HYPOGLYCEMIC EFFECT OF LOTUS (NELUMBO NUCIFERA GAERTN.) FLOWER ETHANOLIC EXTRACT ON ALLOXAN INDUCED DIABETES RAT MODEL

Ho Thi Huynh Thu[1], Pham Thi Thanh Xuan[2], Nguyen Thi Thuy Hang[3], Nguyen Nhut Vu[4], Trinh Huu Phuoc[5,*]

[1,2,3,4,5]Ho Chi Minh City Open University, Vietnam.

*Email: trinhhuuphuoc.ou@gmail.com

(Received: 04 /03/2016; Revised: 28 /03/2016; Accepted: 29/03/2016)

ABSTRACT

The current study was purposed to evaluate an acute toxicity and effects of lotus (Nulumbo nucifera Gaertn.) flower ethanolic extract (LFEE) on alloxan induced diabetic rats. In vivo acute toxicity study of LFEE was carried out via the guideline of the testing of Chemicals (OECD) by oral administration at limit test of 2000 mg/Kg and 5000 mg/Kg, the results showed that, LD50 was greater than the limit dose at 5000 mg/Kg. The various dosages, including 150 and 200 mg/Kg, were enrolled in current study to evaluate its effect on fasting blood glucose reduction. The results revealed that LFEE had hypoglycemic activity, no signs and disorder symptoms were observed in the period of oral administration. Furthermore, the extract at a dose of 150 mg/Kg showed the good effect on reducing of fasting blood glucose. These findings indicated that LFEE had non acute toxicity and hypoglycemic activities, in future, alternative beneficial products in a treatment of diabetes.

Keywords*: Nelumbo nucifera Gaertn; acute toxicity; diabetic rats; alloxan; LFEE.*

1. Introduction

Diabetes mellitus (DM) is a metabolic disorder disease in which characterized by hyperglycemia, constant high levels of blood glucose, resulting from various metabolic derangements, such as defects in insulin secretion, insulin function, or both (American Diabetes Association, 2014). According to International Diabetes Federation, there were total cases of adults (20-79 years) with 3.5 million cases of diabetes in Vietnam in 2015, counting for 5.6% of adult population. Currently, in diabetes treatment, available pharmotherapies have been based on the oral hypoglycemic agents and insulin, however, there are no any effects on restoration of normal glucose homeostasis and not free from side effects (Bandawane *et al*., 2011; Kumar *et al.,* 2012). Therefore, management of diabetes without any side effect is still a challenge to medical system. This leads to increase the demand for complementary and alternative medicine with antidiabetic activity and without side effects (Abd El Sattar El Batran *et al.,* 2006; Sakuljaitrong *et al*., 2013).

Many medical plants have been found and recommended for the treatment of diabetes in many studies. Lotus (*Nelumbo nucifera* Gaertn.) is a perennial water plant grown and is one of traditional folk herbs widely used in Vietnam. Remarkably, its roots, leaves, seeds and other parts are edible and thought to have multiple medical properties (Ono *et al*., 2006). In tradition, lotus flowers are useful to treat many disorder

diseases, such as bleeding disorders, cholera, fever, hyperdipsia, etc. (Sakuljaitrong *et al.*, 2013). However, especially in Vietnam, the study on acute toxicity, the effects on hyperglycemia of *N. nucifera* Gaertn. Flower extract has been poorly studied. Thus, the aims at current study were designed to evaluate acute toxicity and effect of *N. nucifera* Gaertn. Flower on hyperglycemia in alloxan induced diabetic rats. The results from current study could be used for utilization in the traditional herb for the diabetic treatment.

2. Materials and methods

Plant materials, ethanolic extract and alloxan induced diabetic rat preparation

Lotus (*N. nucifera* Gaertn.) were collected from natural resource in Phu Thuan, Hong Ngu, Dong Thap province, Vietnam. The fresh lotus flowers were isolated and cut into small species, then, dried in a temperature of 65^{o}C for 72 hours. The dried flowers were subjected to sized reduction a coarse powder, then, extracted with 95% ethanol for 7 days (1:10 w/v). The extract was evaporated by using a rotary evaporator. The obtained 95% ethanolic extract of lotus flower was stored at -20^{o}C until further being used.

