

## Phytochemical and pharmacological investigations on aerial parts of *Nelumbo nucifera* Gaertn. for haematopoietic activity

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The aim of the study was to investigate effect of ethanolic extract of aerial parts of *Nelumbo nucifera* Gaertn. (Indian Lotus) on haematological parameters in anaemic rats. Haematopoietic activity of ethanolic extract of aerial part of plant was performed using cyclophosphamide (CP) at the dose of 0.3 mg/kg body weight i.p. and haloperidol (HP) at the dose of 0.2 mg/kg body weight induced aplastic and iron deficiency anaemia in rats, respectively. A morphological study of blood cells was performed along with phytochemical screening and iron estimation of extract by qualitative test and spectrophotometric method. The results of evaluation of the haematopoietic activity induced by cyclophosphamide and haloperidol showed that the plant extract diminish the activity of cyclophosphamide and haloperidol at the 200 mg/kg dose. Qualitative test of extract indicated the presence of alkaloids, flavonoids, phenolic compounds, steroids, carbohydrates, protein and iron. Iron content was estimated by using spectrophotometric method. The data from results supports the use of *N. nucifera* Gaertn. in traditional medicine for their haematopoietic activity.

**Keywords:** *Nelumbo nucifera*, Indian Lotus, Haematopoietic activity, Cyclophosphamide, Haloperidol.

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### Introduction

*Nelumbo nucifera* Gaertn. (Family-Nymphaeaceae), commonly known as Indian Lotus, is a native to China, Japan and possibly India. Also known as the sacred water lotus has been used in the indigenous system of medicine<sup>1</sup>. The plant contains alkaloids: nuciferin, romerine, nor-nuciferine; flavonoids, quercetin, myricetin-3-0-(6''-p-coumaroyl) glucoside and two epimeric macrocyclic derivatives, as well as the known myricetin-3-0-rhamnoside and pentagalloyl, benzylisoquinoline, liensine, isoliensinine and neferine oligomeric procyanidins. The ripe seed of plant are very effective in case of neurasthenia, spermatorrhoea and metrorrhoea. The decoction of leaves and seeds are very effective for insomnia, haemorrhage and haematemesis. Filament in the form of decoction is used in treating bloody stool, haematuria, uterine haemorrhage and haematemesis<sup>2-5</sup>. It also has antioxidant, psychopharmacological, antidiabetic, antiobesity, antimalarial and antifungal effect, antidiarrhoeal, hepatoprotective and free radical

scavenging and antipyretic effect<sup>6-13</sup>. The aim of the present study is to investigate haematopoietic properties of aerial parts of *N. nucifera* using cyclophosphamide and haloperidol on animal models.

### Materials and Methods

#### Plant material and preparation of extracts

The fresh aerial parts of plant sample were collected in the month of April and was authenticated (Voucher specimen no.: Bot/Herbarium/727) by botanist Dr. Amarjeet Bajaj, Head Department of Botany. The specimen is deposited in Botany department, C.S.A Government Postgraduate Nodal College, Sehore (M.P.). The plant material comprising of leaves and stem were air dried at room temperature and powdered by using a pestle and mortar. Thereafter 100 g was continuously extracted with 350 ml ethanol at (40-60°C) for 72 h. The ethanolic extracts were collected in a tarred conical flask and the solvent was removed by distillation. The total ethanol extract was concentrated under reduced pressure at temperature 50-60°C (yield 8.453).

#### Drugs and chemicals

Cyclophosphamide (Avalon Pharma, Mumbai, India), haloperidol (Venkat Pharma Ltd. Hyderabad,

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India), 1, 10-phenanthroline (Suvidhinath Laboratories, Baroda, India), Absolute ethanol (Merck), Sulphuric acid (Swastic Sales Corporation, Ahmedabad, India), Hydroquinone (Laxmi Organic Industries, Mumbai, India), Trisodium citrate (Salvi Chemicals Ltd. Mumbai, India), EDTA (Bhagnagar Laboratories, Hyderabad, Mumbai) were used as drug and chemicals.

Dosage form was made in the 2% polyvinyl pyrrolidone solution. 2 g of ethanolic and aqueous extract were taken in the mortar and pestle and triturated with 20 mL of 2% polyvinyl pyrrolidone solution. Suspensions were stored in airtight bottles in a cool place.

