



ORIGINAL ARTICLE

Antihematotoxic Role of *Bunium persicum* Seed Differential Extracts in Animal Model: Reactive Oxygen Species Might Be a Contributor

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Abstract

Objectives: Humans have been using plants as natural medicines since prehistoric times. *Bunium persicum* is a rich source of oils with different biological activities such as antioxidative and antimicrobial activities. The aim of this study is to evaluate the antihematotoxic and antioxidative effects of the differential extracts of *B. persicum* against leukemic blood induced hematotoxicity in an animal model.

Methods: This study was performed on animals, which were divided into several groups: normal control, disease control, and groups that were administered with differential extracts of plants. We measured the concentration of free radical [reactive oxygen species (ROS)] and hematological parameters as blast cells from the tibia and femur in different groups.

Results: The ROS level and blast cells count were higher in disease control groups than in groups treated with varying amounts of *B. persicum* extract and the normal group. Moreover, there was an imbalance in hematological parameters in untreated and treated groups with a correlation between free radicals and plant extract administration.

Conclusion: These findings may indicate a possible link between free radicals and hematotoxicity and blast cells, while depicting a potential therapeutic role for *B. persicum* against ROS-induced hematotoxicity.

1. Introduction

Plants have been used as medicines since the dawn of civilization [1]. *Bunium persicum* is a high-value herbaceous spice of the parsley family (Apiaceae) used as a spice

to add flavor to certain dishes and delicacies, and bakery perennial aromatic and medicinal umbelifer [2]. *B. persicum*, also known as “black cumin” in English, is a common plant that is widely distributed in Gilgit, Baltistan, Chitral, Swat, Hazara, and Baluchistan in Pakistan [2,3].

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B. persicum is a rich source of essential oils including terpenoids and alkaloids with different biological activities such as antioxidant and antimicrobial effects [4]. Essential oils found in *B. persicum* have antifungal [5] and antimicrobial activities [6]. Seeds of *B. persicum* are rich in phenolic compounds and γ -terpinene, which exhibit potential antioxidant properties and can act as efficient free radical scavengers [7].

Reactive oxygen species (ROS) are produced during natural metabolic processes in the form of superoxide (O_2^-), hydrogen peroxide (H_2O_2), hydroxyl radical (OH^\cdot), and singlet oxygen (1O_2), which attack biological molecules leading to cell or tissue injury [8]. ROS have a pivotal role in signal transduction but in excess can contribute to fetal toxicity by deregulation of signal transduction and/or by oxidative damage to cellular macromolecules such as lipids, proteins, DNA, and RNA. Oxidative stress can lead to a number of human diseases such as atherosclerosis, inflammatory diseases, neurodegenerative disorders, and different types of cancers [9,10]. Wan and Winn [11] have reported that ROS play a major role in benzene-mediated fetal hematotoxicity in mice.

These oxidative stresses are encountered by antioxidant compounds that prevent or delay the peroxidation of fats. Different types of synthetic antioxidants such as butylatedhydroxytoluene, butylatedhydroxyanisole, propyl gallate, and tertbutylhydroquinone are used as additives in food industries. These antioxidants, however, have been reported by researchers to be potential carcinogens and can cause external and internal bleeding and finally death owing to their effects on the blood coagulation factor [12]. Because of the hazardous effects of synthetic antioxidant additives, consumers have shown an increasing tendency to use additives from natural resources such as plant essential oils in foods, beverages, and cosmetics. The seeds of *B. persicum* are rich in essential oils, which have a potent antioxidant activity and can be used as natural antioxidants [13].

In the present study, we examined the effects of different fractions of *B. persicum* seed extracts in leukemic blood induced hematotoxicity in rats. To achieve this goal, different seed fractions of *B. persicum* as well as ethyl acetate, butanol, hexane, and aqueous fractions were used, and their effects on various parameters including hematological parameters, ROS, aberrant protein expression, and blast cells of bone marrow were studied.

2. Materials and methods

2.1. Plant materials

Plant materials (seeds) were purchased from a market in Rawalpindi, Pakistan. They were cleaned, washed, and dried under the shade. The seeds were then ground into fine powder with a grinder.