Male *Mus musculus var. Albino* rats weighting 20 – 22 gs., purchased from Pasteur Institute in Ho Chi Minh City, Vietnam, were the animals used in the present study. They were kept in clean cages at 25^{o}C with 12-h light/12-h dark cycles.

The rats were induced to be diabetes by a single intraperitoneal injection with 150 mg/kg of Alloxan monohydrate (Sigma, USA). After injection, they were provided with 5% glucose solution as their drinking water for 72 hours to alleviate the severity after initial hypoglycemic phase. For diabetes confirmed after 3 days' injection, the rats with fasting blood glucose over 150 mg/dl were selected and enrolled into present study.

Acute toxicity study

Acute oral toxicity tests: up-and-down procedure, was carried through the guild line 425 of for Testing of Chemicals, OECD (Organization for Economic Cooperation). The limit test at 2000 mg/kg was firstly carried out at group of five healthy rats. If the LD50 is greater than the test dose (2000 mg/kg) by three or more animal survive observed, conducted the limit test at dose of 5000 mg/kg. Dose one animal at the test dose, if the animal dies conduct the main test to determine the LD50. If the animal services, dose two additional animals. If both animals survive, the LD50 is greater than the limit dose and the test is terminated. Signs or symptoms of acute toxicity and the mortality rats were observed within 24h. (Notably, it was necessary to full 14-day observation without dosing of further animals).

Experiment designed

In current initial study, all the diabetic animals were randomly divided into four groups with seven animals each and treated once a day for 21 days. Group I (Negative control, NG) was administered with distilled water. Group II and group III were administered with lotus flower ethanolic extract (LFEE) 150 and 200 mg/kg, respectively. Group IV (Positive control, PG) was administered with Glibenclamide, dissolved in distilled water, 0.25 mg/Kg. LFEE, distilled water and Glibenclamide were once administered to the rat orally. Blood glucose was measured with LeverChecK TD-4230 glucometer at weekly intervals, i.e. 0, 5, 10, 15 and 20 after daily administration of extract orally. In addition, body weight, total red blood cells were also evaluated to note whether or not the physiological changes after extract oral administration.

Statistical analysis

All the data were expressed as Mean ± SEM. Statistical analysis was carried out using *t-test* to analyze the significance of each groups, and a value of $p < 0.05$ was considered to be significant.

3. Results and discussion

Efficiency of *N. nucifera* Gaertn. ethanolic extraction and alloxan-induced diabetes rats

The small species of lotus flowers were dried in a temperature of 65^{o}C for 72 hours and subjected to coarse powder (Fig. 1). The lotus powder was extracted with 95% ethanol for 7 days, the efficiency of ethanolic extraction was 8.27 ± 1.15%.

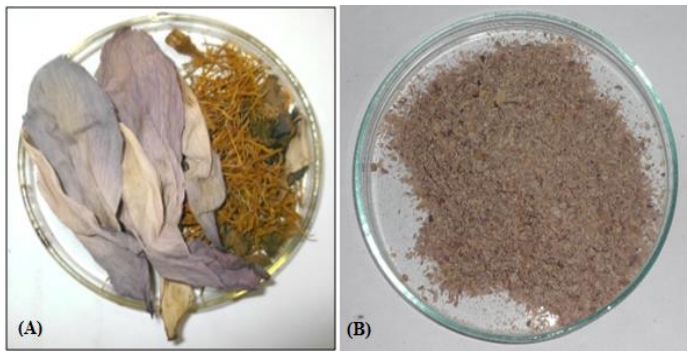

Figure 1. (A). Dried lotus (*N. nucifera* Gaertn.) flower and (B). dried lotus flower powder.

