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Preliminary study on hypoglycemic effect of *Phyllanthus niruri* Linn. (Taung-ze-phyu) on rabbit model

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The aim of this study is to determine phytochemical constituents, acute toxicity and the hypoglycemic effect of aqueous and 95% ethanol extract of whole plant of *Phyllanthus niruri* Linn. (Taung-ze-phyu). Phytochemical results showed that crude powder, aqueous extract and 95% ethanol extract contained alkaloids, flavonoids, tannins, saponins, steroids, amino acids and polyphenols. Acute toxicity study of crude powder, aqueous extract and 95% ethanol extract were evaluated in mice. Both crude powder and 95% ethanol extract showed no evidence of toxicity up to the maximum feasible dose level of 3 gm/kg body weight. In contrast, the maximum feasible dose level of aqueous extract was 6 g/kg body weight. Therefore, the median lethal dose (LD_{50}) of crude powder and 95% ethanol extract was observed to be more than 3 gm/kg body weight. The median lethal dose (LD_{50}) of aqueous extract was observed to be more than 6 gm/kg body weight. Evaluation of hypoglycemic effect of 95% ethanol extract (1.5 gm/kg body weight) and aqueous extract (3 gm/kg body weight) was carried out on adrenaline-induced diabetic rabbits. It was found that 95% ethanol extract significantly lowered the blood glucose levels at 2hr ($p<0.05$). Aqueous extract also lowered the blood glucose level at 1hr, 2hr, 3hr and 4hr ($p<0.005 \sim p<0.05$) respectively when compared with those of the control. Hypoglycemic effect of standard drug glibenclamide (4 gm/kg body weight) was also studied to compare with that of the plant extracts. Between the two extracts, the effect of aqueous extract (3 gm/kg body weight) when compared with that of glibenclamide was not significantly different.

INTRODUCTION

Phyllanthus niruri Linn. (Taung-ze-phyu) is a small annual herb growing widely in the rainy season of Myanmar. It belongs to the family Euphorbiaceae. It is also a well known medicinal plant for hepatitis, diabetes mellitus and hypertension. Decoction of fresh whole plant was taken for diabetes mellitis and hepatitis [1]. Many researchers have observed its hepatoprotetive, hypotensive, hypoglycemic and diuretic effects in laboratory animals [2, 3, 4, 5 & 6]. There have been some reports on the hypoglycemic effect of *Phyllanthus niruri* Linn. which varies in regard to the plant species, the part of the plant used and in the preparation of extracts as well as the animal models. But there has been no systematic research on the hypoglycemic effect of *P. niruri* locally. Hence, this study was conducted.

MATERIALS AND METHODS

This study was done at the Pharmacology Research Division, Department of Medical Research (Lower Myanmar), Yangon. Cross-over study design was used. Experimental animals were obtained from Laboratory Animal Services Division, DMR (Lower Myanmar).

Collection and identification of Phyllanthus niruri Linn.

Fresh whole plants growing in the suburban of Yangon were collected. The plants were identified and authenticated at the Botany Department, Yangon University. A voucher specimen was deposited at the herbarium of Pharmacology Research Division, Department of Medical Research (Lower Myanmar).

Preparation of whole plant extracts of P. niruri Linn.

Aqueous and 95% ethanol extracts of whole plant *P. niruri* Linn. were prepared by the standard method [7]. One hundred grams of whole plant powder was extracted with either 1 litre of distilled water or 95% ethanol on water bath at 60°C for 6 hours. It was filtered through cheese cloth and the filtrate was evaporated to dryness on a boiling water bath. The extract obtained was dissolved in distilled water before administration to respective animals.

Preliminary phytochemical analysis of crude powder, aqueous and 95% ethanol extracts of P. niruri Linn.

Phytochemical analysis of crude powder, aqueous extract and 95% ethanol extract of whole plant was carried out according to the standard methods [7, 8 & 9].

Acute toxicity tests of P. niruri Linn.

Acute toxicity tests of crude powder, aqueous and 95% ethanol extracts were performed by the method of Litchfield and Wilcoxon [10]. Adult healthy albino mice, both male and female, weighing 30-35 gm were selected. They were divided into 5 groups of 10 mice each. Each group of mice was housed separately in mouse cages. They were fasted for 18 hours prior to the experiment, allowing access to water only. Each group received orally crude powder of whole plant in the doses of 0.5, 1, 2 and 3 gm/kg body weight, respectively. One group, served as control, was given 0.1 ml/ 10 gm body weight of distilled water. Food and water were supplied as usual after administration of test sample. They were kept under close observation for 24 hours. Any toxic symptoms and mortality found within 24 hours were recorded. Careful observation was continued up to 14 days in order to detect delayed effects. Acute toxicity tests of aqueous and 95% ethanol extracts were also done. The procedure is same as above. The tested doses for aqueous extract and 95% ethanol extract are 1, 2, 3 and 6 gm/kg and 0.5, 1, 2 and 3 gm/kg body weight, respectively.