2.2. Preparation of crude extract and fractionation

The procedure described by Wube et al [14] was followed for extraction. Briefly, the powdered residue (3 kg) was kept in a closed container and extracted with methanol by maceration for 4 days to obtain the crude extract (175 g). The solvent containing the crude extract was filtered with a Whatman filter paper (Number 1) and evaporated under reduced pressure using a rotary evaporator and allowed to dry at room temperature. The maceration process was repeated three times. The dried extract obtained was suspended in water and extracted with hexane, ethyl acetate, and butanol solvents subsequently using a separating funnel to obtain the ethyl acetate, butanol, hexane, and aqueous fractions. The solvent from each fraction was evaporated and allowed to dry. The dried fractions were kept at -2°C in dark glass bottles for experimentation.

2.3. Ethical considerations

This study was carried out with the approval and under the strict guidelines of the institutional ethics committee for the handling of animals.

2.4. Experimental models

Twenty-four albino neonate rats (birth age < 24 hours) were obtained from the Animal House of the Department of Biological Sciences, Quaid-i-Azam University, Islamabad, Pakistan, and randomly divided into six groups. One group was designated as the control normal group (Group C), whereas the other five groups (A, B, D, E, H) were injected intraperitoneally (i.p.) with fresh leukemic (acute myeloid leukemia) blood (0.2 mL; age, \leq 24 hours) to induce hematotoxicity within 13–38 days [15].

2.5. Experimental design and treatment

After the 40th day of injection, the weight of each rat was accurately measured, and rats were treated as follows: Group A, 300 mg/kg/d aqueous fraction in 0.5 mL Tween; Group B, 300 mg/kg/d butanol fraction in 0.5 mL Tween; Group C, 0.5 mL/d of 5% Tween; Group D, normal with leukemic blood treated, 0.5 mL/d of 5% Tween; Group H, 300 mg/kg/d hexane fraction in 0.5 mL Tween; Group E, 300 mg/kg/d of rat body weight ethyl acetate fraction in 0.5 mL Tween. The dosages were given as i.p. injections for 9 consecutive days. Table 1 shows the treatment schedule.

2.6. Hematological parameters

After the treatment with plant differential extracts was completed, all rats were sacrificed on the 10th day by decapitation, and blood was collected and sent to North Western Laboratory Peshawar, Pakistan, to determine the blood parameters: white blood cells differential (monocytes, basophils, eosinophils, and neutrophils), red blood cells (RBCs; g/dL), and number of platelets.

Table 1. Treatment schedule.

Group	Treatment
A	Injected with leukemic blood; treated with aqueous fraction
B	Injected with leukemic blood; treated with butanol fraction
C	Designated as control group (normal); treated with 0.5 mL of 5% Tween
D	Assigned as disease control group; injected with leukemic blood and treated with 0.5 mL of 5% Tween
E	Injected with leukemic blood; treated with ethyl acetate fraction
H	Injected with leukemic blood; treated with hexane fraction

2.7. Collection of bone marrow

The bone marrow was collected from the both the tibia and the femur within hours after the rats were sacrificed. The bones were cleaned from the tissue, and the tips of each bone were cut down from one side and flushed out from the other side with 25-gauge needle with phosphate buffer saline into a falcon tube. The flushing was repeated several times until the bone turned pale. The filtrate was centrifuged at 1000 rpm for 10 minutes, and the supernatant was discarded. The fixative (1:3) acetic alcohol was added to the falcon tube and was gently mixed then centrifuged again to obtain a fresh cell button end; next, fresh fixative was added to obtain cell suspension. A drop was made from the cell suspension into a clear slide and allowed to dry. The air-dried smears of bone marrow aspirate were fixed in methanol for 8 minutes. The slides were then immersed in Giemsa stain for 10 minutes and washed with distilled water to remove the extra stain. Slides were observed under a light microscope (Meiji Techno MT6200) at 100× magnification.

