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Effect of Flower Extract from Lotus (Nelumbo nucifera) on Haematological Values and Blood Cell Characteristics in Streptozotocin-induced Diabetic Rats

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ABSTRACT

Lotus (Nelumbo nucifera Gaertn.) is a well known medicinal plant. The extracts of rhizomes, seeds, flowers and leaves have been reported to have varied therapeutic potential. Flowers are traditionally used to treat diarrhoea, cholera, fever, hepatopathy, hyperdipsia and many bleeding disorders. Furthermore, N. nucifera can be used for antidiabetic. This study was aimed to investigate the effect of Nelumbo nucifera flower extract (NNFE) on hematological values and blood cell characteristics in diabetic rats. NNFE at a dose of 250 mg/kg was administered orally and daily to streptozotocin-induced diabetic rats for eight weeks. Blood cell characteristics and hematological values including red blood cell count (RBC), white blood cell count (WBC), hemoglobin (Hb), hematocrit (Hct), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), platelet count (PLT), and different white blood cell counts including lymphocytes, monocytes, neutrophils, and eosinophils of the rats were examined. The result showed that NNFE had no effect on hematological values and blood cell character. However, its recovered the WBC count in diabetic rats closely to normal controls. NNFE has a beneficial effect on the improvement of some hematological values in diabetes. The results suggest that NNFE can be used for diabetic treatment.

Keywords: *Nelumbo nucifera, Hematological values, blood cell characteristics, diabetic rats.*

1. INTRODUCTION

Diabetes mellitus is a metabolic disorder disease characterized by high blood glucose levels, which result from defects in pancreatic insulin secretion and/or impaired target cell responsiveness to insulin [1]. It is often associated with several complications, such as cataract and retinopathy, gastrointestinal diseases with a high recurrence of pancreatitis, neuropathy, nephropathy, myocardial ischaemia, and dermatitis, as well as various infectious diseases, both in human and in veterinary medicine [2]. Hematological complications consist mainly of abnormalities in the function, morphology and metabolism of erythrocytes, leukocytes and platelets [3]. Heart rate of diabetes is higher, but red and white blood cells counts are lower than in non-diabetes [4]. Literature has shown that ingestion of medicinal compounds or drugs can alter the normal range of hematological parameters [5].

Lotus (Nelumbo nucifera Gaertn.) a perennial aquatic plant, also known as sacred lotus, is a well known medicinal plant. Leaves, rhizomes, seeds and flowers of this plant are traditionally used for the treatment of pharyngopathy, pectoralgia, spermatorrhoea, leucoderma, small pox, dysentery, cough, haematemesis, epistaxis, haemoptysis, haematuria, metrorrhagia, hyperlipidaemia, fever, cholera, hepatopathy, and hyperdipsia [6], [7], [8]. The extracts of rhizomes, seeds, flowers and leaves have been reported to have varied therapeutic potential which all have their own therapeutic impact [8]. Some pharmacological activities of N. nucifera have shown antioxidant, anticancer, antiviral, anti-obesity, lipolytic, hypocholesterolaemic, antipyretic, hepatoprotective, antidiarrheal, antifungal, antibacterial, anti-inflammatory, diuretic activities, and anti-diabetes [9], [10], [8].

Although this plant has been used for treatment of diabetes. However, any side effect of NNFE on the hematological study and morphology of the blood cell characteristics is still unclear. Therefore, this study was designed to determine the hematological values and the blood cell characteristics in streptozotocin-induced diabetic rats recived 95% ethanolic flower extract of N. nucifera.

2. MATERIALS AND METHODS

2.1 Plant Materials

Lotus flowers were collected from natural source in Khon Kaen Province, Northeastern, Thailand. The specimen was identified by The Plant Varieties Protection Division, Department of Agriculture, Ministry of Agriculture and Cooperatives. A voucher specimen is deposited in the Department of Biology, Faculty of Science, and Mahasarakham University, Thailand (Code: MSU.Sc-BI001).

