Effects of Aqueous Extract of *Basella alba* L. leaves on Blood Cell Count in Rats

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Abstract

The effect of aqueous extract of *Basella alba* (puishak) leaves on blood cell count of rats was studied. Forty rats of both sexes weighing between 100-150 gm were used. The rats were divided into four groups (7 rats in each group), with group A as the control group and experimental groups were denoted as B, C and D. With all aseptic precautions, aqueous extract of *Basella alba* (Puishak) leaves was administered into the three different doses (For group B: 60 mg/kg bw, group C: 80 mg/kg bw and group D: 100 mg/kg bw). Control group A also received distilled water as a placebo at the dose of 10 mg/kg bw for 14 days. At day 15, blood samples were collected and sent for haematological analysis. Data analysis of blood count profile of 28 rats revealed that there is an increased number of RBC, WBC and platelet count in experimental groups than in control group. ANOVA test revealed that increased blood cell counts following administration of aqueous leaves extract of keeves of *B. alba* were statistically significant (p value for each case was .0001<.05) than control group. Paired samples *t* test was performed to compare between baseline hematological parameters and parameters after 14 days of intervention. Then comparison between Group A & Group B, between Group A & Group C and between Group A & Group D were done. In all cases, probability (p) value < 0.05 was considered as statistically significant.This it is clearly evident that aqueous extract of *B.alba* has positive stimulant effect on blood cells count of rats. Moreover, it was found that increment of doses also increases the cell count that is positively correlated with the hypothesis.

Key words: *Basella alba, blood cell count, rats, red blood cell, platelet, white blood cell.*

Introduction

With the advent of the information age, technology and the explosion of social media, the world's populations are better informed than ever. Social conscience and environmental awareness are at the forefront of our progress as a developed world (Bent, 2008). In keeping with this direction, there is a global trend both in the developing and in the developed countries towards natural health products before their pharmaceutical alternatives. They are seen as safer, more natural and healthier. The rediscovery of the connection between plants and health is responsible for launching a new generation of botanical therapeutics that include plant-derived pharmaceuticals, multi-component botanical drugs, dietary supplements, functional foods and plant-produced recombinant proteins (Rashrash *et al.*, 2017).

Blood is a good indicator to determine the health of an organism. It is also a good pathological mirror of the entire body. Cellular component of blood is valuable in immunotoxicology to evaluate

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immunotoxic potential of a compound. To this end, haematological parameters are important in establishing the body's functional status as a result of exposure to toxicants (Joshi *et al.*, 2002). The human body is known to produce billions of new red blood cells, and other blood components which replace blood cells that are lost due to normal cell turnover processes, illness or trauma (Akashi *et al.*, 1999). All the mature blood cells in the body are generated from a relatively small number of haematopoietic stem cells (HSCs) and progenitors. Each blood cell, red blood cells, white blood cells, and platelets play important roles in the body's normal physiological functions (Ajugwo *et al.*, 2017).

Basella alba, one of the traditional medicinal plant, is a fast growing vegetable commonly found in the tropical Asia (Bamidele *et al.*, 2010). It was thought that this species was originated from India or Indonesia. Due to geographical variations, *B. alba* also familiar into different name in different countries. In Bangladesh it is known as "Puishak" whereas it is known as "Puiki Bhaji" "Potaki" "Indian spinach" and "Malaber spinach" in Gujarati, Sanskrit, India and in English, respectively (Swati, 2005). As this herb is heat tolerant, it can grow throughout the tropics as a perennial and in warmer temperate region as an annual crop. It is a succulent, branched, smooth, twining herbaceous vine, several meters in length. Mainly leaves and stems are used for the medicinal purposes (Kumar *et al.*, 2013).

The aerial part (leaves, stems) of the plant serves as edible vegetable in many parts of the world (Kirtikar and Basu, 2000). The cooked roots and leaves have been reported to be used as a laxative for treatment of constipation. Besides these, this plant has several nutritious values (Siriwatanametanon *et al.*, 2010). Data suggest that, the leaves of this plant contains proteins, fat, vit A, vit C, vit E, vit K which acts as an antioxidant. Moreover, it possesses several vitamins like thiamin, riboflavin and niacin (Swati, 2005). Several studies evidenced that it also contains folic acid which is a maturation factor of RBC (Siriwa-tanametanon *et al.*, 2010). It is also a source of several minerals such as calcium,

magnesium and iron which can act as a haematinic particularly iron (Arokoyo *et al.*, 2015). Due to its enrich content it has proven effective as an antioxidant, anti-inflammatory agent, antiulcerant and antibacterial activities (Krishna, 2012; Sivasarkar *et al.*, 2011; Vijender *et al.*, 2011). In addition, it has roles in wound healing and androgenic effect (Rathee *et al.*, 2010; Venkatalakshmi and Senthamaraiselvi, 2012). It has also been observed by the scientists that this medicinal plant has some anti-depressant effect on CNS and nephroprotective role on kidney (Anandarajgopal *et al.*, 2011; Moundipa, 2014; Mohammed *et al.*, 2012).

