



## Antidiabetic and Long-term Effects of *Elaeocarpus grandiflorus*

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### Abstract

This study aimed to investigate the hypoglycemic effect of *Elaeocarpus grandiflorus* water extract in alloxan-induced diabetic rats. Chronic effects of the extract on plasma glucose, lipids, blood biochemistry, some drug-metabolizing enzymes and internal organ weights were also studied. Diabetes was induced in Wistar rats by subcutaneous injection of alloxan. After being confirmed as diabetic, the animals were orally administered with distilled water or the extract at 0.0001, 0.001 or 0.01 g/kg body weight (BW) daily for 30 days. The results showed that hypoglycemic effect of the extract was dose-dependently observed on day 7. The glucose lowering effect was also continuously observed at the highest dose until the second week. The diabetic rats treated with the extract at 0.0001 and 0.001 g/kg BW possessed greater alanine aminotransferase (ALT) activity than the diabetic control rats. The diabetic rats treated with the extract at 0.001 g/kg BW possessed increased plasma triglyceride compared with the diabetic control rats. The diabetic control rats tended to possess decreased aminopyrine-N-demethylase (APD) activity. Insulin or the extract could not reverse the enzyme activity to the baseline value. However, the aniline hydroxylase (AH) activities were unchanged in all groups. The diabetic-induced BW lost was minimized by the extract. Studies for long-term effects of the extract on internal organ weights revealed that the normal rats treated with extract at 0.01 g/kg BW possessed increased weights of heart and pancreas. The extract at 0.001 g/kg BW significantly reduced the diabetic effect on the kidney and liver weights but failed to return them to the baseline values. The extract at 0.01 g/kg BW significantly reversed the heart and lung weights to the normal values. It is concluded that *E. grandiflorus* water extract possesses a hypoglycemic effect. The use of the extract should not be longer than two consecutive weeks. A close follow-up for any possible toxicity should be performed during the chronic use of *E. grandiflorus*.

**Keywords:** *Elaeocarpus grandiflorus*; Diabetes; Hypoglycemia

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### Introduction

*Elaeocarpus grandiflorus* belongs to the family Elaeocarpaceae. Previous studies show that *E. grandiflorus* possesses antibacterial (Rahman et al., 1998), anti-diuretic (Van Der Woerd, 1950) and antiviral (Kurokawa et al., 1993; Nawawi et al., 1999; Xu et al., 1996) activities. Water extract of leaves, fruit and twigs of *E. grandiflorus* has been traditionally used to treat diabetic patients while none of scientific data is available. Tannin, geraniin and 3, 4, 5-trimethoxy geraniin have been isolated from *E. grandiflorus* leaves (Rahman et al., 1998).

Cytochrome P450 is composed of a number of CYP enzyme families that metabolize a large number of compounds such as steroids, chemical carcinogens and drugs (Gibson & Skett, 1994). The CYP enzymes may be induced or suppressed in many pathophysiological conditions, such as diabetes, cancer and inflammation (Po-Yung & Edward, 2001). It was reported that the activities of some drug metabolizing enzymes were altered in chemical-induced diabetes animals (Po-Yung & Edward, 2001). Therefore, this study aimed to determine the chronic effect of *E. grandiflorus* on plasma glucose levels, on some hepatic enzyme functions and on plasma lipid in alloxan-induced diabetes rats. In addition, long-term effects of *E. grandiflorus* extract on rat internal organs were also studied by measuring the organ weights.

## Materials and Methods

### Animals

Adult male Wistar rats (190-260 g) were obtained from the National Laboratory Animal Centre of Mahidol University, Thailand. They were housed in a room with controlled temperature ( $23\pm 2$  °C) and a 12-hr light/ 12-hr dark cycle. The animals had free access to food and water except on the day of plasma glucose measurement. The study protocol was approved by the Naresuan University Ethical Committee.