Alloxan (2, 4, 5, 6-pyrimidinetetrone) is an oxygenate pyrimidine derivative, a toxic glucose analogue, which selectively destroys insulin-producing cells in the pancreas when administered to rodents, was used to induce diabetes rats. As the results of alloxan administration, the percentage of success in diabetic induction was 32.14% with the average of fast blood glucose was 418.4 mg/dL. All of the rats with fasting blood glucose over 150 mg/dl were collected and randomly divided into four groups for LFEE testing.

Effect of LFEE on acute toxicity test

The limit test was the primarily used in situation where the experimenter has information indicating that the test animal is likely to be nontoxic or toxic below regulatory limit doses. A dose of five healthy rats was enrolled into a first limit test at the dose of 2000 mg/Kg. As the result, all of five animals were surviving, especially, not produced any signs or symptoms of toxicity during the period of 24-hour observation. For full period observation, the body weight was measured each day to have an overview of the any changes in administration period (Table 1). According to the results, the body weights significant increased from 19.71 ± 0.58 at day 1 to 23.72 ± 0.59 ($p < 0.05$). Therefore, the LD50 was greater than the test dose of 2000 mg/kg. According to the guideline for the testing of Chemicals (OECD), it was necessary to continuously carry out the limit test at the dose of 5000 mg/kg. Dose one animal at the test dose, as the result, the animal survived, thus, dose two additional animals were continuously tested. The result showed that both animals survived, the LD50 was greater than the limit dose, thus, the test was terminated. On the LFEE experiment designed, the dose at 150 and 200 mg/kg were under the dose at 2000 mg/kg, thus, those were concluded to be safe and non-toxicity on rats.

Table 1. The body weight of rat during limit test at dose of 2000 mg/kg

Day	1	2	3	4	5	6	7	8	9	10
Weight (g)	19.71 ± 0.58	20.34 ± 0.30	20.59 ± 0.29	21.46 ± 0.44	21.84 ± 0.52	22.48 ± 0.59	23.04 ± 0.77	23.45 ± 0.60	23.72 ± 0.60	23.72 ± 0.59

Effect of LFEE on fasting blood glucose of alloxan diabetes rat

In present study, two doses of LFEE at 150, 200 mg/Kg were oral administrated, and distilled water, Glibenclamide were used as negative and positive control, Hspeetively. As shown in Table 1 and Fig.1, repeated oral administration of LFEE at a dose of 150, 200 mg/Kg to the diabetic rats for 20-day period showed that both 150 and 200 mg/Kg had effected on fasting blood glucose of diabetes rats. Remarkably, the dose at 150 mg/Kg, it showed that there significantly reduced fasting blood glucose from 466.14 mg/dL at day 0 to 176.29 mg/dL at day 20 ($p < 0.05$), shifted to normal fasting blood glucose (under 200 mg/dL). Whereas, in the control group, orally distilled water, the high blood glucose was remained at the level of round 517 mg/dL and no significant differences between day 0 and day 20. Compared to positive group, Glibenclamide, also known as Glyburide, is an anti-diabetic drug, showed the significant reduction of blood glucose in diabetic rats from 463.00 mg/dL at day 0 to 267.42 mg/dL at day 20 ($p < 0.05$). Even though the level of blood glucose was not reduced at normal level, but there was a reduction of blood glucose level to 267.42 mg/dL.

Table 2. The effect on LFEE, negative control and positive control on fasting blood glucose (mg/dL) in present study

	Day 0	Day 5	Day 10	Day 15	Day 20
Group 1: NC	538.86 ± 35,94a	502.86 ± 26.61a	560.43 ± 16,4a	516.00 ± 30.37a	517.43 ± 28.87a
Group 2: 150 mg/Kg	466.14 ± 48.24c	321.71± 51.03b	262.14 ± 40.35ab	222.29 ± 23.37ab	176.29 ± 16.93a
Group 3: 200 mg/Kg	524.00 ± 34.49b	399.14 ± 57.61a	364.43 ± 32.83a	346,29 ± 34.65a	285.57 ± 31.41a
Group 4: PC	463.00 ± 32.79c	369.43 ± 25.59b	374.85 ± 24.37ab	312.42 ± 25.03ab	267.42 ± 26.71a