#### Phytochemical studies

The ethanolic extract was subjected to phytochemical study by using various chemical qualitative tests<sup>14</sup>. Iron estimation was done by using UV spectroscopy (Shimadzu, Japan)<sup>15</sup>.

#### Animals

Male Wistar albino rats (110-120 g) were procured from Central Animal House of Pharmacology division, VNS Institute of Pharmacy, Bhopal, India. The animals were housed in standard conditions of temperature (22±2°C) and 12 h light-dark cycle. The rats were fed with commercial diet and water *ad libitum*. The experimental protocol was approved by the institutional Animals Ethical Committee (IAEC) (Registration No. 778/03/c/CPCSEA). All experimental procedures were conducted in accordance to the ethical guidelines of International Association for the study of anemia.

#### Acute toxicity studies

Toxicological study was done by the study of LD<sub>50</sub>. The test animals were divided into six groups of six rats in each group. The extract was administered intraperitoneally as according to body weight. Group I, II, III, IV, V, and VI were received extracts at different doses as 150, 300, 450, 600, 750, and 900 mg/kg i.p., respectively. It was found that groups I, II, III were found to be healthy after seven days. In Group IV, one rat died after a day of extract administration while the remaining five died after three days. In Group VI, three albino rats died after 24 h, while two died after three days. The LD<sub>50</sub> studies revealed that albino rats tolerated a considerable dose of ethanol extract at 450 mg/kg body weight, i.p.) without any manifestations<sup>16</sup>.

#### Studies on haematopoietic activity

##### *Cyclophosphamide induced aplastic anemia model*

Cyclophosphamide (CP)-induced aplastic anaemia model was used for the study of haematopoietic activity. Five groups were made with six animals in each group. Group I was kept as the control group, Group II was given CP control group at the dose of 0.3 mg/kg. Group III was given ethanolic extract alone at the dose of 200 mg/kg, Group IV was given CP at the dose of 0.3 mg/kg along with ethanolic extract at dose of 100 mg/kg and Group V was given CP 0.3 mg/kg along with ethanolic extract at dose of 200 mg/kg body weight i.p.

Anaemia was induced by CP at the dose of 0.3 mg/kg given i.p. for seven days. On the seventh days, blood samples were collected from the retro-orbital plexus vein of the rat's eyes in vials containing EDTA as the anticoagulant. These samples were evaluated for hematological parameters using hematology cell counter (ERMA, Japan), repeatedly (five times) to check the reproducibility of results. Blood samples were collected on day 7, a significant lowering in blood parameters was observed. After seven days, CP was withdrawn from all groups and treated with only ethanol extract of once a day, at the doses of 100 and 200 mg/kg body weight continuously up to next 15 days. Blood sample were collected on the 22<sup>nd</sup> day and evaluated for hematological parameters<sup>17</sup>.

##### *Haloperidol induced iron deficiency anemia model*

Haloperidol (HP)-induced iron deficiency anaemia model was used for the study of haematopoietic activity. Five groups were made with six animals in each group. Group I was kept as the control group, Group II was the HP control group 0.2 mg/kg. Group III was given ethanolic extract alone 200 mg/kg, Group IV was given HP 0.2 mg/kg along with ethanolic extract at dose of 100 mg/kg, and Group V was given HP 0.2 mg/kg along with ethanolic extract at dose of 200 mg/kg body weight. Anaemia was induced by HP 0.2 mg/kg given i.p. for four days. On the fourth days, blood samples were collected from the retro-orbital plexus vein of the rats' eyes in vials containing EDTA as the anticoagulant. These samples were evaluated for hematological parameters using hematology cell counter (ERMA, Japan) repeatedly (five times) to check the reproducibility of results. Blood samples were collected on day 4<sup>th</sup> a significant lowering in blood parameters was observed. After four days, HP was withdrawn from all groups and treated with only ethanolic extract of once a day, at

the doses of 100 and 200 mg/kg body weight continuously up to next 17 days. Blood sample was collected on the 21<sup>st</sup> day and evaluated for hematological parameters as well as serum iron and serum protein<sup>18</sup>.