From the insight of several studies it was hypothesized that *B. alba* may enhance hematological parameters, though it was locally practiced for treatment of anemia in several regions (Alada, 2000; Bolarinwa*et al.*, 1991; Saleh, 2011). But very limited study underwent to investigate the overall effects of *B. alba* on different hematological parameters. As no scientific investigation has been conducted in Bangladesh regarding these issues, this study was designed to evaluate the effects of *B. alba* leaf extract on blood cells count in rats.

Materials and Method

Collection of plant material: The leaves of *B. alba* (Puishak) were collected from Dhaka, Bangladesh in July, 2016. The plant was authenticated by a botanist of Bangladesh National Herbarium, Mirpur, Dhaka (Accession number: DACB 45089).

Extraction: The collected leaves (3 kg) of *B. alba* were cut into small pieces and dried in air under shade at room temperature for 5 days. Then aqueous extract of that air dried leaves was prepared in Drug Research Laboratory of Centre for Advanced Research of Sciences (CARS) University of Dhaka. There the dried leaves (150 g) were soaked in distilled water (2000 ml) in a round bottom flask with continuous shaking (50 rpm) at 25°C for 3 days to allow aqueous extraction. The liquid extract was then filtered through cotton and evaporated by Rotavapor (70 rmp) for 5 hours to obtain a semisolid residue

(12 g). The extract in this form kept in air tight glass container at 4°C until used. Before use, the concentrated extract was diluted in adequate amount of distilled water to obtain fresh preparation.

Animals: Total 40 rats of both sex weighting about 100-150 gm were purchased from the Animal Research Branch of the International Center for Diarrhoeal Disease and Research, Bangladesh (icddr,b), Dhaka. They were acclimatized for 7 days in well ventilated room in metallic cages with optimum temperature and relative humidity in the animal house of the Department of Pharmacology, Dhaka Medical College, Dhaka. They were allowed to feed standard laboratory diet and to drink water *ad libitum.* Blood samples of 12 rats were collected at the beginning of the study that represented the baseline values of blood cell parameters. Rest 28 rats were used to carry out the experiment. After wearing protective gloves, with the help of infant feeding tube (5 size) and plastic disposable syringe (3 ml size) distilled water and aqueous extract were administered into the stomach of respective rat.

Experimental design: Rats were first separated into following four groups (n=7) and treated orally for 14 days: A: 10 ml/kg bw distilled water treated rat ; B:ratsreceived the extract 60 mg/ kg body weight; C: rats treated the extract (80 mg/ kg body weight)and D: rats received the extract 100 mg/ kg body weight.

Collection of blood samples and hematological analysis: On 15th day, blood samples were collected from each rat aseptically by sterile disposable syringe (3 ml size) through cardiac puncture. Then collected blood was taken rapidly in anticoagulant (EDTA) containing test tube. After labeling, blood samples were sent for hematological analysis by Automated Hematology Analyzer SYSMEX XT-2000i in the Department of Hematology, Dhaka Medical College Hospital, Dhaka.

Data collection and analysis technique: Following laboratory analysis of blood samples, data collections for each group of rats were done in a data collection sheet. Obtained data on red blood cell count, total & differential count of white blood cell

and platelet count were recorded and compiled. All the values were expressed as the mean ± SEM (Standard Error of Mean). ANOVA test revealed that increased blood cell counts following administration of aqueous extract of *B. alba* were statistically significant (p value for each case was .0001) than control group. Paired samples *t* test was performed to compare between baseline haematological parameters and parameters after 14 days of intervention. Then comparison between Group A & Group B, between Group A & Group C and between Group A & Group D were done. In all cases, Probability (p) value < 0.05 was considered as statistically significant.

Results and Discussion

This experimental study was carried out on 28 rats. These rats were distributed into 4 different groups: A, B, C and D. Group A was considered control group. It was given distilled water 10 mg/kg body weight. Group B, Group C and Group D were given *B. alba* extract at 60, 80 and 100- mg/kg body weight, respectively. All of the groups were given their respective food for 14 days. Blood sample from each group was collected at 15th day. Baseline RBC, WBC and platelet count among the experimental groups are shown in table 1.

After 14 days of experiment, the changes of RBC count are shown in table 2. In comparison to group A, the mean RBC count showed a significant dose dependent increase across groups. Here *p* value was significant across groups (<0.001) and between groups (<0.001 for all comparisons). This was consistent with previous findings (Bamidele *et. al.,* 2010).

We also observed in table 3 that there was a gradual and significant increase in WBC count in groups B, C and D in comparison to group A (p <0.001 each). However, the difference between group C and group D was not significant. Mean WBC in group C was 10.06±0.50 and in group D was 10.13 ± 0.23 (p <0.761). In the study done in Nigeria, (Bamidele *et al.* 2010) found a similar pattern of dose-dependent increase in WBC count. In their study the increase was not significant in group II and

III (similar to group B and C in this study), but the increase was significant in group IV (similar to group D in this study).