2.8. ROS measurement

The protocol described by Hayashi et al [16] was followed for the standard curve formation and ROS detection in all groups using spectrometric methods. To make standard solutions, in 0.1M sodium acetate buffer (pH 4.8), DEPPD (R1) was dissolved to obtain a final concentration of 100 µg/mL, and ferrous sulfate (R2) was dissolved in sodium acetate buffer to attain a final concentration of 4.37µM. R1 and R2 were mixed at a ratio of 1:25 prior to use to make a mixed solution. Then, 1200 µL of the mixed solution was added as a starter in a 3-mL cuvette followed by adding 1000 µL sodium acetate buffer and 60 µL hydrogen peroxide (H₂O₂) as positive control. Instead of H₂O₂, serum was used for ROS detection in the rat blood serum. After free incubation of 1 minute, absorbance at 505 nm was observed using the Agilent 8453 UV–Visible Spectrophotometer (UK).

2.9. Statistical analysis

Data are expressed as mean ± standard deviation. SPSS version 16 (SPSS Inc., Chicago, IL, USA) was used for all statistical analyses. A *p* value < 0.05 was considered statistically significant.

3. Results

3.1. Hematological parameters and plant extracts

Hematological parameters in various study groups treated with differential extracts of *B. persicum* seeds are presented in Table 2 and Figure 1. Group D (disease control group) showed an increase in monocytes, basophils, and eosinophils % values and a decrease in % neutrophils, RBC (g/dL), and platelets count when compared with their corresponding values among normal rats. Among the treated (butanol, ethyl acetate fraction) groups, % monocytes and % basophils decreased significantly when compared to Group D (normal leukemic blood induced group). Similarly, a significant increase was observed in RBCs (g/dL) and platelet concentration/counts compared with those of disease controls (Table 2, Figure 1). In the case of Group A (leukemic treated with aqueous fraction), platelet levels were observed to be elevated compared to the disease control group (Group D; Table 2, Figure 1), whereas % of monocytes and RBCs decreased.

3.2. Effects of plant extracts on ROS level

Elevated concentrations of free radicals are considered toxic for biological systems.

The level of free radicals in the hematotoxic group (Group D) was high compared with that of normal rats in Group C (Table 2). After the administration of plant extracts, a significant decrease in ROS was observed only in Group E, whereas in Group B the decrease was nearly significant (*p* = 0.059).

3.3. Effects of plants extracts on blast cells

Large numbers of blast cells were observed in the disease control group as compared with the normal control group. The number of blast cells seen in EFT and BFT was lower compared with that of the disease control group but higher than that of the normal control group (Figure 2).

4. Discussion

Regulation of ROS level is essential for maintenance of normal populations of hematopoietic stem cells and their differentiation in normal hemopoetic cell lines [17,18]. ROS in cancer can be targeted by the use of antioxidant molecules to suppress the high levels of

Table 2. Variation of differential blood parameters and comparative analysis of ROS mean values among normal control, disease control, and treated groups.

Groups	WBC differential				RBC (g/dL)	Platelet counts ($\times 10^9/L$)
	% Monocytes	Basophils %	Eosinophils %	Neutrophils		
A	29.7 \pm 1.25	6.48 \pm 0.91	5.75 \pm 0.5	6.69 \pm 0.76	5.70 \pm 0.76	716 \pm 28
B	5.4 \pm 0.7	4.95 \pm 0.88	5.69 \pm 0.86	7.47 \pm 0.91	5.68 \pm 0.52	102 \pm 109
C	6.57 \pm 0.40	1.0 \pm 0.50	5.18 \pm 1.1	13.63 \pm 0.9	7.6 \pm 0.86	851 \pm 89
D	31.1 \pm 0.90	6.87 \pm 1.51	9.43 \pm 0.7	6.10 \pm 0.30	4.84 \pm 0.76	411 \pm 44
E	11.2 \pm 1.71	4.3 \pm 1.02	5.2 \pm 0.36	9.77 \pm 0.3	6.48 \pm 0.37	739 \pm 50
H	30.1 \pm 0.76	6.48 \pm 0.91	5.38 \pm 0.58	6.99 \pm 0.6	5.35 \pm 0.22	538 \pm 62
Mean value of absorbance (ROS)						
A	0.24 \pm 0.025					
B	0.25 \pm 0.007					
C	0.21 \pm 0.083					
D	0.41 \pm 0.067					
E	0.24 \pm 0.025					
H	0.35 \pm 0.029					

Data are expressed as mean \pm SD. RBC = red blood cell; ROS = reactive oxygen species; WBC = white blood cell.

