HYPOGLYCEMIC ACTIVITY OF WATERY EXTRACT OF THE LEAVES OF *THUNBERGIA LAURIFOLIA* LINDL. (PAN-YE-SUT-NWE)

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Abstract

Thunbergia laurifolia Lindl. is commonly known as Rang Chuet belongs to the family Acanthaceae and Myanmar name is pan-ye-sut-nwe. The plant samples were collected from Daik-U Township, Bago Region. The present work was done to investigate the acute toxicity and hypoglycemic activity. The acute toxicity study of watery extracts of *Thunbergia laurifolia* Lindl. leaves were carried on albino mice by using OECD guideline 423 method. There were no lethality and toxic effect of the albino mice observed up to the dose of 5 g/kg body weight during observation period of two weeks. Hypoglycemic activity of watery extract of the leaves of *Thunbergia laurifolia* Lindl. were tested on adrenaline-induced hyperglycemic rats by the method of Agrawal & Paridhavia, 2007. The rats with watery extract (0.75 g/kg and 1.5 g/kg) showed significant decrease in blood glucose concentration at 2 hr, 3 hr and 4 hr ($p<0.01$- $p<0.001$) whereas watery extract (3 g/kg) showed significant decrease in blood glucose concentration at 3 hr ($p<0.05$) and 4 hr ($p<0.01$). The results indicated that watery extracts of the leaves possessed significant hypoglycemic effect on adrenaline-induced hyperglycemic rats. From the experimental data, it can be concluded that *Thunbergia laurifolia* Lindl. could be used as potential herbal medicine for hypoglycemic activities. It also can be safe to eat the leaves of *Thunbergia laurifolia* Lindl. because the leaves are non-toxic.

Keywords *: Thunbergia laurifolia* Lindl., hypoglycemic activity, acute toxicity.

Introduction

Thunbergia laurifolia Lindl. belongs to the family Acanthaceae, under the order Lamiales. The generic name *Thunbergia* commemorates Carl Peter Thunberg, Swedish physician and Professor at Upsala. The specific epithet *laurifolia* refers to its laurel shaped leaves (Gledhill, 2008). Its English name is laurel-clock vine or blue trumpet vine, the common name in Myanmar is pan-ye-sut-nwe or kyikan-hnokthi or kyininwe.

Chuthaputti (2010) reported that the leaves of *Thunbergia laurifolia* Lindl. contains sterols such as β-sitosterol, stigmasterol, alphaspinasterol and lupeol.

Thunbergia laurifolia Lindl. contained phenolic acids such as caffeic acid, gallic acid, procateuchuic acid and chlorogenic acid (Oonsivilai *et al*., 2007).

The chromatographic separation of secondary compounds has been investigated such as grandifloric acid and 8-epi-grandifloric acid (Kanchanapoom *et al*., 2002).

Diabetes is an important public health problem. Globally, an estimated 422 million adults were living with diabetes in 2014, compared to 108 million in 1980. Diabetes caused 1.5 million deaths in 2012. The effects of diabetes mellitus include long term damage, dysfunction and failure of various organs. It may present with characteristic symptoms such as thirst, polyuria, blurring of vision and weight loss (WHO, 2016).

Currently available therapies for diabetes include insulin and various oral antidiabetic agents such as sulfonylureas, biguanides and alpha glucosidase inhibitors which are used as

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monotherapy or in combination to achieve better glycemic regulation. Many of these oral antidiabetic agents have a number of serious adverse effects. Thus, managing diabetes without any side effects is still a challenge. Plant drugs and herbal formulations are considered to be less toxic and free from side effects than synthetic ones. Based on the WHO recommendations, hypoglycemic agents of plant origin used in medicine which play an important role in future drug development programs. Easy availability, least side effects and low cost make the herbal preparations which are the main key player of all available therapies especially in rural areas. (www.diabete.qc.ca)

Thunbergia laurifolia Lindl. leaves have been reported to have antioxidant, antimicrobial, detoxifying, anti-diabetic activites and non-toxic effects (Chan *et al*., 2011). The biological activitiessuch as antioxidant, antimicrobial, anti-inflammatory, hepatoprotective and antidiabetes activitieswere found in *Thunbergia laurifolia* Lindl. (Junsi & Siripongvertikorn, 2016).