#### Morphological studies of blood cells

The blood smear was prepared by placing a small drop of blood near an end of a slide and bringing the edge of another slide in contact with the drop and allows the drop to bank evenly behind the spreader. Fixed for at least 30 seconds in absolute methanol and removed the methanol by tilting the slide then applied staining solution (Wright stain) for 2 minutes on a horizontally positioned slide after that added aliquot of the buffer solution without any of the stain running off the slide. Gently mixed the buffer (Sorensen's buffer solution) and stain without touching the surface of the blood film on the slide let stand for 3 minutes and rinse the slide with distilled water for 30 seconds. Dried the slide in a tilted position mounts a cover glass and examined under light microscope (Labomed CX R3)<sup>19</sup>.

#### Statistical Analysis of Data

Data obtained from animal experiments were expressed as mean  $\pm$  S.E.M. (standard error mean). Statistical differences between the treated and the control groups were evaluated by ANOVA, followed by the Dunnett's test.  $P < 0.05$  was considered significant.

#### Results

The ethanolic extract showed presence of alkaloids, carbohydrates, proteins, phenolic compounds,

glycosides, flavonoids and iron. The plant selected for study (*Nelumbo nucifera*) contained iron in crude drug which is found to be 10.7  $\mu\text{g}/50 \text{ mg}$  of ethanolic extract. The extract did not show any sign and symptom of toxicity and mortality intraperitoneally dose up to 450 mg/kg.

#### Effect of ethanolic extract on Cyclophosphamide induced anaemia

The CP control group (Group II) showed significant ( $P > 0.05$ ) decrease in blood parameters as compared to the vehicle control group (Group I), without any drug treatment. The comparison of Group II with Groups III, IV and V exhibited a slight significant ( $P < 0.05$ ) increase in haematological parameters after seven days. CP-induced aplastic anaemia in Group II was not restored to normal counts even after the discontinuation of the drug after seven days. Upon continuation of ethanolic extract at the dose of 100 and 200 mg/kg, body weight, respectively, for the next 15 days there was a significant ( $P < 0.05$ ) improvement in haematological parameters in Groups III, IV and V as compared to Group II. The result is shown in Table 1.

#### Effect of ethanol extract on Haloperidol induced anaemia

The HP control group (Group II) showed significant ( $P > 0.05$ ) decrease in blood parameters as compared to the vehicle control group (Group I), without any drug treatment. The comparison of Group II with Groups III, IV and V exhibited a slight significant ( $P < 0.05$ ) increase in haematological parameters after four days. HP-induced iron deficiency anaemia in Group II was not restored to

Table 1—Effect of ethanolic extract of *Nelumbo nucifera* on cyclophosphamide (CP) treated albino rats (After 7 days & on 22 day)