### Plant materials

*E. grandiflorus* was collected from Poi Waterfall area, Wangthong District, Phitsanulok, Thailand. A voucher specimen (NO.6863) was deposited at Royal Forest Department, Ministry of Agriculture and Cooperatives.

### Extract preparation

Water extract of *E. grandiflorus* was prepared according to the traditional use. Dried *E. grandiflorus* leaves, twigs and fruits were weighed in the ratio of 3:4:1 by weight and then boiled with 3 liter of distilled water for 3 hr. The aqueous extract was filtered and freeze-dried.

### Induction of diabetes

A freshly prepared solution of alloxan (120 mg/kg body weight (BW), Sigma-Aldrich, USA) in 0.9% NaCl was subcutaneously injected to 24-hr fasted rats (Poopat, 1993). After 24 hr of the treatment, blood glucose was measured using Glucostrip read on a Glucometer. The animals having blood glucose exceeded 200 mg/dl were judged to be diabetic and then chosen for the subsequent experiments.

### Treatment protocol

The animals were divided into seven groups (five rats in each group). The first three groups were: normal control rats orally receiving distilled water (DW); normal control rats orally receiving the extract (0.01 g/kg BW); and diabetic rats orally receiving DW. The other four groups were diabetic rats intraperitoneally injected with insulin (6 U/kg BW) and diabetic rats orally receiving the extract at the doses of 0.0001, 0.001 or 0.01 g/kg BW. The treatment was performed daily for 30 days.

### Collection of blood samples

The rats were fasted for 15 hr before blood collection. Blood was collected from the tail vein and the extract was administered immediately. After 30 min, equal volume of blood was collected again and glucose (1.25 g/kg) was orally administered. Then blood was further collected for five times at every 30 min.

### Plasma glucose determination

Plasma glucose was determined by glucose oxidase method (Peungvicha et al., 1998). Regarding long-term treatment, plasma glucose was determined on days 7, 14, 21 and 30 after the alloxan injection (Nagappa et al., 2003 with some modification).

### Enzyme activity assay

At the end of the treatment, the animals were sacrificed and liver microsomes were prepared by  $\text{CaCl}_2$  precipitation (Lake, 1987). Protein concentration was measured by the method

of Lowry et al (1951). Aniline hydroxylase (AH, CYP2E1) activity was measured by the method of Schenkman et al (1967). Aminopyrine-N-demethylase (APD, CYP2C11) and *p*-Nitroanisole-*O*-demethylase (*p*-NAOD, CYP1A1/2) activities were measured by the method of Nash (1953) and Bidlack & Lowery (1982), respectively.

#### Long-term effects of *E. grandiflorus* on blood biochemistry and organ weight

Blood samples were collected at the beginning and the end of the study. They were centrifuged at 5000 rpm for 5 min. Plasma was obtained and stored at room temperature for biochemical analysis. Plasma aspartate aminotransferase (AST), alanine aminotransferase (ALT), triglyceride, cholesterol and creatinine were determined by an automatic chemistry analyzer (Roche Diagnostics, Switzerland).

This study investigated long-term effects of the extract on internal organs by determining organ weights. At the end of the study, all the rats were sacrificed. The liver, kidney, heart, lung, spleen, urinary and pancreas were weighed. The organ weights are expressed as percentages of the body weights.

#### Statistical analysis

The statistical analysis was performed by one-way analysis of variance (ANOVA) followed by the least significant different (LSD) test. When the number of groups was 2, Student's t-test was used for comparison. Data are expressed as mean±standard error of mean (SEM). A P-value of less than 0.05 was considered as statistically significant.

## **Results**

#### Effect of *E. grandiflorus* on plasma glucose in normal rats

Plasma glucose reached the maximum level at 30 min after the glucose administration (Table 1). The same phenomena were observed with the extract-treated normal rats and DW-treated diabetic rats. Therefore, this time point was employed for blood collection in the subsequent long-term study. The diabetic control rats possessed significant increased glucose levels compared with the normal control rats.