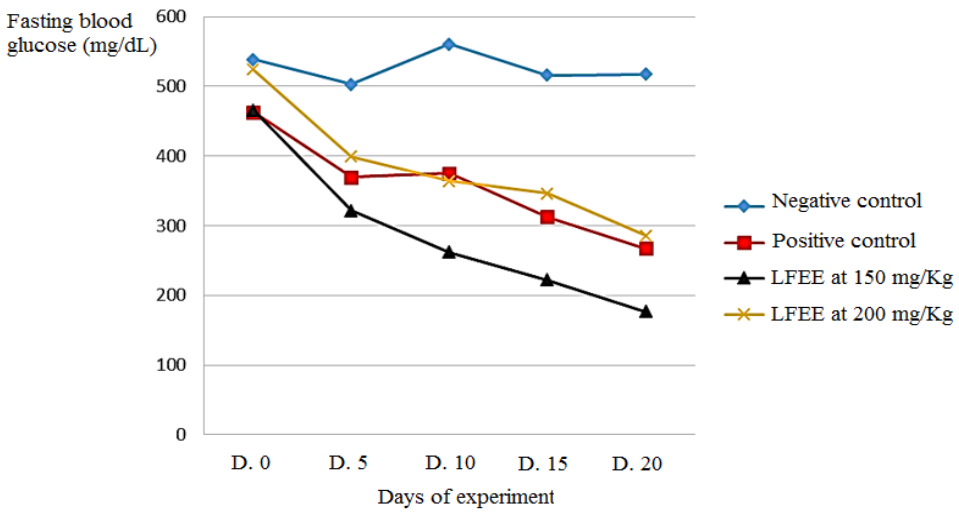

Figure 2. The fasting blood glucose on experiment designed.

Effect on total of red blood cells

The body weights, total red blood cells were also evaluated to note whether or not the physiological changes after extract oral administration. According to table 3, figure 3, the body weights were no significant changes in all of these experiment groups, and no difference changes between day 0 and day 20. Moreover, based on table 4, figure 4, the total of red blood cells at the dose of 150, 200 mg/Kg and positive control were reduced to the normal level of total of red blood cells, whereas in negative control, the total of red blood cells was no any significant differences between day 0 and day 20.

Based on those data, LFEE exhibited the potential reduction of blood glucose level in alloxan induced diabetes rats, significantly the dose of 200 mg/Kg showed greater efficiency than 150 mg/Kg.

Table 3. The effect on LFEE, negative control and positive control on body weight (gram)

	Day 0	Day 5	Day 10	Day 15	Day 20
Group 1: NC	27.13 ± 1.36a	25.01 ± 0.98a	24.06 ± 1.08a	24.35 ± 1.44a	24.03 ± 1.33a
Group 2: 150 mg/Kg	27.73 ± 1.05a	26.92 ± 1.21a	26.98 ± 0.72a	26.12 ± 0.26a	25.74 ± 0.14a
Group 3: 200 mg/Kg	28.44 ± 1.82a	27.33 ± 1.82a	26.54 ± 1.35a	27.63 ± 1.43a	27.93 ± 1.45a
Group 4: PC	27.22 ± 0.79a	26.46 ± 1.04a	26.93 ± 1.32a	27.19 ± 1.64a	26.99 ± 1.61a