Groups	Treatment	RBCs	PCV	Hb	WBCs	N	E	L	B	M
<b>Day 7</b>										
I	Control	7.9 $\pm$ 0.2	50.2 $\pm$ 1.7	15.6 $\pm$ 0.6	13.9 $\pm$ 1.5	30.3 $\pm$ 0.0	2.67 $\pm$ 0.66	6.03 $\pm$ 1.20	0.6 $\pm$ 0.18	2.0 $\pm$ 0.44
II	CP Control (3mg/kg)	5.4 $\pm$ 0.9	42.4 $\pm$ 1.2	12.8 $\pm$ 0.9	11.6 $\pm$ 1.0	22.6 $\pm$ 0.46	1.92 $\pm$ 0.62	4.12 $\pm$ 1.3	0.32 $\pm$ 0.4	0.92 $\pm$ 0.31
III	EE (200mg/kg)	8.1 $\pm$ 1.1 <sup>a</sup>	51.1 $\pm$ 1.6 <sup>a</sup>	15.9 $\pm$ 0.8 <sup>a</sup>	14.2 $\pm$ 1.3 <sup>a</sup>	34.8 $\pm$ 2.1 <sup>a</sup>	2.98 $\pm$ 0.26 <sup>a</sup>	6.89 $\pm$ 1.2 <sup>a</sup>	0.69 $\pm$ 0.2 <sup>a</sup>	2.2 $\pm$ 0.26 <sup>a</sup>
IV	CP+EE (100mg/kg)	7.8 $\pm$ 1.0 <sup>a</sup>	51.1 $\pm$ 2.1 <sup>a</sup>	15.2 $\pm$ 0.9 <sup>a</sup>	13.2 $\pm$ 1.2 <sup>a</sup>	32.8 $\pm$ 1.9 <sup>a</sup>	2.89 $\pm$ 0.32 <sup>a</sup>	6.52 $\pm$ 1.26 <sup>a</sup>	0.69 $\pm$ 0.2 <sup>a</sup>	1.8 $\pm$ 0.18 <sup>a</sup>
V	CP+EE (200mg/kg)	8.0 $\pm$ 1.0 <sup>a</sup>	51.6 $\pm$ 2.2 <sup>a</sup>	15.6 $\pm$ 1.0 <sup>a</sup>	14.0 $\pm$ 1.1 <sup>a</sup>	2.9 $\pm$ 1.8 <sup>a</sup>	2.96 $\pm$ 0.24 <sup>a</sup>	6.78 $\pm$ 1.42 <sup>a</sup>	0.68 $\pm$ 0.22 <sup>a</sup>	1.9 $\pm$ 0.20 <sup>a</sup>
<b>On Day 22</b>										
VI	CP Control (3mg/kg)	6.9 $\pm$ 1.1	47.2 $\pm$ 2.1	14.6 $\pm$ 0.8	13.0 $\pm$ 0.7	28.2 $\pm$ 1.8	2.52 $\pm$ 0.28	6.0 $\pm$ 0.9	0.5 $\pm$ 0.1	2.0 $\pm$ 0.3
VII	EE (200mg/kg)	7.9 $\pm$ 0.3 <sup>b</sup>	50.8 $\pm$ 1.2 <sup>b</sup>	16.0 $\pm$ 0.4 <sup>b</sup>	13.7 $\pm$ 0.9 <sup>b</sup>	31.2 $\pm$ 2.2 <sup>b</sup>	2.8 $\pm$ 0.26 <sup>b</sup>	6.2 $\pm$ 1.10 <sup>b</sup>	0.6 $\pm$ 0.18 <sup>b</sup>	2.0 $\pm$ 0.18 <sup>b</sup>
VIII	CP+EE (100mg/kg)	7.6 $\pm$ 0.6 <sup>b</sup>	50.9 $\pm$ 1.3 <sup>b</sup>	15.8 $\pm$ 0.6 <sup>b</sup>	13.9 $\pm$ 0.9 <sup>b</sup>	31.2 $\pm$ 2.2 <sup>b</sup>	2.61 $\pm$ 0.29 <sup>b</sup>	6.12 $\pm$ 1.12 <sup>b</sup>	0.60 $\pm$ 0.16 <sup>b</sup>	1.9 $\pm$ 0.20 <sup>b</sup>
IX	CP+EE (200mg/kg)	7.7 $\pm$ 0.8 <sup>b</sup>	50.8 $\pm$ 2.1 <sup>b</sup>	15.2 $\pm$ 1.2 <sup>b</sup>	13.7 $\pm$ 0.8 <sup>b</sup>	30.9 $\pm$ 1.8 <sup>b</sup>	2.71 $\pm$ 0.30 <sup>b</sup>	6.1 $\pm$ 1.26 <sup>b</sup>	0.61 $\pm$ 0.15 <sup>b</sup>	1.9 $\pm$ 0.22 <sup>b</sup>

n=6 albino rats per group; <sup>a</sup> $P < 0.05$  when compared to CP control (7days); <sup>b</sup> $P < 0.05$  when compared to CP control (22days). All values are expressed as mean $\pm$ SEM. RBC=total red blood cell count (X million/ml); PCV=packed cell volume (%); Hb= Haemoglobin concentration gm%; WBC=total white blood cell count (X 1000/ml); N= Neutrophils; E= Eosinophils; L= Lymphocytes; B= Basophils; M= Monocytes.

Table 2—Effect of ethanolic extract of *Nelumbo nucifera* on haloperidol (HP) treated albino rats (After 4 days & on 21day)