2.2 Preparation of NNFE

The fresh lotuses flowers were chopped and then dried in an hot air oven at a 50°C for 72 h. dried flowers were macerated with 95% ethanol (Merck, Germany) for 7 days (1:10 w/v). The extract was evaporated by using a rotary evaporator (Heidolph Laborota 4000, Germany) and dried using a freeze dryer (Christ Alpha 1-4, Germany) to get a powder (15.26 w/w of dry flowers). The obtained extract was stored at -20°C until being used.

2.3 Animals

Male albino Wistar rats weighing 200-250 g purchased from the National Laboratory Animal Centre (NLAC), Mahidol University, Thailand were used in this study. The rats were acclimatized in an air conditioned room at 25±2°C, 12-h light/12-h dark cycle and relative air humidity of 40-60% for 7 days. They were given a standard chow and water ad libitum prior to the commencing experiment. The rats were maintained in accordance with the guidelines of the Committee Care and Use of Laboratory Animal Resource, National Research Council Thailand and preformed in accordance with the advice of the Institutional Animal Care and Use Committee, MSU, Thailand (License number 0001/2011).

2.4 Induction of Diabetes

The rats were induced by intra-peritoneally injection with a single dose of 65 mg/kg b.w. of streptozotocin (Sigma Chemicals, St. Louis, MO) dissolved in fresh and cold 20 mM citrate buffer pH 4.5 After injection, the rats were provided with 2% sucrose solution as their drinking water for 48 h to alleviate the severity after initial hypoglycemic phase [1]. Three days after injection, fasting blood glucose (FBG) of the rats were examined to confirm diabetic stage. The rats with FBG higher than 126 mg/dL were used as diabetic rats [11], [12].

2.5 Experimental Design

The rats were randomly assigned in to four groups, eight rats in each; group I: normal controls, group II: diabetic controls, group III: diabetic rats treated with glibenclamide (Gb) (0.25 mg/kg), and group IV: diabetic rats treated with NNFE (250 mg/kg). The normal and diabetic control rats were administered orally with 0.05% ethanol. NNFE and Gb were suspended with 0.05% ethanol and administered orally once daily for eight weeks by using an orogastric tube. After eight weeks of treatments, the rats were fasted overnight and sacrificed by cervical dislocation technique. The blood sample were then drawn from the rat heart for the determination of hematological values and the blood cell characteristics.

2.6 Hematological Values Study

The hematological values including white blood cell count (WBC), red blood cell count (RBC), hemoglobin concentration (Hb), hematocrit (Hct), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), platelet count (Plt) and differential white blood cell count including lymphocytes, momocytes, neutrophils, and eosinophils. Were determined by using an automatic blood analyzer (Swelab Alfa, Biozen, Sweden).

2.7 Blood Cell Characteristics Study

The blood cell characteristics were monitored using Light Microscope (LM) and Scanning Electron microscope (SEM).

LM Specimen Preparation: Blood smear was fixed in 95% ethanol for 5 min and stained with Wright-Geimsa. Stained blood smear were examined and photographed under a light microscope.

SEM Specimen Preparation: Ultrastructure of blood cells was investigated using SEM. Blood sample were dropped in 2.5% glutaraldehyde in 0.1 M phosphate buffer, pH 7.2 overnight at 4°C and then washed in the same buffer. Samples were postfixed with 1% osmium tetroxide for 2 h, rinsed with distilled water, dehydrated in acetone:water (20:80, 40:60, 60:40, 80:20 and 100:0 v/v), left and dried in room temperature. Dried specimens were mounted on stups, coated with gold and investigated under a SEM (JSM6460LV).

2.8 Statistical Analysis

Statistical analyzed using F-test (One-way ANOVA) followed by Scheffe's test. The criterion for statistical significance was p-value less than 0.05. All data were expressed as mean±standard error of mean (SEM).