Antioxidant supplementation derived from medicinal plants is more interested. Due to high antioxidant activity and low toxicity, *Thunbergia laurifolia* Lindl. is used as natural antioxidant supplementation to prevent oxidative stress-related pathology (Palipoch *et al*., 2013).

However, the scientific investigations for acute toxicity and hypoglycemic activity from *Thunbergia laurifolia* Lindl. are still lacking in Myanmar. Therefore, the aims of the present research were to investigate the acute toxicity and hypoglycemic activity of watery extract of the leaves of *Thunbergia laurifolia* Lindl.

Figure 1 Habit of *Thunbergia laurifolia* Lindl.

Materials and Methods

Acute toxicity test of watery extract of leaves of *Thunbergia laurifolia* Lindl. on albino mice

The acute toxicity test on albino mice was carried out according to the method of OECD Guideline 423 at Department of Medical Research (DMR).

Materials

Test animals	-	18 female albino mice (ddy strain, body weight 25– 30 g)
Test agents	-	Distilled water, watery extract of *Thunbergia laurifolia* Lindl.
Apparatus	-	Mice cages, animal balance, 18 gauge dosing cannula, disposal syringes (1 ml and 5 ml), rubber glove and mask
Dose Schedule	-	2 g/kg and 5 g/kg (body weight) of albino mice
Period of observation	-	14 days

Methods

Female albino mice, weighing between 25-30 g were randomly selected and kept in their cages for at least 5 days prior to dosing to allow for acclimatization for laboratory condition. The mice were kept fasting overnight for 18 hours but were allowed with free access to water. Following period of fasting, mice were weighed and test substance was administered. Group (1) served as control and only 10 ml/kg of distilled water was given orally by using intragastric needle. In this study, starting dose 2 g/kg was chosen. The extract was dissolved in distilled water and required doses were given orally with 6 animals (3 animals per step). After administration of the test agent orally, they were allowed to have food and water. The sign of toxicity such as changes in skin and fur, eyes and mucous membrane, respiratory, circulatory, central nervous system were observed on test animals. Mice were observed individually after dosing at one time during the first 30 minutes hourly up to 4 hours for first day. After that all mice were monitored daily and weighed weekly. They were found to survive at the dose of 2 g/kg during observation period of 14 days. So, another 3 albino mice were administered with of watery extract 5 g/kg. The mice were observed for toxic sign by using the method described above. No toxic signs and lethality were observed in these 3 mice at the dose of 5 g/kg. So, another 3 mice were administered with watery extract 5 g/kg. A total of 6 albino mice were used at the dose level of 5 g/kg of the watery extract.

Body weight analysis

Individual weights of animals were recorded before the administration of drug on 1st day of the study and on 14th days of the experiment. Weight changes were recorded and calculated.

Figure 2 Groups of mice in acute toxicity study

Figure 3 Administration of extract suspension to mice

Hypoglycemic activity of watery extract of leaves of *Thunbergia laurifolia* Lindl.

The hypoglycaemic activity of watery extract was studied on adrenaline induced hyperglycaemic rats model (Gupta *et al*., 1967).

Materials

Test animals - 8 Wistar albino rats of both sexes (body weight 180- 250 g)

Test agents - Distilled water, watery extract, Glibenclamide tablets (B. P 5 mg, India), Adrenaline injection (1 mg/ml) (Myanmar Pharmaceutical Factory)

Apparatus - Aluminium cages, Animal balance, Spirit cotton wools, disposable syringes with needle (1 ml and 5 ml), Glucometer, Test strips, 18 gauge dosing needles, rubber gloves and masks

Dose Schedule - Watery extract of *Thunbergia laurifolia* Lindl. 0.75 g/kg, 1.5 g/kg and 3g/kg body weight

Method

In this experiment, adult albino rats (Wistar strain) of both sexes (body weight 180 - 250 g) were used. Eight rats were kept without food for 18 hours before the experiment but water was orally allowed freely. They were served as control group and oral administration of distilled water (10 ml/kg) body weight was given. Before the oral administration of distilled water to the control group, blood sample was collected from tail vein and blood glucose level was determined by glucometer. After 30 minute, the rats were made hyperglycemic by injection with adrenaline (0.4 ml/kg) subcutaneously to nape of the neck. The sample of blood were collected from the tail veins by cutting the tail (1 mm) at 1 hr, 2 hr, 3 hr and 4 hr intervals after subcutaneous injection of adrenaline. After taking the blood samples from tail vein, the tail was rubbed with cotton wool soaked in absolute alcohol to protect the puncture against infection. The result was read on the glucometer which was expressed in (mg/dl) of blood glucose level on the glucometer screen. Then, washout period of 1 week was done.