Groups	Treatment	RBCs	PCV	Hb	WBCs	Serum iron	Serum protein
<b>Day 4</b>							
I	Control	7.9 ± 0.2	50.2 ± 1.7	15.6 ± 0.6	13.9 ± 1.5	7.89 ± 0.8	38.9 ± 0.6
II	HP Control (3mg/kg)	4.8 ± 0.8	44.4 ± 1.3	12.2 ± 0.8	11.0 ± 1.2	5.61 ± 1.2	34.4 ± 0.9
III	EE (200mg/kg)	8.1 ± 1.1 <sup>a</sup>	51.1 ± 1.6 <sup>a</sup>	15.9 ± 0.8 <sup>a</sup>	14.2 ± 1.3 <sup>a</sup>	7.35 ± 0.7 <sup>a</sup>	36.0 ± 0.8 <sup>a</sup>
IV	HP+EE (100mg/kg)	7.9 ± 1.2 <sup>a</sup>	51.8 ± 2.1 <sup>a</sup>	15.8 ± 0.8 <sup>a</sup>	13.9 ± 0.9 <sup>a</sup>	60.11 ± 1.1 <sup>a</sup>	35.8 ± 1.2 <sup>a</sup>
V	HP+EE (200mg/kg)	8.0 ± 1.1 <sup>a</sup>	50.9 ± 1.6 <sup>a</sup>	15.9 ± 0.9 <sup>a</sup>	13.7 ± 0.8 <sup>a</sup>	6.87 ± 0.9 <sup>a</sup>	36.8 ± 1.1 <sup>a</sup>
<b>Day 21</b>							
VI	HPControl(3mg/kg)	7.6 ± 0.9	47.5 ± 1.9	15.0 ± 0.7	13.1 ± 0.6	7.19 ± 1.2	36.6 ± 0.8
VII	EE (200mg/kg)	7.9 ± 0.3 <sup>b</sup>	50.8 ± 1.2 <sup>b</sup>	16.0 ± 0.4 <sup>b</sup>	13.7 ± 0.9 <sup>b</sup>	7.59 ± 1.7 <sup>b</sup>	38.0 ± 1.4 <sup>b</sup>
VIII	HP+EE (100mg/kg)	7.8 ± 0.2 <sup>b</sup>	5 0.6 ± 1.9 <sup>b</sup>	15.9 ± 1.1 <sup>b</sup>	13.4 ± 0.7 <sup>b</sup>	7.12 ± 0.8 <sup>b</sup>	38.2 ± 1.9 <sup>b</sup>
IX	HP+EE (200mg/kg)	7.6 ± 0.3 <sup>b</sup>	50.2 ± 1.2 <sup>b</sup>	15.2 ± 1.2 <sup>b</sup>	13.2 ± 0.7 <sup>b</sup>	7.4 ± 1.4 <sup>b</sup>	38.4 ± 0.3 <sup>b</sup>

N = 6 albino rats per group. <sup>a</sup>P<0.05 when compared to HP control (4days); <sup>b</sup>P<0.05 when compared to HP control (21days); RBC=total red blood cell count (X million/ml); PCV=packed cell volume (%); Hb= Haemoglobin concentration gm%; WBC=total white blood cell count (X 1000/ml), serum iron (µg/dl), and serum protein(g/100ml) All values are expressed as mean ± SEM.

normal counts even after the discontinuation of the drug after four days. Upon continuation of ethanolic extract at the dose of 100 and 200 mg/kg, body weight, respectively, for the next 17 days there was a significant (P<0.05) improvement in haematological parameters in groups III, IV and V as compared to group II. The result is shown in Table 2.

**Effect of CP and HP on morphological characteristics of RBCs**

Photomicrograph of blood smear of aplastic anaemia on 7<sup>th</sup> day in CP treatment group. Photograph shows less number of red blood cells (RBCs) but normal in shape named normocytic-normochromic anaemia (Plate 2) when compared with control (normal) (Plate 1). Photomicrograph of blood smear of iron deficiency anemia on 4<sup>th</sup> day in HP treatment group. shown red blood cells (RBCs) in microcytic, hypochromic and anisocytosis shape (Plate 3) when compared with control (Normal) (Plate 1).

**Effect of ethanolic extract on morphological characteristics of RBCs**

Photomicrograph of blood smear on 22<sup>nd</sup> day in CP and ethanolic extract of NN treatment group shown increased number of red blood cells (RBCs) and normal in shape (normocytic) (Plate 4) when compared to CP treated group (Plate 2). Photomicrograph of blood smear on 21<sup>st</sup> day after HP and ethanolic extract of NN treatment shown increased number of red blood cells (RBCs) is normal in shape (normocytic) (Plate 5) when compared to HP treated group (Plate 3).