3. RESULTS AND DISCUSSION

3.1 Hematological Values

The hematological values including RBC, Hb, Hct, MCV, MCH, MCHC and plt in normal controls, diabetic controls, diabetic rats treated with Gb and diabetic rats treated with NNFE were not different. However, WBC in diabetic controls was significantly less than those in normal controls. Interestingly, WBC in diabetic rats treated with NNFE was significantly higher than those in diabetic controls and diabetic rats treated with Gb. NNFE recovered WBC in diabetic rats close to those in normal controls (Table 1).

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Table 1: Effect of NNFE on hematological values.

Hematological values	Groups			
	Normal controls	Diabetic controls	Diabetic rats+Gb	Diabetic rats+NNFE
WBC ($x10^3$cell/mm^3)	3.64± 0.09b	2.15± 0.10a	2.27± 0.20a	3.06± 0.21b
RBC ($x10^6$cell/mm^3)	8.89± 0.14	8.93± 0.10	9.08± 0.12	9.14± 0.15
Hb (g/dL)	15.91±0.06	15.95± 0.22	15.95± 0.36	16.08± 0.11
Hct (%)	46.75±0.83	49.54± 1.13	49.88± 1.22	48.43± 1.01
MCV (fL)	51.94±0.69	53.31± 0.75	53.72± 0.43	53.07± 1.16
MCH (pg)	17.71±0.17	17.65± 0.16	17.67± 0.22	17.48± 0.32
MCHC (g/dL)	34.02±0.30	33.09± 0.24	32.86± 0.25	33.10± 1.03
Plt ($x10^5$ mm^3)	651.12±12.23	628.88±33.66	641.00±30.64	684.00± 61.13

Data were expressed as mean ± S.E.M. of eight rats. Within the same low, the mean values followed by a different letters were significantly different ($p<0.05$) analyzed by Scheffe's test.

The differential white blood cell count including lymphocytes, monocytes, neutrophils, and eosinophils from all groups was not different. However, lymphocytes from diabetic controls were significantly less than those from normal controls. In contrast, neutrophils from diabetic controls were significantly higher than those from normal controls (Table 2).

Table 2: Effect of NNFE on differential white blood cell counts.

Hematological values	Groups			
	Normal controls	Diabetic controls	Diabetic rats+Gb	Diabetic rats+NNFE
Lymphocytes	77.50± 0.82b	70.90± 2.04a	71.91± 1.65ab	76.74± 1.34ab
Monocytes	4.60± 0.33	4.50± 0.66	4.67± 0.14	4.57± 0.46
Neutrophis	17.07± 0.74a	23.56± 1.97b	22.53± 1.69ab	17.74± 1.18ab
Eosinophils	0.83± 0.16	1.04± 0.08	0.90± 0.04	0.95± 0.11

Data were expressed as mean ± S.E.M. of eight rats. Within the same low, the mean values followed by a different letters were significantly different ($p<0.05$) analyzed by Scheffe's test.

3.2 Blood Cell Characteristics

The diameter of red blood cells from NNFE treated rats was not significantly different ($p<0.05$) from diabetic controls, diabetic rats treated with Gb and normal controls. However, the diameter of red blood cells from diabetic controls was less than normal controls (Table 3).

Table 3: Diameter of the red blood cells from normal controls, diabetic controls, diabetic rats treated with Gb, and diabetic rats treated with NNFE.

Groups	Diameter of red blood cells (µm)
Normal controls	4.63±0.04b
Diabetic controls	4.44±0.04a
Diabetic rats + Gb	4.48±0.03a
Diabeteic rats + NNFE	4.50±0.04ab

Data were expressed as mean ± S.E.M. of eight rats. Within the same column, the mean values followed by a different letters were significantly different ($p<0.05$) analyzed by Scheffe's test.

Figure 1 scanning electron micrographs of RBC presented different characteristics of the immature and mature RBC from normal controls, diabetic controls, diabetic rats treated with Gb and diabetic rats treated with NNFE. The RBC were no nucleated biconcave disk and contain hemoglobin. The cytoplasm was filled with electron dense hemoglobin without any organelles. The mature ones were smaller and had a prominent central pallor than the immature ones. However, the RBC characteristics from all experimental rats were not significantly different.