#### Hypoglycemic effect of *E. grandiflorus* in diabetic rats

The subcutaneous injection of alloxan induced diabetes in all rats. The plasma glucose levels of the diabetic rats at 30 min after glucose administration are shown in Table 2. In the diabetic rats treated with DW, hyperglycemia existed for the whole period of the study. Insulin injection significantly reduced plasma glucose levels compared with the DW-treated diabetic rats. Hypoglycemic effect of the *E. grandiflorus* extract was dose-dependently observed on day 7. The glucose lowering effect was also continuously observed at the highest dose until the second week.

#### Effect of *E. grandiflorus* on plasma lipid and blood biochemistry

Effect of the *E. grandiflorus* extract on plasma cholesterol and triglyceride were investigated. The plasma lipids and biochemistry were measured at the beginning and the end of the treatment. Long-term treatment of the normal control rats with DW or the extract revealed unchanged plasma lipids and biochemistry parameters (Table 3). Although they were not statistically significant, the diabetic control rats tended to possess greater plasma cholesterol and triglyceride levels compared with the initial values.

Treatment of the diabetic rats with the extract had no effect on the plasma cholesterol and triglyceride. However, the diabetic rats treated with 0.001 g/kg BW extract exhibited higher plasma

triglyceride than that of the initial value. The diabetic control rats tended to possess greater ALT activity compared with that of the normal control rats although it was not statistically significant. The diabetic rats treated with the extract at the doses of 0.0001 and 0.001 g/kg BW possessed greater ALT activity than those of the normal and the diabetic control rats. The AST activities of the diabetic rats treated with the extract at the dose 0.001 g/kg BW was significantly increased compared with the normal and the diabetic control rats.

The DW-treated diabetic control rats possessed lower creatinine level compared with the normal control rats. Treatment of the diabetic rats with the extract had no effect on the creatinine level.

#### Effect of *E. grandiflorus* on hepatic enzyme function

Activities of AH, APD and p-NAOD were investigated at the end of the treatment. The results show that the AH activity of all the treatment groups were not significantly different (Table 4). The diabetic control rats tended to possess decreased APD activity compared to the normal control group although this was not statistically significant. Decreased APD activities were observed in the diabetic rats treated with insulin and the extract at the dose of 0.0001 and 0.001 g/kg BW. Regarding the p-NAOD activity, the normal rats treated with the extract tended to possess an increase in the p-NAOD activity although this was not statistically significant.

#### Effect of *E. grandiflorus* on rat organ weights

After being sacrificed, the organ weights were recorded and the data are shown in Table 5. Treatment of the normal control rats with the extract 0.01 g/kg BW significantly increased weights of heart, kidney and pancreas. The diabetic control rats possessed increased all the organ weights, except spleen weight, compared with the normal control group. Insulin treatment of the diabetic rats restored the heart, lung and urinary bladder weights to the baseline values. The extract at the dose of 0.001 g/kg BW significantly reduced the diabetic effect on the kidney and liver weights but failed to return them to the baseline values. The extract at the dose of 0.01 g/kg BW significantly reversed the heart and lung weights to the normal values.

#### Effect of *E. grandiflorus* on body weight

The normal control rats progressively gained weight while the diabetic rats lost weight during the study (Table 6). The normal rats treated with the extract tended to have higher weight gain compared with the water-treated normal rats although this was not statistically significant. Insulin treatment reduced the diabetic effect on body weight lost. Effects of the extract on the body weight gain were significantly observed on the third week. The diabetes-induced body weight lost was minimized by the extract treatment.