In this study, three different doses of watery extract of *Thunbergia laurifolia* Lindl. leaves (0.75 g/kg, 1.5 g/kg and 3 g/kg) and standard drug, glibenclamide (4 mg/kg) were tested for hypoglycemic activity in the rats. One week wash out period was done in between the determination of hypoglycemic activity of the different doses of the extracts and glibenclamide.

The detail procedure for testing of hypoglycemic activity was the same as mentioned above by using the same eight rats.

Data Analysis

The results were shown in (Mean ± SE). Student “t” test (Paired “t” test) was used for statistical comparison between blood glucose concentrations of the control group and experimental groups. P-value <0.05 was considered to be significant. Percent reduction of hyperglycemia was calculated by following formula:

$$\text{Percent reduction} = \frac{C-T}{C} \times 100$$

C = Rise in blood glucose of control

T = Rise in blood glucose level of tested animals

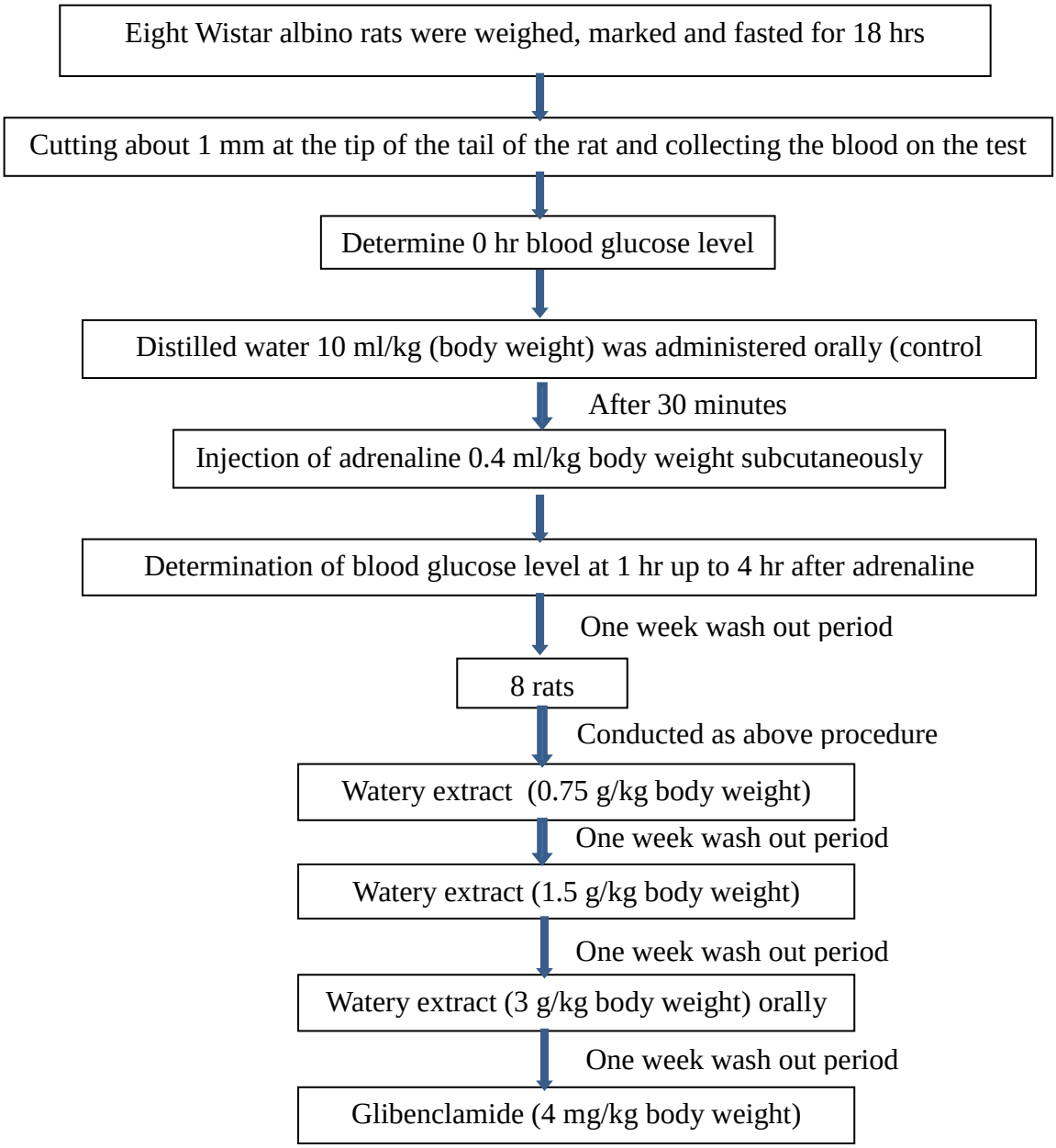

Figure 4 Flow chart for hypoglycemic activity testing in albino rats

Figure 5 Albino rats in cages and each contains 2 rats

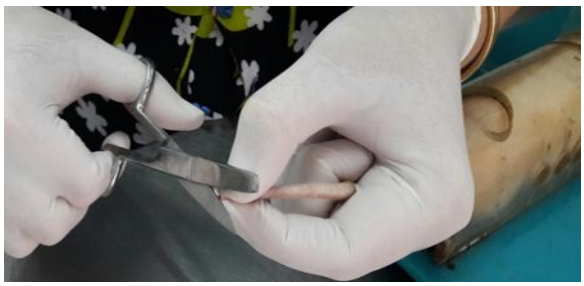

Figure 6 Cutting the tip of tail of the rats

Figure 7 Determining of blood glucose level in rats by using glucometer