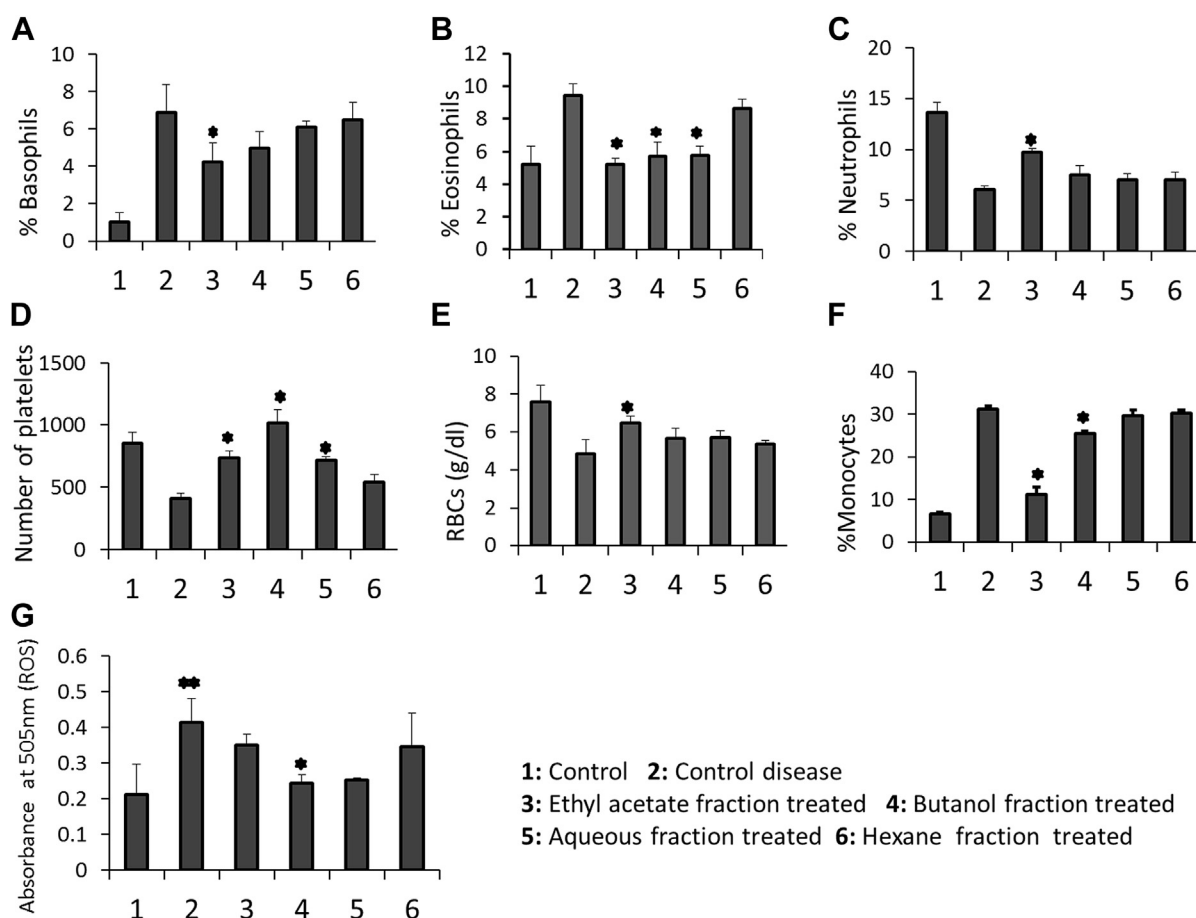


Figure 1. Effect of differential fractions extracted from the seeds of *Bunium persicum* on different hematological parameters compared from CBC results and free radical concentration in serum. Groups A, B, E, and H were administered intraperitoneally (i.p.) with nontoxic dose of 300 mg/kg of rat body weight of each fraction in 5% Tween solution for 9 consecutive days. Groups C and D were assigned as vehicle controls and were only injected (i.p.) with 0.5 mL/d of 5% Tween solution. Mean values were compared using one-way ANOVA followed by Tukey test. *A *p* value < 0.05 indicates significant difference from the control disease (group D) values. (A) Percentage of basophils in different groups. (B) % of eosinophils. (C) % of neutrophils. (D) Numbers of platelets. (E) RBC counts. (F) % of monocytes. (G) Level of ROS in different groups. ANOVA = analysis of variance; CBC = complete blood count; RBC = red blood cell; ROS = reactive oxygen species.