## **Discussion**

The water extract of *E. grandiflorus* was investigated for its antidiabetic activity and long-term effects on the organ weights. The diabetic state was induced by intraperitoneal injection of alloxan, one of the two most commonly used diabetogenic chemicals (Szkudelski et al., 1998; Szkudelski, 2001). In this study alloxan effectively induced diabetes in all animals studied. The plasma glucose levels observed were above the standard range for diabetes. The previous studies reported that some animals died upon alloxan injection due to its potent effect on pancreatic cells and some other organs (Alarcon et al., 2005; Gupta et al., 2005). However, our results did not correspond to the previous studies since none of animals died during the study. This may be a result of the differences in the animal strains and sources. The rats used in this study may withstand to the alloxan damaging effect more than the others.

**Table 1.** Effect of *E. grandiflorus* water extract on glucose tolerance in fasted and glucose loaded normal rats

Animal	Treatment (number of animal)	Mean plasma glucose (mg/dl)						
		Fasting	Time after glucose administration (min)					
			0	30	60	90	120	150
Normal rats	DW (5)	113.8 ± 9.7	111.0 ± 10.3	170.6 ± 16.9	125.2 ± 13.3	104.8 ± 12.2	94.0 ± 9.8	96.2 ± 12.0
	Extract at 0.01g/kg BW (5)	91.5 ± 2.3	114.1 ± 3.0	251.0 ± 10.2*	189.0 ± 14.6*	163.7 ± 17.9*	150.5 ± 16.0*	113.2 ± 1.9
Diabetic rats	DW (7)	297.9 ± 41.3	274.2 ± 41.6	351.8 ± 36.9	336.7 ± 54.6	326.4 ± 53.1	346.8 ± 46.1	329.4 ± 64.3

\* P<0.05 compared to the control. Data represent means ± S.E.M.

**Table 2.** Long-term effects of *E. grandiflorus* water extract on plasma glucose in normal and diabetic rats

Animal	Treatment (number of animal)	Mean plasma glucose (mg/dl)			
		Day 7	Day 14	Day 21	Day 30
Normal rats	DW (5)	142.7 ± 25.2	232.3 ± 25.0	189.9 ± 30.2	214.4 ± 8.8
	Extract at 0.01 g/kg <sup>a</sup> (5)	166.1 ± 9.8	206.3 ± 8.1	294.8 ± 6.8	204.1 ± 8.4
Diabetic rats	DW <sup>a</sup> (6)	443.5 ± 41.2**	558.9 ± 13.2**	439.4 ± 43.3**	388.9 ± 2.8**
	Insulin <sup>b</sup> (4)	216.7 ± 28.9**	237.0 ± 77.6**	250.7 ± 54.0**	191.1 ± 56.9**
	Extract at 0.0001 g/kg <sup>b</sup> (7)	349.9 ± 29.4*	475.6 ± 26.0	451.7 ± 29.5	410.0 ± 12.7
	Extract at 0.001 g/kg <sup>b</sup> (5)	285.3 ± 24.5**	460.5 ± 28.3	431.3 ± 13.6	376.5 ± 11.2
	Extract at 0.01 g/kg <sup>b</sup> (6)	271.1 ± 48.9**	424.6 ± 39.4*	359.3 ± 62.8	376.3 ± 34.0

Data represent means ± S.E.M.

<sup>a</sup> compared with the normal rats treated with DW

<sup>b</sup> compared with the diabetic rats treated with DW

\*\* P<0.005.

\* P<0.05.