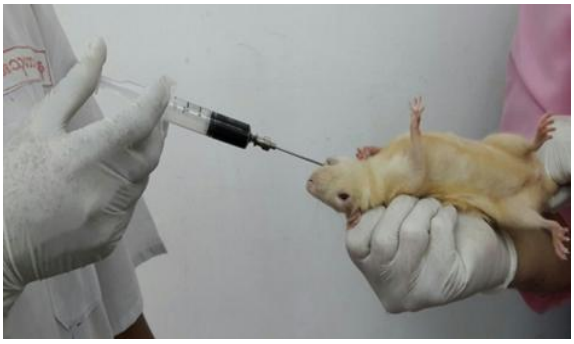

Figure 8 Administration of extract suspension to rat

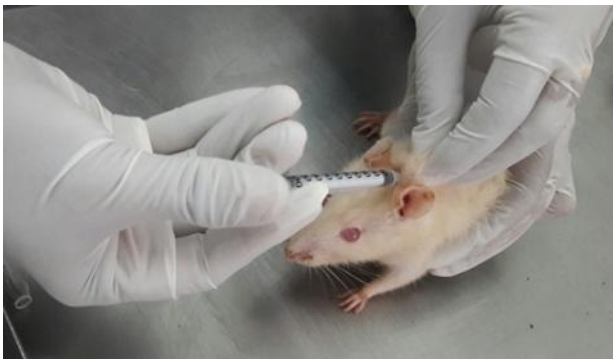

Figure 9 Adrenaline injection into nape of neck of albino rat

Results

Acute toxicity test of watery extract of the leaves of *Thunbergia laurifolia* Lindl. on albino mice

The acute toxicity test was carried out according to the guideline of OECD-423 method. In this experiment, the mice were administered with the does of 2 g/kg and 5 g/kg (body weight) of watery extract of the leaves of *Thunbergia laurifolia* Lindl. No lethality and toxic signs of the mice were observed up to 14 days observation period. There were no significant differences in body weight of the test groups when compared with control group. Therefore, the medium lethal dose (LD_{50}) was observed to be more than 5 g/kg (body weight). The results of acute toxicity were shown in Table 1.

There were no significant differences in body weights between the control group and watery extract of *Thunbergia laurifolia* Lindl. leaves (2 g/kg) receiving group at one week and two week after given the extracts. There was significant increase in body weight of watery extract (5 g/kg) receiving group at two week after extract administration when compared with control group. The results were shown in Table 1.

