses of 400 mg/kg body weight (high dose) and 300 mg/kg body weight (low dose) respectively.
- Group III received a single oral administration of glibenclamide at a dose of 0.5 mg/kg body weight and it served as a standard.
- Group IV served as an antihyperglycemic control group and received 4 ml/kg of distilled water only.

One hour after giving the corresponding drugs or vehicles, subcutaneous injection of adrenaline 0.2 ml/kg was given to all four groups. Then fasting blood sugar levels of all rats were measured again at 1, 2, 3 and 4 hours serially after giving injection adrenaline and recorded the results separately and safely. Percent inhibition was calculated according to the formula mentioned here [8].

Percent inhibition of requested drug = {blood glucose level of (control-requested drug) / blood glucose level of control} x 100%

Acute toxicity study

Seven albino mice in each four groups (three tested groups and one control group) were fasted overnight before administration of the ethanolic extract. Increasing doses of 0.4, 0.5 and 0.6 gm/kg of ethanolic extract of *A. paniculata* were orally administered respectively. The mice were housed separately in individual cage with free access to food and water and observed clinically for 1 week. LD_{50} was determined from the number of animals surviving at the end of 1 week period [9].

Statistical analysis

Data were checked and entered using Epidata software 3.1. Data were expressed as means of FBS at 0hr and blood sugar level at 1hr up to 4 hours. The comparison between test extract and western drug along with distilled water, using paired t-test was done. The results were expressed in figure and tables.

RESULTS

Phytochemical analysis

Phytochemical data of dried *A. paniculata* are shown in Table 1. It constituted various compounds, extracted from *A. paniculata*.

Table 1. Types of compound present in *Andrographis paniculata*

No.	Types of compound	Presence(+) / Absence(-)
1	Alkaloid	+
2	Amino acid	+
3	Carbohydrate	+
4	Phenol	+
5	Flavonoid	+
6	Glycoside	+
7	Resin	-
8	Saponin	+
9	Steroid	+
10	Tannin	+

Altogether 9 chemical compounds were identified qualitatively. They were alkaloid, amino acid, carbohydrate, phenol, flavornoid, glycoside, saponin, steroid and tannin. The yield percentage of *Andrographis paniculata* was 4.0% with the usage of 96% ethanol.

Hypoglycemic action

The hypoglycemic effect of ethanolic extract of *Andrographis paniculata* Nees. was studied and found to be effective on albino rats. The results of extracts (low dose and high dose), standard drug and negative control (distilled water) are shown in Figure 1.

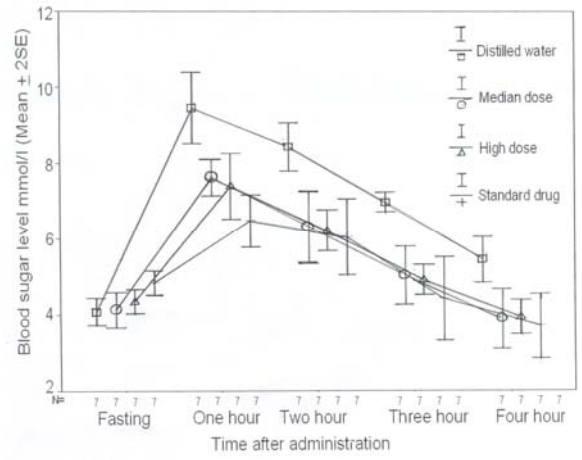

Fig 1. Mean blood sugar level after giving ethanolic extract of *Andrographis paniculata* Nees.

The ethanolic extract of the whole plant of *A. paniculata* produced a dose-dependent acute hypoglycemia in adrenaline-induced hyperglycemic rats. Two dose of extracts ie., 400 mg/kg (high dose) and 300 mg/kg (low dose) produced a significant reduction in blood glucose level, compared to control (distilled water) ($p<0.05$). In other words, the extract had definite hypoglycemic effect on hyperglycemic rats.

However, it was found that high dose extract was as effective as standard drug. There was no significant difference in means blood glucose level at all four hours in comparison with standard drug (glibenclamide) and high dose of ethanolic extract ($p<0.5$). It indicated that the hypoglycemic effects of ethanolic extract of *A. paniculata* and standard drug (glibenclamide) were the same.

On contrary, low dose of extract was found to be less effective because there was significant difference in blood glucose reduction at 1 hour when compared with standard drug ($p<0.02$). But the glucose levels in successive hours showed no significant difference. It indicated that low dose extract was inferior to standard drug. Percent inhibition of glibenclamide, high and low dose of ethanolic extract of *A. paniculata* were calculated and shown in Table 2.

Table 2. Percent inhibition of ethanolic extract and glibenclamide

Type of drug	1 hour %	2 hour %	3 hour %	4 hour %
Glibenclamide	31.57	28.57	37.14	32.72
High dose of ethanolic extract	22.10	26.19	30.00	29.09
Low dose of ethanolic extract	18.94	25.00	28.57	29.09

The percent inhibition of glibenclamide was higher than that of high and low dosage levels at all four hours. Maximum inhibition was found at 3 hour for standard drug and high dose extract, but at 4 hour for low dose extract. However, the trend of percent inhibition showed fluctuation in standard drug, smooth or even in high dose extract and low dose extract.

Acute toxicity test

Acute toxicity test indicated no death in all doses tested. Thus ethanolic extract of the drug was safe up to 0.6 gm/kg when administered orally.

DISCUSSION

A. paniculata was used traditionally in Myanmar. There were many literatures concerning the anti-diabetic activity of this

plant on alloxan-induced or streptozotocin-induced rats. One study showed a significant anti-diabetic acute activity of ethanolic extract of whole plant with the dosage of 0.1, 0.2 and 0.3 mg/kg body weight against the streptozotocin-induced rat models [9]. But here we conducted upon adrenaline-induced model for acute activity.

In this study, our results indicated the potent action in acute activity of ethanolic extract with the dosage of 400 mg/kg and 300 mg/kg body weight in adrenaline-induced hyperglycemic rats. In other study of Zhang XF and Tan BK, *A. paniculta* has significant effect on both streptozotocin- and adrenaline-induced hyperglycemic rats [10].

Another study reported that andro-grapholide (1.5 mg/kg), an active principle in the leaves of *A. paniculata*, had significant anti-diabetic activity [11] and suggested that andrographolide can increase glucose utilization to lower the plasma glucose level in diabetic rats lacking insulin. In our study, we used crude extract of ethanolic extract form only since we have limitations to separate the active principle from these extracts even though the active principle (andrographolide) was found to contain in the extract.

Thus *A. paniculata* is shown to have significant anti-hyperglycemic activity and it is suggested that andrographolide can increase glucose utilization to lower plasma glucose in diabetic rats lacking insulin but other possibility of extra pancreatic action such as increased glucose uptake by *A. paniculata* cannot be ruled out. This study revealed that ethanolic extract of *A. paniculata* was useful in diabetes for its prompt action. Further work on identifying active principle, chronic anti-hyperglycemic action of this plant is obviously required.

According to the results shown in Table 2, this herbal plant has strong antihyper-glycemic activity as compared to the western drug (glibenclamide), thus studies on pre-clinical and clinical trial should be continued to complete the full picture of this precious herbal plant. It is needed to know the chronic activity of indigenous anti-hyperglycemic drug and try to study the experimental trial with alloxan-induced model.

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