**Table 3.** Effect of chronic exposure to *E. grandiflorus* water extract on plasma lipid and biochemistry

Animal	Treatment (number of animal)	Day	Cholesterol (mg/dl)	Triglyceride (mg/dl)	ALT (U/L)	AST (U/L)	Creatinine (mg/dl)
Normal rats	DW (40)	initial	82.5 ± 2.3	167.6 ± 22.3	50.8 ± 2.5	204.7 ± 10.4	0.59 ± 0.05
	DW (5)	final	87.80 ± 8.35	136.20 ± 22.25	48.80 ± 2.80	194.40 ± 45.75	0.64 ± 0.02
	Extract at 0.01 g/kg BW (5)	final	84.00 ± 4.28	113.20 ± 19.70	71.60 ± 15.84	477.40 ± 154.54	0.68 ± 0.04
Diabetic rats	DW (5)	final	136.60 ± 17.64	254.80 ± 65.38	67.60 ± 8.85	181.00 ± 22.43	0.42 ± 0.10*
	Insulin (5)	final	116.60 ± 10.25	210.00 ± 13.15	48.60 ± 7.44	190.00 ± 28.04	0.50 ± 0.03
	Extract at 0.0001 g/kg BW (6)	final	128.00 ± 35.65	239.17 ± 99.50	140.33 ± 50.61 <sup>*,#</sup>	259.80 ± 34.99	0.58 ± 0.12
	Extract at 0.001 g/kg BW (5)	final	134.40 ± 18.78	335.40 ± 74.65*	144.80 ± 27.92 <sup>*,#</sup>	1057.00 ± 490.61 <sup>*,#</sup>	0.56 ± 0.02
	Extract at 0.01 g/kg BW (6)	final	102.83 ± 11.98	190.67 ± 33.00	65.33 ± 9.00	178.17 ± 22.38	0.58 ± 0.03

Data represent means ± S.E.M.

\* significant difference from the normal rats treated with DW on the final day.

# significant difference from the diabetic rats treated with DW on the final day.

**Table 4.** Effect of chronic exposure to *E. grandiflorus* water extract on hepatic enzymes

Animal	Treatment (number of animal)	Protein (mg liver/g BW)	AH (nmol/min/mg protein)	APD (nmol/min/mg protein)	<i>p</i> -NAOD (nmol/min/mg protein)
Normal rats	Distilled water (5)	32.44 ± 1.42	0.039 ± 0.004	1.24 ± 0.20	0.427 ± 0.068
	Extract at 0.01 g/kg BW (5)	18.49 ± 5.25*	0.067 ± 0.016	1.09 ± 0.19	0.732 ± 0.163
Diabetic rats	Distilled water (5)	42.80 ± 3.64	0.047 ± 0.016	0.73 ± 0.22	0.487 ± 0.065
	Insulin (5)	28.40 ± 3.70	0.051 ± 0.010	0.47 ± 0.11*	0.711 ± 0.083
	Extract at 0.0001 g/kg BW (6)	31.05 ± 3.03	0.053 ± 0.007	0.49 ± 0.13*	0.800 ± 0.252
	Extract at 0.001 g/kg BW (5)	36.50 ± 7.34	0.038 ± 0.013	0.57 ± 0.41*	0.643 ± 0.114
	Extract at 0.01 g/kg BW (6)	30.24 ± 4.61	0.045 ± 0.008	0.91 ± 0.13	0.644 ± 0.155

Data represent means ± S.E.M.

\* $p < 0.05$  compared with the normal rats treated with DW.

AH=Aniline hydroxylase

APD=Aminopyrine-*N*-demethylase

*p*-NAOD=*p*-nitroanisole-*O*-demethylase

**Table 5.** Organ weights of normal and diabetic rats treated with distilled water, insulin or *E. grandiflorus* extract