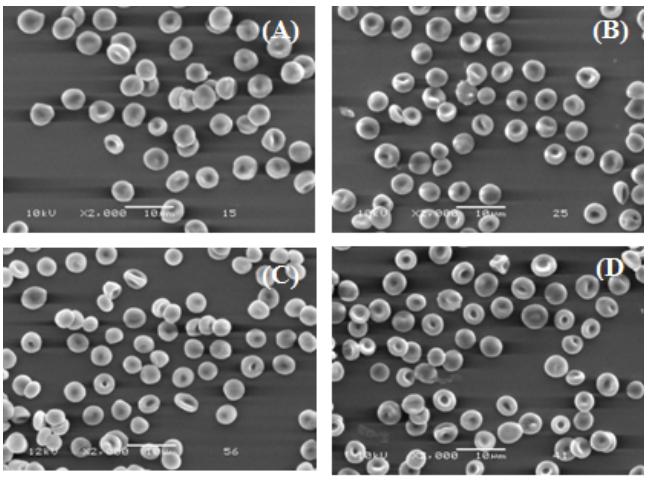


Fig 1: Scanning electron micrographs (Bars = 10 µm) of red blood cells from normal controls (A), diabetic controls

(B), diabetic rats treated with Gb (C), and diabetic rats treated with NNFE (D).

Figure 2 scanning electron micrographs of red and white blood cells showed the smooth membrane of red blood cells and the knobby white blood cells. Nevertheless, the red and white blood cells of all experimental rats were not different.

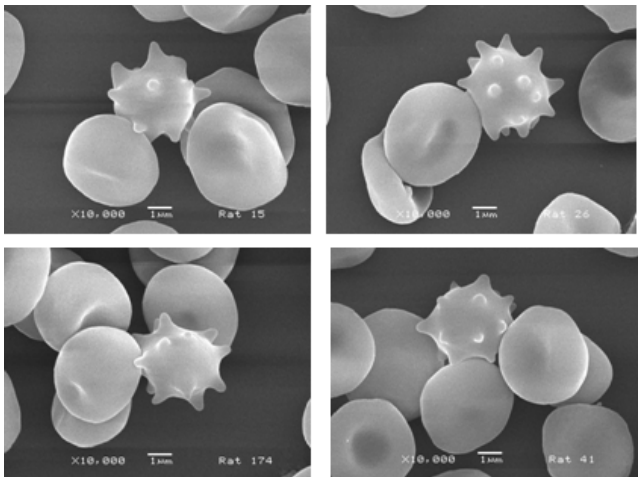


Fig 2: Scanning electron micrographs (Bars = 1 µm) of red and white blood cells from normal controls (A), diabetic controls (B), diabetic rats treated with Gb (C), and diabetic rats treated with NNFE (D).

Figure 3 light micrographs illustrated of RBC from the normal controls, diabetic controls, diabetic treated with Gb and diabetic treated with NNFE. The RBCs are very numerous in the blood and without nucleus. They are round and flattened like a donut with a depression in the middle instead of a hole (biconcave) as scanning electron micrographs. They appear pink to red in color with a pale center with Wright-Giemsa's stained. However, the RBC characteristics from all experimental rats were not found.

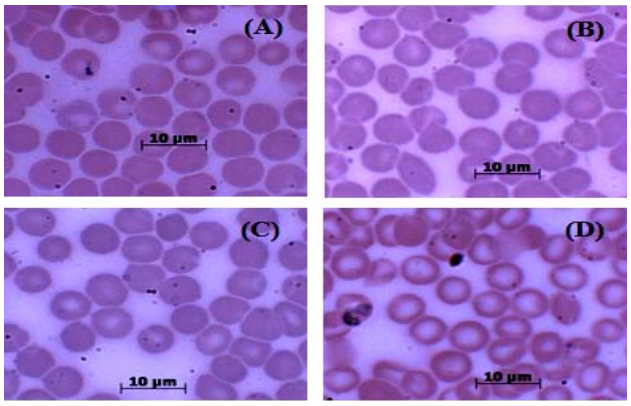


Fig 3: Light micrographs (Bars = 10 µm) of red blood cells from normal controls (A), diabetic controls (B), diabetic rats treated with Gb (C), and diabetic rats treated with NNFE (D).