Table 1 Acute toxicity test of watery extract of *Thunbergia laurifolia* Lindl. in albino mice

No of Group	Type of drug administration	No of mice tested	Dosage	Observed period	No. of death
1	Control (distilled water)	6	10 ml/kg	14 days	0/6
2	Watery extracts	6	2 g/kg	14 days	0/6
3	Watery extracts	6	5 g/kg	14 days	0/6

Hypoglycemic activity of watery extract of the leaves of *Thunbergia laurifolia* Lindl. on the adrenaline induced hyperglycemic rats

Hypoglycemic activity of watery extract was tested by using adrenaline induced hyperglycemic rat model which was described in (Gupta *et al*., 1964 and Agrawal & Paridhavia, 2007). In this study, both sexes (body weight 180-250 g) of Wistar strain albino rats were used. Eight albino rats were served as control group with oral administration of distilled water (10 ml/kg) body weight. Then, washout period for a week was done. After the washout period of one week, the same eight rats were tested again with oral administration of each dose level of watery extract (0.75 g/kg, 1.5 g/kg and 3 g/kg) of the leaves of *Thunbergia laurifolia* Lindl. and standard drug (glibenclamide - 4 mg/kg). The results of mean blood glucose concentrations of 8 albino rats treated with watery extract of the leaves (0.75 g/kg) at 0 hr, 1 hr, 2 hr, 3 hr and 4 hr after subcutaneous injection of adrenaline (0.4 mg/kg) were 59 ± 2.96 mg/dl, 157 ± 4.24 mg/dl, 175.5 ±6.01mg/dl, 160.38±6.44mg/dl and 126.13± 7.81 mg/dl respectively. Significant decreases in blood glucose were observed at 2 hr, 3 hr and 4 hr ($p<0.05$ - $p<0.001$).

The results of mean blood glucose concentrations of 8 albino rats treated with watery extract of the leaves (1.5 g/kg) at 0 hr, 1 hr, 2 hr, 3 hr and 4 hr after subcutaneous injection of adrenaline (0.4 mg/kg) were 59.5 ± 1.94 mg/dl, 154 ± 7.35 mg/dl, 170.63 ± 6.22 mg/dl, 166.75 ± 12.49 mg/dl and 137.25 ±13.34 mg/dl respectively. Significant decrease in blood glucose were observed at 2 hr, 3 hr and 4 hr ($p<0.05$ - $p<0.001$). The results of mean blood glucose concentrations of 8 albino rats treated with watery extract of the leaves (3 g/kg) at 0 hr, 1 hr, 2 hr, 3 hr and 4 hr after subcutaneous injection of adrenaline (0.4 mg/kg) were 62.88 ± 2.06 mg/dl, 164.88 ± 8.82 mg/dl, 187.25 ± 11.16 mg/dl, 169.88 ± 10.36 mg/dl and 132.88 ± 10.71 mg/dl respectively. Significant decreases in blood glucose were observed at 3 hr and 4 hr ($p<0.05$ - $p<0.01$).

The results of mean blood glucose concentrations of 8 albino rats treated with standard drug (glibenclamide - 4 mg/kg) at 0 hr, 1 hr, 2 hr, 3 hr and 4 hr after subcutaneous injection of adrenaline (0.4 mg/kg) were 66.5 ± 4.5 mg/dl, 148.63 ± 6.21 mg/dl, 164.13 ± 6.62 mg/dl, 135.38 ± 8.01 mg/dl and 105.25 ± 7.47 mg/dl respectively. Significant decreases in blood glucose were observed at 3 hr and 4 hr ($p<0.01$- $p<0.001$). The results were shown in Table 2-3 and Figure 10-11.

Table 2 Mean blood glucose concentration (Mean ± SE) of watery extract and standard drug, glibenclamide to adrenaline-induced hyperglycemic rat model

	Blood glucose concentration (mg/dl)				
	0 hr	1 hr	2 hr	3 hr	4 hr
Control	64.88 ± 2.42	165.38 ± 7.43	205.75 ± 6.71	227.13 ± 10.43	204.5 ± 14.39
watery extract 0.75 g/kg	59 ± 2.96	157 ± 4.24	175.5 ± 6.01	160.38 ± 6.44	126.13 ± 7.81
watery extract 1.5 g/kg	59.5 ± 1.94	154 ± 7.35	170.63 ± 6.22	166.75 ± 12.49	137.25 ± 13.34
watery extract 3 g/kg	62.88 ± 2.06	164.88 ± 8.82	187.25 ± 11.16	169.88 ± 10.36	132.88 ± 10.71
Glibenclamide 4 mg/kg	66.5 ± 4.5	148.63 ± 6.21	164.13 ± 6.62	135.38 ± 8.01	105.25 ± 7.47