Animal	Treatment (number of animal)	Organ weights (g)						
		Heart	Kidney	Liver	Lung	Urinary bladder	Spleen	Pancreas
Normal rats	DW (5)	0.29 ± 0.01	0.54 ± 0.02	2.71 ± 0.06	0.38 ± 0.03	0.03 ± 0.01	0.18 ± 0.01	0.08 ± 0.03
	Extract at 0.01 g/kg BW (5)	0.35 ± 0.02*	0.58 ± 0.01*	2.95 ± 0.03	0.41 ± 0.02	0.05 ± 0.01	0.20 ± 0.02	0.19 ± 0.01*
Diabetic rats	DW (5)	0.40 ± 0.03*	1.47 ± 0.12*	5.39 ± 0.18*	0.56 ± 0.02*	0.14 ± 0.03*	0.21 ± 0.03	0.21 ± 0.01*
	Insulin (5)	0.36 ± 0.04	0.91 ± 0.05 <sup>*,#</sup>	4.42 ± 0.30 <sup>*,#</sup>	0.44 ± 0.04 <sup>#</sup>	0.07 ± 0.00	0.23 ± 0.03	0.19 ± 0.03*
	Extract at 0.0001 g/kg BW (6)	0.35 ± 0.02	1.21 ± 0.07*	4.68 ± 0.21*	0.49 ± 0.03*	0.09 ± 0.01	0.19 ± 0.01	0.24 ± 0.04*
	Extract at 0.001 g/kg BW (5)	0.35 ± 0.01	1.12 ± 0.14 <sup>*,#</sup>	4.41 ± 0.48 <sup>*,#</sup>	0.44 ± 0.03 <sup>#</sup>	0.20 ± 0.10*	0.21 ± 0.01	0.14 ± 0.01
	Extract at 0.01 g/kg BW (6)	0.34 ± 0.02 <sup>#</sup>	1.01 ± 0.17 <sup>*,#</sup>	4.11 ± 0.46 <sup>*,#</sup>	0.43 ± 0.05 <sup>#</sup>	0.08 ± 0.02	0.19 ± 0.01	0.16 ± 0.05

Data represent means ± S.E.M.

\*  $p < 0.05$  compared to the normal rats treated with DW.

#  $p < 0.05$  compared to the diabetic rats treated with DW.



**Table 6.** Effect of *E. grandiflorus* on the % body weight change in diabetic rats in 30 days

Animal	Treatment (number of animal)	% Body weight change			
		Day 7	Day 14	Day 21	Day 30
Normal rats	DW (5)	4.9 ± 0.8	22.9 ± 9.1	35.6 ± 2.0	53.1 ± 10.2
	Extract at 0.01 g/kg <sup>a</sup> (5)	9.8 ± 1.1	31.0 ± 2.3	48.2 ± 1.6	72.1 ± 2.1
Diabetic rats	DW (5)	-8.2 ± 3.5*	-5.9 ± 4.0*	-7.1 ± 7.2*	-13.1 ± 7.2*
	Insulin <sup>b</sup> (5)	1.2 ± 5.2	15.4 ± 3.7	26.0 ± 6.3 <sup>#</sup>	42.0 ± 11.7 <sup>#</sup>
	Extract at 0.0001 g/kg <sup>b</sup> (6)	-3.7 ± 3.4	4.8 ± 5.9	4.2 ± 7.8*	9.5 ± 10.1*
	Extract at 0.001 g/kg <sup>b</sup> (5)	-0.1 ± 4.7	12.6 ± 7.4	17.2 ± 12.4 <sup>#</sup>	25.5 ± 16.7 <sup>#</sup>
	Extract at 0.01 g/kg <sup>b</sup> (6)	-1.1 ± 4.1	12.2 ± 12.6	14.9 ± 7.6 <sup>#</sup>	22.2 ± 19.8

Data represent means ± S.E.M.

<sup>a</sup>compared with the normal rats treated with DW.

<sup>b</sup>compared with the diabetic rats treated with DW.

\*  $p < 0.05$  compared to the normal rats treated with DW.

<sup>#</sup> $p < 0.05$  compared to the diabetic rats treated with DW.

Alloxan-induced diabetic rats were used as a model for studying hypoglycemic effects of the *E. grandiflorus* water extract. The results demonstrate that the water extract possessed an antidiabetic activity in the diabetic rats. The hypoglycemic activities were dose-dependently observed on the first week of the treatment with a longer effect when the highest dose was used. The hypoglycemic activity was unobserved during the rest of the study. However, the extract was not able to restore the plasma glucose to the baseline value. This indicates that if the *E. grandiflorus* extract is going to be used to treat diabetic patients, it should be used with other means for diabetic control such as food restriction and hypoglycemic drugs.