Figure 4 light micrographs showed the differential of WBCs; lymphocytes, monocytes, neutrophils and eosinophils. WBCs have a nucleus. It is easily visible under the microscope, but only after stainning the smear. However, all type of WBCs from all experimental rats were not significantly different.

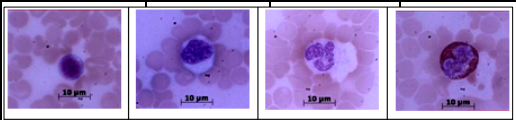
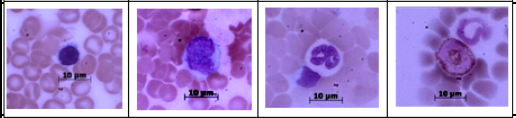
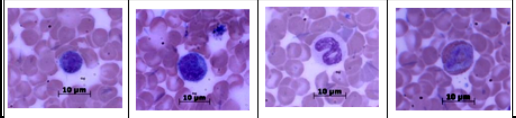
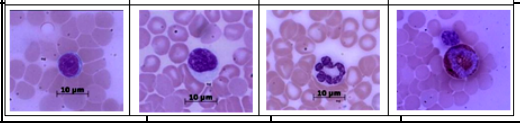
Groups	Differential of white blood cells (WBCs)			
	Lymphocytes	Monocytes	Neutrophils	Eeosinophils
Normal controls				
Diabetic controls				
Diabetic rats + Gb				
Diabetic rats + NNFE				

Fig 4: Light micrographs (Bars = 10 µm) of differential of white blood cells including lymphocytes, monocytes, neutrophils and eosinophils from normal controls, diabetic controls, diabetic rats treated with Gb, and diabetic rats treated with NNFE.

4. DISCUSSION

This project was designed to determine the hematological values and blood cell characteristics promoted by 95% ethanolic flower extract of Nelumbo nucifera in diabetic rats. Our previous study revealed that the flower extract of the plant was decreased fasting blood glucose, but increased serum insulin in diabetic rats with similar potent to glibenclamide [13]. Diabetes consist with abnormalities on hematological functions such as blood cell morphology and decreasing of red or/and white blood cells counts [14], [11], [2]. Hematological complications consist mainly of abnormalities in the function, morphology and metabolism of erythrocytes, leukocytes and platelets [3]. In Wistar rats, the mean normal value range of PCV and total leukocytes count are documented to be 40.5 - 53.1% [15] and 7,063 - 8,760 cell/mm^3, respectively [16]. However, literature has shown that oral

ingestion of medicinal compounds or drugs can alter the normal range of hematological parameters [17], [5], [18]. Effects of NNFE from this study on hematological values and blood cell characteristics in diabetic rats was not difference from normal controls cause NNFE have varied therapeutic potential which all have their own therapeutic impact [8] like many herbal medicine. Herbal medicine is based on the fact that plants contain natural substances that can promote health and alleviate illness. [19]. They make an enormous contribution to primary health care and have shown great potential in modern phytomedicine against numerous ailments and the complex diseases and ailments of the modern world. There will always be risks when appropriate regulations do not mandle the appropriate formulation of the remidies or when self medication fosters abuse [20]. Significant increase in the WBC counts in diabetic rats possible immunomodulatory effects of NNFE in diabetic rats. Results from this study have been used to confirm animal health safety in usage of NNFE in diabetic rats. On the other hand, NNFE recovered WBC counts, lymphocytes and neutrophils in diabetic rats closely to normal control rats.

5. CONCLUSIONS

NNFE prevent the complications of diabetes and improve the hematologic disease. In summary, the overall results show that long-term treatment of some hematologic changes NNFE But the intensity of the blood cells in diabetic rats.

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