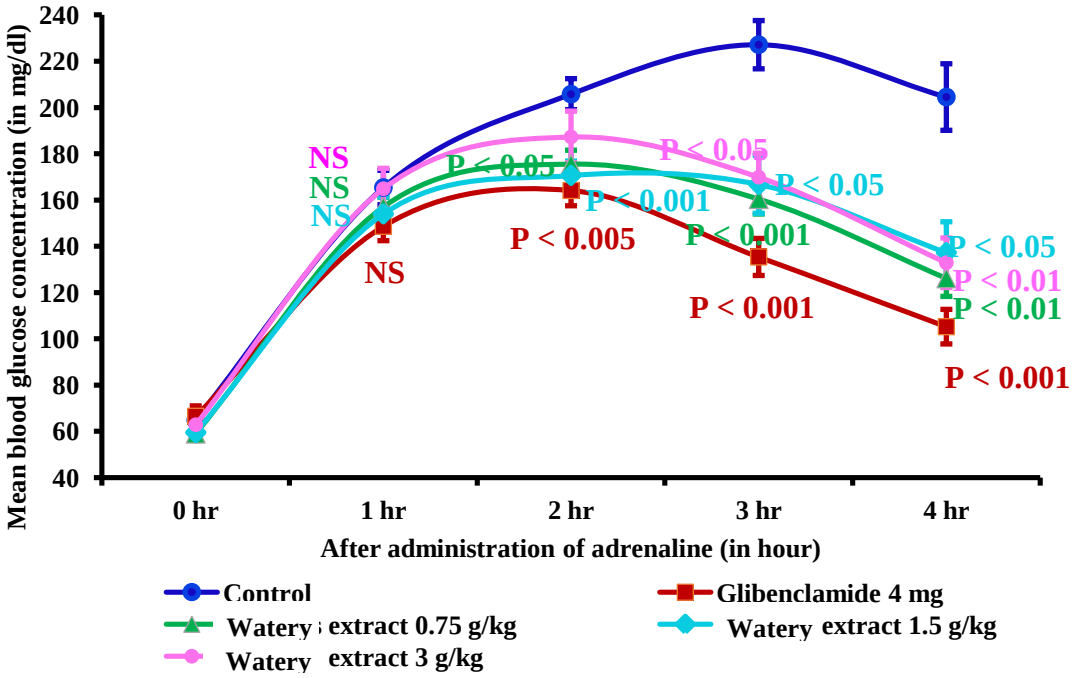

Figure 10 Time course of the effects of watery extract of *Thunbergia laurifolia* Lindl. leaves (0.75 g/kg, 1.5 g/kg and 3 g/kg) and glibenclamide (4 mg/kg) to adrenaline induced hyperglycemic rat model

Table 3 Percent reductions (Mean ± SE) of hyperglycemic with watery extract and glibenclamide (4 mg/kg) to adrenaline-induced hyperglycemic rat model

	Percent reduction of hyperglycemic			
	1 hr	**2 hr**	**3 hr**	**4 hr**
Glibenclamide 4 mg/kg	13.72 ± 10.8	30.09 ± 6.05	56.22 ± 5.8	71.36 ± 5.03
Watery extract 0.75 g/kg	-3.33 ± 11.72	15.83 ± 7.39	36.56 ± 4.69	49.64 ± 7.21
Watery extract 1.5 g/kg	0.98 ± 12.1	20.78 ± 4.21	30.93 ± 11.16	40.92 ± 11.71
Watery extract 3 g/kg	-7.23 ± 15.31	10.43 ± 9.72	31.1 ± 9.31	46.63 ± 10.54