**Discussion**

The study aimed to evaluate the effect of ethanolic extract of *N. nucifera* Gaertn. on the aplastic and iron

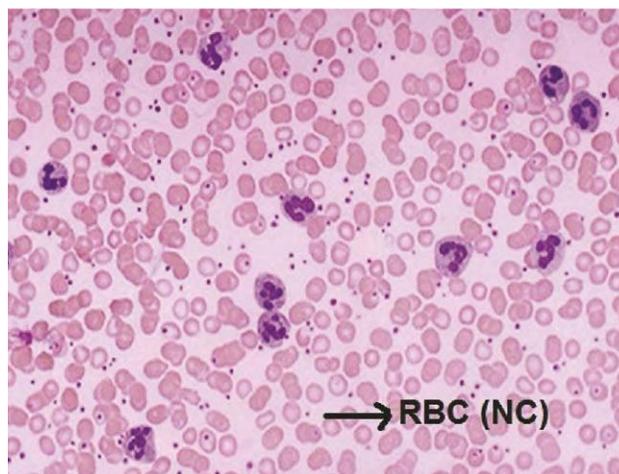


Plate 1—Control (vehicle only) [Photomicrograph of normal peripheral blood smear on 7<sup>th</sup> day administered vehicle only. Photograph shows red blood cells (RBCs) are normocytic with good haemoglobin content-Leishman, X40].

deficiency anaemia induced by CP and HP in Swiss albino rat.

CP has a bone marrow suppressive effect and induces aplastic anaemia. CP treatment at the dose of 0.3 mg/kg, body weight and i.p. resulted in the significant lowering of haematological parameters on the seventh day<sup>17</sup>. All the haematological indices as aplastic anaemia and iron deficiency anaemia were restored to almost normal counts after continuous administration of the extract via repairing of bone marrow (Erythropoiesis) and iron recovering mechanism in RBCs.

HP has an antipsychotic drug and induced iron deficiency anaemia. The peripheral blood smear has been reported to reveal characteristic changes in the

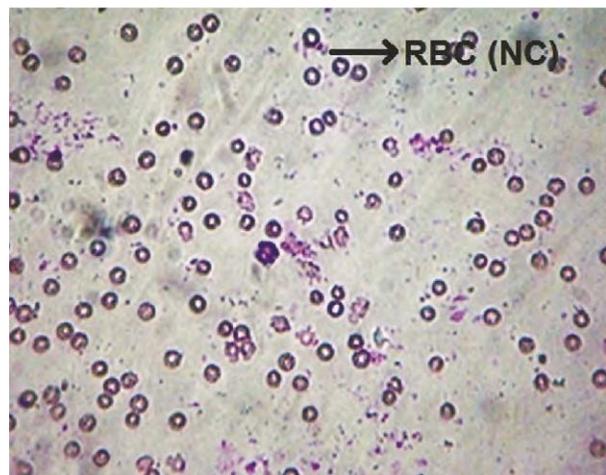


Plate 2—Cyclophosphamide control (0.3 Mg/Kg) treated [Photomicrograph of normal peripheral blood smear of aplastic anemia on 7<sup>th</sup> day after cyclophosphamide treatment. Photograph shows less number of red blood cells (RBCs) but normal in shape named normocytic-normochromic anemia-Leishman, X40].

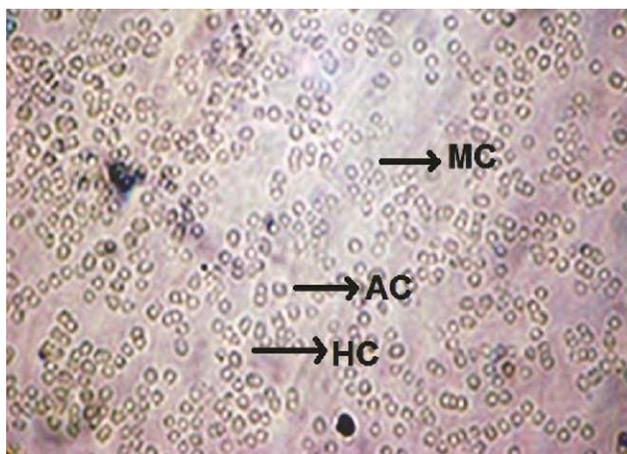


Plate 3—Haloperidol control (0.2 mg/kg) treated [Photomicrograph of normal peripheral blood smear of iron deficiency anemia on 4<sup>th</sup> day after haloperidol treatment. Photograph shows red blood cells (RBCs) in microcytic, hypochromic and anisocytosis shape-Leishman, X40].