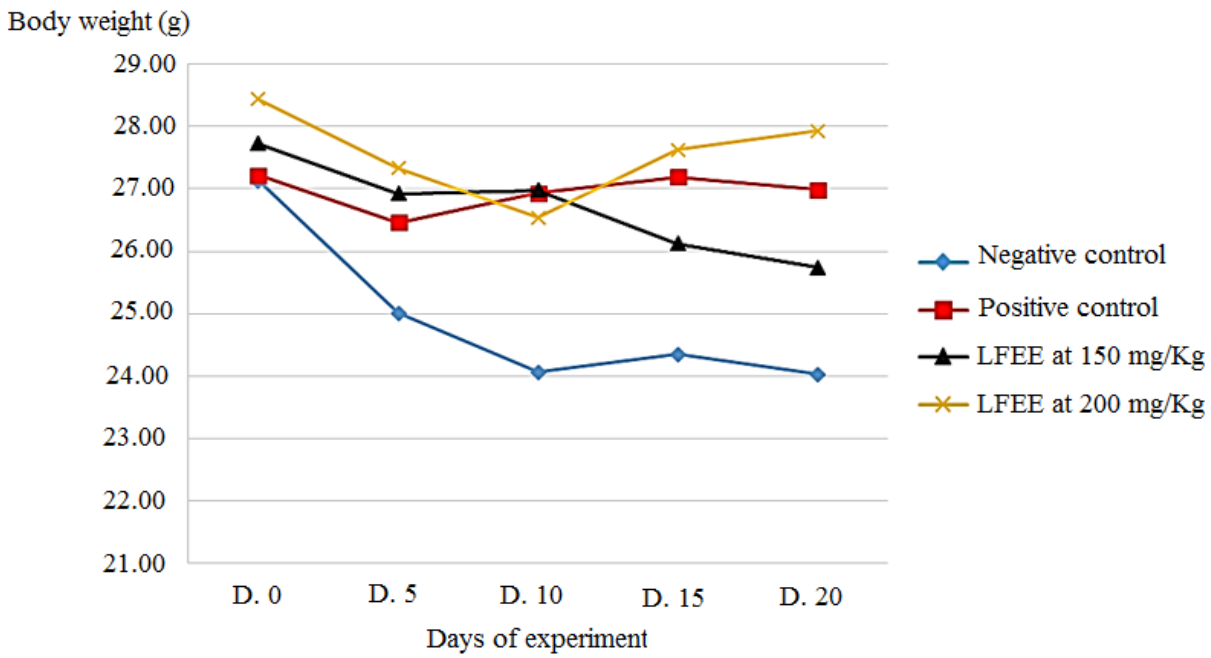

Figure 3. The body weight on experiment designed.

Table 4. The effect on LFEE, negative control and positive control on total of red blood cells (x10^6 cells/mm^3)

	Day 0	Day 5	Day 10	Day 15	Day 20
Group 1: NC	10.81 ±0.25a	10.79 ±0.27a	10.76 ±0.21a	10.90 ±0.3a	11.40 ±0.25a
Group 2: 150 mg/Kg	10.72 ±0.41b	9.49 ±0.36ab	9.38 ±0.42a	9.06 ±0.26a	8.68 ±0.61a
Group 3: 200 mg/Kg	11.06 ±0.63b	9.62 ±0.50a	8.98 ±0.29a	9.39 ±0.27a	9.45 ±0.33a
Group 4: PC	10.72 ± 0.30d	8.95 ±0.18c	7.51 ±0.35ab	7.39 ±0.28a	8.25 ±0.22bc