Hypoglycemic effect of aqueous extract on adrenaline-induced hyperglycemic rabbit models

Six adult healthy rabbits of Japanese White strain, weighing 2.5-3.0 kg were used. They were deprived of food for 18 hours before the experiment. On the experiment day, 0 hr blood glucose level of all rabbits was determined with Glucometer (Ascensia ELITE XKL, Bayer Corporation, U.S.A) by taking blood samples from the marginal ear vein with blood glucose test strips (Ascensia ELITE XKL, Bayer Corporation, U.S.A). It was then immediately followed by oral administration of distilled water (10 ml/kg body weight), for control, by using a Ryle's tube No. 6. After administration of distilled water, all rabbits were induced to mimic hyperglycemia by injecting them subcutaneously with 0.2 mg/kg body weight of adrenaline tartrate B.P. using the method of Gupta *et al.* [11].

Serial blood glucose levels were measured at 1, 2, 3 and 4 hours after administration of adrenaline. And then all the rabbits were allowed to rest for a week. After a week's rest, the fasting blood glucose levels (0 hr) of all the rabbits were determined. The aqueous extract of whole plant 3gm/kg body weight was administered orally instead of distilled water. Adrenaline tartrate was injected subcutaneously as in control group. The blood glucose levels at 1, 2, 3 and 4 hours after adrenaline injection were recorded. The mean blood glucose levels of distilled water treated group (control group) and aqueous extract treated group (test group) were then compared and analyzed by the student '*t*' test.

The above procedure was repeated after a week's rest of wash out period using standard drug glibenclamide (4 mg/kg body weight) orally instead of aqueous extract. The mean blood glucose levels of control group and glibenclamide treated group (standard group) were compared and analyzed as above.

Hypoglycemic effect of 95% ethanol extract on adrenaline-induced hyperglycemic rabbit models

A new group of six adult healthy rabbits of JW strain weighing 2.5-3.0 kg were used. The test procedure used was the same as described above. Crossover study design was also used. The tested dose of the extract was 1.5 gm/kg body weight.

Statistical analysis

Data were expressed as mean ± SE (Standard Error) and the mean differences calculated using Student '*t*' test. $p<0.05$ was chosen as a significant level.

RESULTS

Phytochemical constituents of crude powder, aqueous extract and 95% ethanol extract of *Phyllanthus niruri* Linn. were determined (Table 1).

Table 1. Phytochemical constituents of *Phyllanthus niruri* Linn.

No.	Phytochemical constituents	Results		
		Crude powder	Aqueous extract	95% ethanolic extract
1	Alkaloides	+	+	+
2	Flavonoids	+	+	+
3	Glycosides	+	+	+
4	Saponins	+	+	+
5	Tannins	+	+	+
6	Phenols	+	+	+
7	Steroids/Terpenes	+	+	+
8	Amino acid	+	+	+
9	Reducing sugar	+	+	+
10	Cyanogenic glycosides	-	-	-

Acute toxicity tests of crude powder, aqueous extract and 95% ethanol extract showed that no lethality of the mice was observed up to 14 days even with the maximal permissible doses. Therefore, LD_{50} for the crude powder, aqueous extract and 95% ethanol extract are more than 3 gm/kg, 6 gm/kg and 3 gm/kg body weight, respectively.

Hypoglycemic effects of aqueous and 95% ethanol extract of *Phyllanthus niruri* Linn. were observed. In adrenaline-induced hyperglycemic rabbits, aqueous extract (3 gm/kg body weight) could effectively reduce the blood sugar levels at 1 hr ($p<0.005$), 2 hr ($p<0.01$), 3 hr ($p<0.05$) and 4 hr ($p<0.05$), respectively, after drug administration (Fig.1). It was highly significant at 2 hours after drug administration.

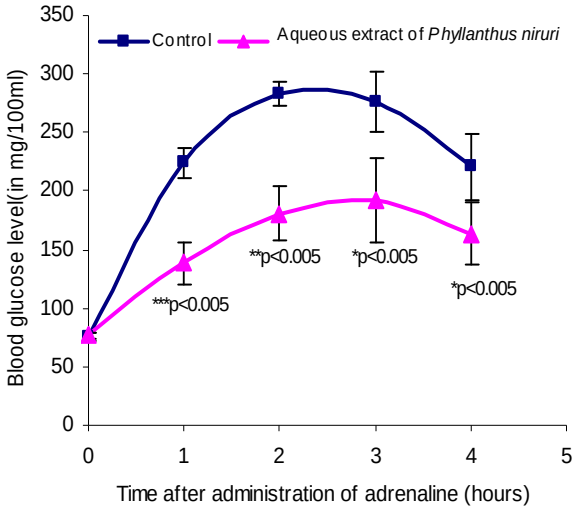

Fig 1. Times course of the effect of aqueous extract of *Phyllanthus niruri* on adrenaline-induced hyperglycemic rabbit model

Each point represents the mean of observations and the vertical bars indicate standard errors of the means.