size and the haemoglobin content of the red cells, observed in some types of anemia although the degree of anisocytosis is correlated with the severity of iron deficiency anaemia<sup>20</sup>. In addition to the clinical assessment, the differential diagnosis based on morphologic examination of blood smear was carried out. The rats treated with CP injection showed less number of red blood cells but normal in shape (Plate 2) when compared to their respective (normocytic-normochromic) normal control rats (Plate 1). In dose-dependent study, ethanolic extract treated group shown increased number of RBCs,

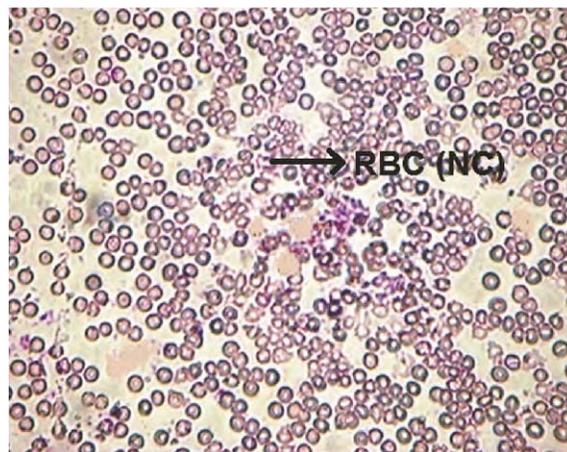


Plate 4—Cyclophosphamide + Ethanolic extract (0.3 mg/kg+ 200mg/kg) treated [Photomicrograph of normal peripheral blood smear on 22<sup>nd</sup> day after CP and ethanolic extract of NN treatment. Photograph shows increased number of red blood cells (RBCs) and normal in shape (normocytic)-Leishman, X40].

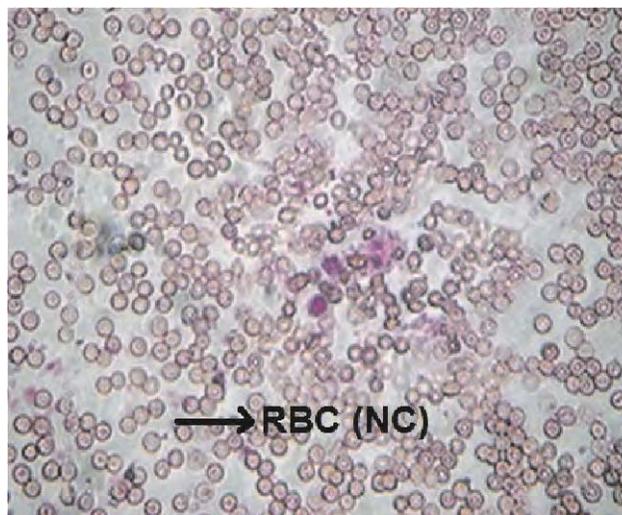


Plate 5—Haloperidol + Ethanolic extract (0.2 mg/kg+ 200 mg/kg) treated [Photomicrograph of normal peripheral blood smear on 21<sup>st</sup> day after HP and ethanolic extract of NN treatment. Photograph shows increased number of red blood cells (RBCs) are normal in shape (normocytic)- Leishman, X 40].

WBCs, Hb content, etc. and observed normal shape (Plate 4). The rat treated with HP showed anisocytosis, hypochromic and microcytic cells when compared to their respective (normocytic-normochromic) normal control rats (Plate 1). A decrease in the blood level of iron was previously reported with the HP treatment<sup>21</sup>. In dose-dependent manner, ethanolic extract treated group showed increased number of RBCs, WBCs, Hb content, etc. and observed normal shape (Plate 5). The extract could stimulate erythropoiesis process. Further

investigations are needed to understand the mechanism involved in the anti-anaemic action of *N. nucifera* Gaertn. and haematological parameters of test animals indicating their boosting effects on the synthesis of haemoglobin and formation of red blood corpuscles due to their richness in iron and other phytoconstituents as flavonoids, proteins, carbohydrates and terpenoids hence these plants might have a promising role in the treatment and/or prevention of anaemia.

### Conclusion

It is concluded that the haematopoietic activity of ethanolic extract of aerial parts of *N. nucifera* were found to be statistically significant biological activity without inducing any apparent acute toxicity on dose dependent study and it may reduce the risk of aplastic and iron deficiency anaemia.

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