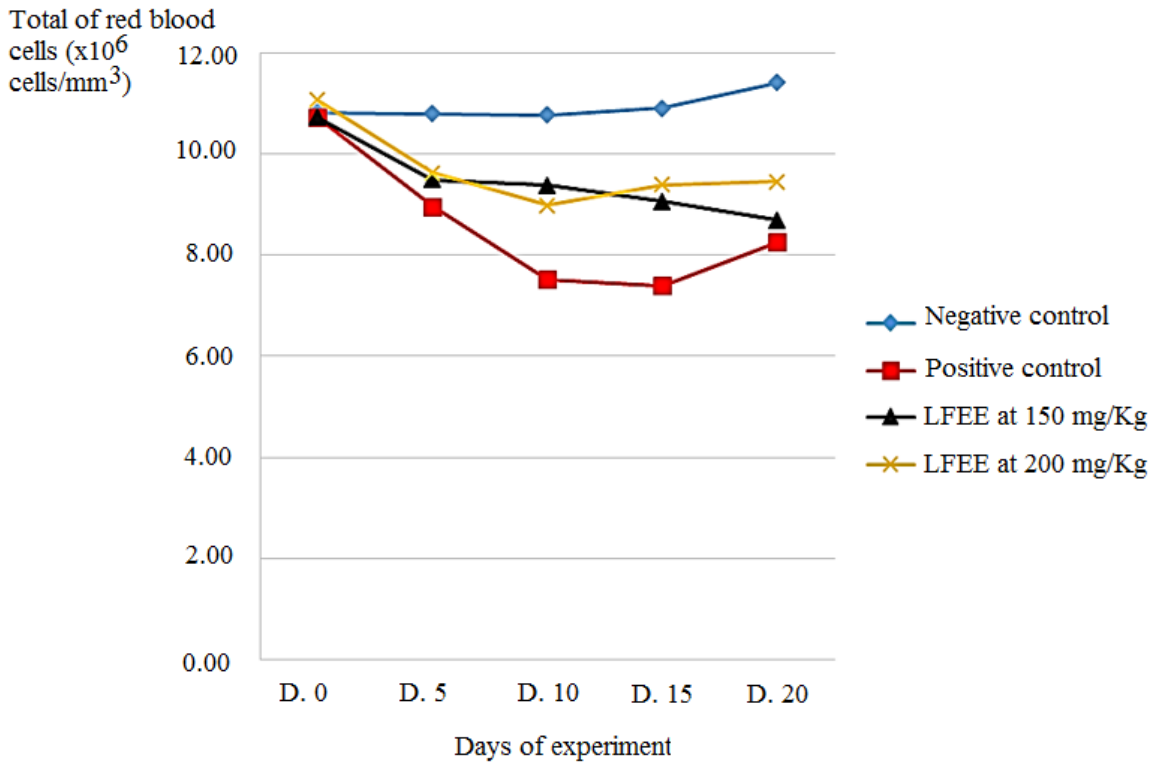

Figure 4. The total of red blood cells on experiment designed.

4. Conclusion

It could be clearly concluded that LFEE had hypoglycemic activity. Based on acute toxicity test, it was nontoxic acute study and no effect on total of red blood cells and body weight. Our findings suggested that LFEE at both doses 150 mg/Kg and 200 mg/Kg had reduced the fasting blood glucose in alloxan induced diabetic rat. Significantly, at a dosage of 150 mg/Kg was a good choice for controlling the blood glucose level in diabetic rat. The results from current study could be developed and used as alternatives for the diabetic treatment.

REFERENCES

Abd El Sattar El Batran, S., El-Gengaihi S, E. , El Shabrawy, O. A. (2006). Some toxicological studies of Momordica charantia L. on albino rats in normal and alloxan diabetic rats. J Ethnopharmacol. 108(2), 236-42.

American Diabetes Association. (2014). Diagnosis and classification of diabetes mellitus. *Diabetes Care.*, Suppl 1:S81-90.

Bandawane D, Juvekar A, Juvekar M. (2011). Antidiabetic and antihyperlipidemic effect of *Alstonia scholaris* Linn bark in streptozotocin Induced diabetic rats. *Indian J Pharm Educ Res*., 45(2), 114-120

International Diabetes Federation, http://www.idf.org/membership/wp/vietnam.

Kumar, S., Kumar, S., Prakash, O. M. (2012). Antidiabetic and hypolipidemic activities of *Kigelia pinnata* flowers extract in streptozotocin induced diabetic rats. *Asian Pac J Trop Biomed*. 2(7), 543-546

OCED Guidelines for the testing of Chemicals. http://www.oecd-ilibrary.org/environment/oecd-guidelines-for-the-testing-of-chemicals-section-4-health-effects_20745788

Ono, Y., Hattor,i E., Fukaya, Y., Imai, S., Ohizumi, Y. (2006). Anti-obesity effect of Nelumbo nucifera leaves extract in mice and rats. J Ethnopharmacol., 106(2), 238-4

Sakuljaitrong, S., Buddhakala, N., Chomko, Sanong., Talubmook, C. (2013). Effects of Flower extract from Lotus (*Nelumbo nucifera*) on hypoglycemic and hypolipidemic in streptozotocin-induced diabetic rats. IJSER., 4(7), 1441-1446.