Regarding the effective period of the hypoglycemic activity, long-term use of the extract does not provide better control of the plasma glucose. Its effect is observed only during the first two weeks. Therefore, it is recommended that the use of the *E. grandiflorus* should not be longer than two consecutive weeks. This recommendation would also prevent the patients from the adverse effects seen from the chronic use. However, more studies are required before any conclusion on human use of *E. grandiflorus* as an antidiabetic can be made.

Generally, body weights are reduced in the diabetic rats as well as in type I diabetic patients (Bwititi et al., 2001; McDermott et al., 2003; Musabayane et al., 2005). In this study, the decrease of body weights were diminished by the extract treatment, thus this effect may be useful for the diabetic patients. Chronic diabetes usually results in disturbance of the plasma lipid profile including increased plasma cholesterol and triglyceride (Safak et al., 2002). This study confirms the previous finding that the plasma lipids were increased in the diabetic rats. However, long-term exposure with *E. grandiflorus* water extract or insulin could not improve the plasma lipids of the diabetic rats.

A number of herbal medicines are hepatotoxic and nephrotoxic (Nyarko et al., 2005). Damages to the liver and kidney often result in elevations in the serum biochemistry parameters such as plasma AST and ALT and creatinine (Handerson, 2001). Increases in the plasma ALT are observed in the condition in which pancreas, liver, kidney and heart are destroyed by alloxan (Szkudelski et al., 1998; Szkudelski, 2001). This study observed an increase in the plasma ALT and a decrease in the creatinine level which correspond with the previous study (Hwang et al., 2005). Treatment with the extract tended to increase plasma AST and ALT levels in both normal and diabetic rats indicating possible unwanted effects of the extract on the internal organ.

The hepatic microsomal CYP enzymes are involved in the metabolism of various drugs and xenobiotics (Gibson & Skett, 1994). Chronic diabetes results in the increased activities of AH and *p*-NAOD and the decrease in APD activity (Poopat, 1993; Po-Yung & Edward, 2001). This study found that the activity of AH in diabetic rats treated with water, insulin and extract did not differ from the normal control rats. A previous study found that the activity of AH began to decline during weeks 4 and 8 after the diabetic induction (Barnett et al., 1994). Therefore, the appearance of the AH activity may be the result of the disease pattern itself. Regarding the *p*-NAOD activity, this study failed to detect any changes in the enzyme activity. However, the APD activity tended to be decreased which corresponds with the previous report. In addition, long-term treatment of the diabetic rats with insulin or the extract had no improving effect on the enzyme activity.

An alteration in the internal organ weights may primarily indicate toxicity or pathology occurring to those organs. This study investigated long-term effects of *E. grandiflorus* through recording the organ weights at the end of the treatment in addition to the hepatic enzyme activity and plasma biochemistry measurement. It was found that most of the organ weights studied, except the spleen weight, were increased by the diabetic state. The insulin treatment could prevent or minimize the increases in the weight of some organs. Chronic exposure did not correct the increased organ weights in the diabetics. Regarding the renal system, extract treatment reduced weights of the kidney and urinary bladder. This may be the result of antidiuretic effect of *E. grandiflorus* as

reported previously. In the normal control rats treated with the extract, increased organ weights were identified in the heart and pancreas. Regarding the plasma ALT levels, the diabetic rats treated with the extract tended to have a greater amount of ALT compared to that of the diabetic control rats. These results together may suggest some possible toxic effects on the liver. This should be also taken into consideration when *E. grandiflorus* is going to be used in long periods.

## Conclusion

*E. grandiflorus* water extract possesses an antidiabetic activity. It should not be used for longer than 14 consecutive days. A close follow-up for any possible toxicity should be performed during the chronic use. Further studies are required to identify the active fractions that are responsible for hypoglycemic activity and to clarify mechanisms of their actions.

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