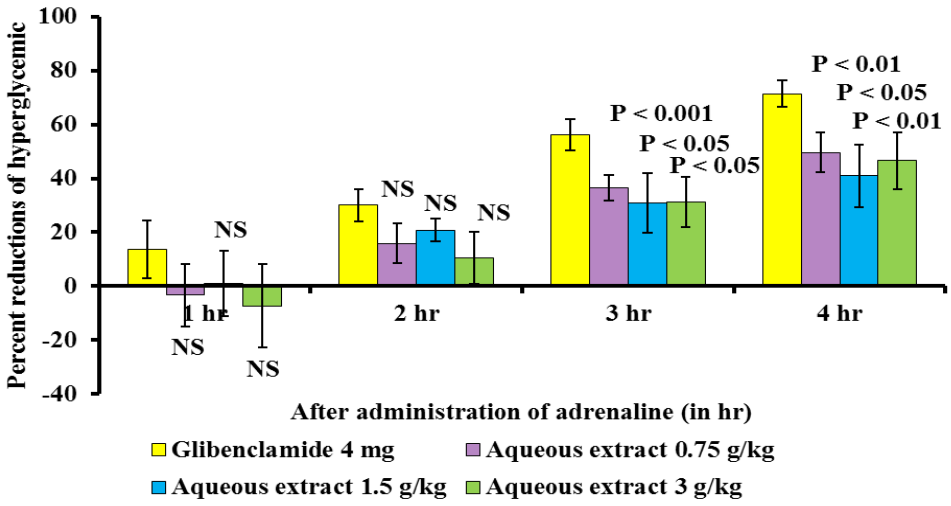

Figure 11 Percent reductions of hyperglycemic with watery extract of *Thunbergia laurifolia* Lindl. leaves (0.75 g/kg, 1.5 g/kg and 3 g/kg) and glibenclamide (4 mg/kg) to adrenaline induced hyperglycemic rats model (NS = not significant)

Discussion and Conclusion

In this study, the acute toxicity of watery extract of the leaves of *Thunbergia laurifolia* Lindl. were studied on albino mice by using OECD guideline 423. Oral route of administration was used because it is the route intended to be used in human. There were no toxic effect and lethality even with the maximum dose of 5 g/kg body weight. Therefore, the median lethal dose LD_{50} of the watery extract of *Thunbergia laurifolia* Lindl. leaves was found to be more than 5 g/kg body weight.

In an earlier acute toxicity study of watery leaf extract of *Thunbergia laurifolia* Lindl. in mice at 1, 2, 4 and 8 g/kg, it was reported that the extract is non toxicand safe for consumption (Usanawarong *et al.*, 2000). These observations are agreed with the results of the present study.

In this study, hypoglycemic activity of watery extract of the leaves of *Thunbergia laurifolia* Lindl. was investigated on adrenaline-induced hyperglycemic rats. The effect of the test extracts were compared to the standard drug, glibenclamide.

Aritajat *et al*. (2004) stated that the hypoglycemic activity of the watery extract from the leaves of *Thunbergia laurifolia* Lindl. was evaluated in normoglycemic and alloxon-induced diabetic rats. Watery extract of the leaves of *Thunbergia laurifolia* Lindl. caused significant decrease ($p<0.001$) levels of blood glucose in diabetic rats.

In the present results, the rats treated with watery extract (0.75 g/kg) showed significant decrease in the blood glucose concentration at 2 hr ($p< 0.05$), 3 hr ($p< 0.001$) and 4 hr ($p< 0.01$). The rats treated with watery extract (1.5 g/kg) showed significant decrease in the blood glucose concentration at 2 hr ($p< 0.001$), 3 hr($p< 0.05$) and 4 hr ($p< 0.05$) whereas at the dose of (3 g/kg) of watery extract, significant decreases in the blood glucose concentration were found at 3 hr ($p< 0.05$) and 4 hr ($p< 0.01$). These results are agreed with Aritajat *et al.,* 2004.

Mean percent reductions of hyperglycemia with watery extract of the leaves of *Thunbergia laurifolia* Lindl. ranged from (36.56 % to 49.64 %) at the dose of 0.75 g/kg, from (20.78 % to 40.92 %) at 1.5 g/kg and from (31.1 % to 46.63 %) at3 g/kg. Mean percent reductions of hyperglycemia with standard drug, glibenclamide ranged from (30.09 % to 71.36). Therefore, watery extract of the leaves of *Thunbergia laurifolia* Lindl. possessed significant hypoglycemic effect in hyperglycemic rats and the extract was less effective than glibenclamide.

The phytochemical constituents (β-Sitosterol, lupeol) were present in the leaves of *Thunbergia laurifolia* Lindl. (Chuthaputti, 2010). Hypoglycemic activity of the leaves of *Thunbergia laurifolia* Lindl. may be due to the presence of phytochemical constituents in the leaves. Therefore, the leaves of *Thunbergia laurifolia* Lindl. could be used as herbal medicine for hypoglycemic activities. It also can be safe to eat the leaves of *Thunbergia laurifolia* Lindl. because the leaves are non-toxic.

Acknowledgement

The authors would like to express their profound gratitude to the Department of Higher Education (Yangon Office), Ministry of Education, Yangon, Myanmar, for provision of opportunity to do this research and Myanmar Academy of Arts and Science for allowing to present this paper.

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