In table 4, the mean platelet count also showed a significant dose dependent increase in B, C and D groups in comparison to group A (p <0.001 each). But, the difference in mean platelet value between group B and C is minimal. Mean platelet in group B was 202.51 ± 1.52 and in group C was 203.47 ± 1.61. This increase was not significant (p > 0.274). On the other hand Bamidele *et al.* (2010) found that mean platelet count in group II was not significantly different from the control group but group III and IV showed significant increase (p < 0.05).

Table 1. Comparison of baseline haematological parameters at the beginning of experiment.

Haematological parameters	Experimental Groups				*p* value
	Group A (n = 3)	Group B (n = 3)	Group C (n = 3)	Group D (n = 3)	
RBC (x 10^6/µl)	7.50 ± 0.27	7.65 ± 0.19	7.60 ± 0.19	7.61 ± 0.18	0.65
WBC (x10^3/ µl)	6.78 ± 0.24	6.86 ± 0.12	6.93 ± 0.27	6.94 ± 0.23	0.57
Platelet (x 10^3/ µl)	198.42 ± 0.60	198.34 ± 0.18	198.48 ± 0.21	198.62 ± 0.36	0.56

Values are expressed as mean ± SEM (n=12). Probability (*p*) value < 0.05 considered as statistically significant.

Table 2. Change in RBC (x 10^6/µl) count in different groups after 14 days of feeding.

Group	Baseline	After 14 day	*p* value
A (n= 7)	7.50 ± 0.27	7.53 ± 0.28	0.095
B (n= 7)	7.64 ± 0.19	8.27 ± 0.31	0.006
C (n= 7)	7.60 ± 0.19	9.27 ± 0.48	< 0.001
D (n= 7)	7.61 ± 0.18	10.33 ± 0.28	< 0.001

Values are expressed as mean ± SEM (n=28). Probability (P) value < 0.05 considered as statistically significant and was determined by paired samples *t* test.

Table 3. Change in WBC (x10^3/ µL) count in different groups after 14 days of feeding.

Group	Baseline	After 14 day	*p* value
A (n= 7)	6.78 ± 0.24	6.83 ± 0.27	0.258
B (n= 7)	6.86 ± 0.12	8.89 ± 0.26	< 0.001
C (n= 7)	6.93 ± 0.27	10.06 ± 0.51	< 0.001
D (n= 7)	6.94 ± 0.23	10.13 ± 0.23	< 0.001

Values are expressed as mean ± SEM (n=28). Probability (p) value < 0.05 was considered as statistically significant and was determined by paired samples *t* test.

Table 4. Change in platelet (x10^3/ µL)count in different groups after 14 days of feeding.

Group	Baseline	After 14 day	*p* value
A (n = 7)	198.42 ± 0.60	198.57 ± 0.19	0.395
B (n = 7)	198.34 ± 0.18	202.51 ± 1.52	<0.001
C (n = 7)	198.48 ± 0.21	203.47 ± 1.61	<0.001
D (n = 7)	198.62 ± 0.36	205.79 ± 1.13	<0.001

Values are expressed as mean ± SEM (n=28). Probability (p) value < 0.05 was considered as statistically significant and was determined by paired samples *t* test.

Table 5. Comparison of haematological parameters among experimental groups.

Haematological parameters	Experimental Groups				*p* value
	Group A (n=7)	Group B (n=7)	Group C (n=7)	Group D (n=7)	
RBC (x 10^6/µl)	7.53 ± 0.28	8.27 ± 0.31	9.26 ± 0.48	10.33 ± 0.28	< 0.001
WBC (x10^3/µl)	6.83 ± 0.27	8.89 ± 0.26	10.06 ± 0.50	10.13 ± 0.23	< 0.001
Platelet (x10^3/µl)	198.57 ± 0.51	202.51 ± 1.52	203.47 ± 1.61	205.79 ± 1.13	< 0.001

Values are expressed as mean ± SEM (n = 28). Probability (p) value < 0.05 considered as statistically significant.

Table 5 showed the comparison of hematological parameters among experiment groups. In the control group (i.e., group A) mean values of RBC, WBC and platelet after 14 days were 7.53 ± 0.28 x 10^6/µl, 6.83 ± 0.27 x 10^3/µl and 198.57 ± 0.51 x 10^6/µl, respectively. Previous study (Bamidele *et al*., 2010) carried out to see the effect of *B.alba* leaf extract on hematological and biochemical parameters found RBC, WBC and platelet count in control group as 4.61 ± 0.06, 6.70 ± 0.50 and 199.87 ± 0.25, respectively. This is similar to the findings of present study except in RBC count which is higher in the present study.

The results of the present study confirm that use of the *B. alba* leaves in traditional medicine for the treatment of anemia is scientifically justifiable. Leaf extract of the plant might have a promising role in the treatment and/or prevention of anaemia. It may also be used to enhance the immunological capacity of body and to ameliorate bleeding tendencies.

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Conflict of interest

Author has declared that he has no conflict of interest.

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