An oral hypoglycemic drug, glibenclamide (4 mg/kg body weight) was observed to lower the blood sugar level significantly at 2 hrs ($p<0.05$) and 3 hr ($p<0.05$) and 4 hrs ($p<0.01$), respectively. It was highly significant at 4 hr after drug administration (Fig. 2). The extent of hypoglycemic effect produced by the aqueous extract when compared with that of glibenclamide was not significantly different.

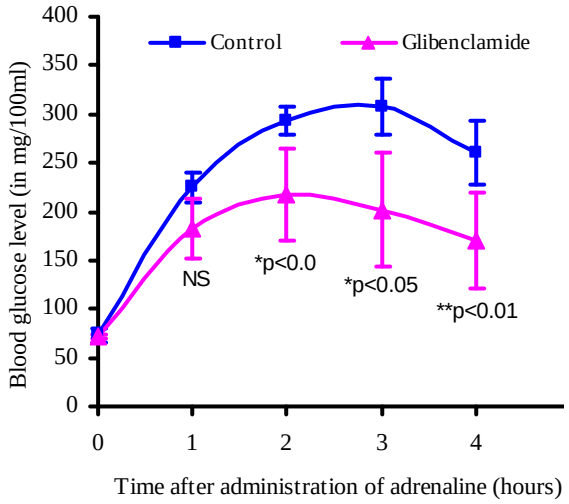

Fig 2. Time course of the effect of glibenclamide on adrenaline-induced hyperglycemic rabbit model

Each point represents the mean of observations and the vertical bars indicate standard errors of the means.

In 95% ethanol extract treated test, 1.5 gm/kg body weight of ethanol extract significantly lowered the blood glucose level at 2 hour ($p<0.05$) after drug administration (Table 2). It was not effective as glibenclamide or aqueous extract (data not shown). This may be due to short duration of action.

Table 2. Effect of 95% ethanolic extract of *Phyllanthus niruri* on blood glucose levels (Mean ± SE) of adrenaline-induced hyperglycemic rabbits model

Test sample	Blood glucose level (mg/100ml)				
	0 hr	1 hr	2 hr	3 hr	4 hr
Control (n=6)	73.6 ± 4.5	181.3 ± 17.5	277.8 ± 16.5	238.2 ± 46.4	250.5 ± 32.7
Ethanolic extract (n=6)	76.5 ± 2.9	164.8 ± 17.5	220.3 ± 28.5*	228.8 ± 40.2	212.5 ± 41.4

*=p<0.05

DISCUSSION

Chemical constituents of the aqueous and 95% ethanol extract of *P. niruri* indicated the presence of alkaloids, flavonoids and saponins. Some plants that contain these alkaloids have been reported to have hypoglycemic activity [12], so the hypoglycemic effect caused by these extracts may be attributed to the presence of alkaloids and flavonoids. These are mainly phenolic compounds, which have been reported to have antidiabetic effects [13]. The acute toxicity studies showed that LD_{50} of the aqueous extract and 95% ethanol extract were more than 6 gm/kg and 3 gm/kg body weight.

In this study we observed that the administration of aqueous extract (3 gm/kg) and 95% ethanol extract (1.5 gm/kg body weight) of *P. niruri* to adrenaline-induced hyperglycemic rabbits caused a significant decrease in blood glucose level. This confirms the claims by traditional medical practitioners and herbalists that the *P. niruri* has blood glucose lowering properties. The precise mechanism by which these extracts lower blood glucose is, however, not clear. It may be due to increased insulin secretion arising from pancreatic stimulation and probably increased utilization of peripheral glucose [13]. It is also believed that some of these hypoglycemic plants perform this function by removing the insulin-inactivating compounds through the SH groups in these inactivating compounds. Nicotinic acid is known to be insulin's inhibitor [14].

Similarly, other hypoglycemic plants containing anthocyanocides appear to act by improving vascularization of the pancreas. Others act by blocking oxidative enzyme of the Kreb cycle (succinic dehydrogenase and cytochrome oxidase), thus increasing anaerobic glycolysis and decreasing gluconeogenesis and entailing an increased rate of transfer of glucose from the blood to the tissue [12]. Nwanjo also reported that the aqueous crude extract of *P. niruri* may have hypoglycemic effect in streptozotocin-induced diabetic Wistar rats and that there was no evidence of hepatotoxicity [5]. Thus, it can be concluded that these obervations showed the aqueous and 95% ethanol extracts of *Phyllanthus niruri* Linn. may have hypoglycemic effect in adrenaline-induced hyperglycemic rabbits.

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