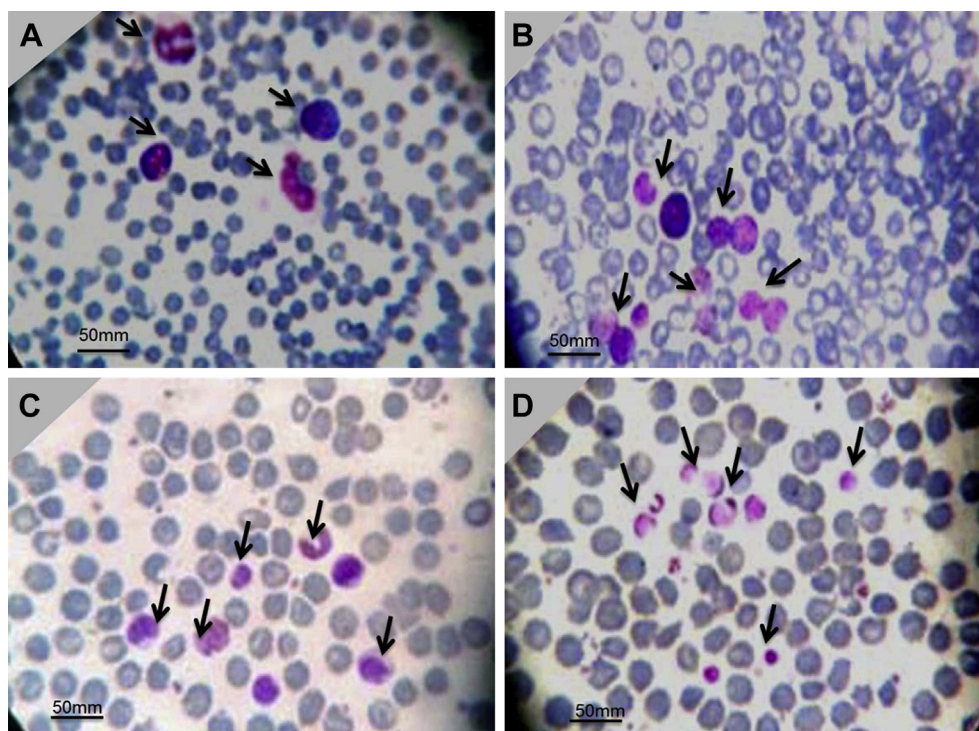


Figure 2. Bone marrow smear stained with Giemsa stain. The blast cells are indicated by an arrow in each figure. (A) Control disease. (B) normal. (C) Blast cell in EFT. (D) Blast cells in BFT. BFT = .

ROS [19]. *B. persicum* is a rich source of essential oils including terpenoids, phenolic compounds, and alkaloids, and is known to have antioxidant and antimicrobial properties [4,7]. Different types of synthetic antioxidants such as butylatedhydroxytoluene, butylatedhydroxyanisole, propyl gallate, and tertbutylhydroquinone are used as additives in food industries. However, researchers have shown that these antioxidants are potential carcinogens and can cause internal and external bleeding and even death owing to their effects on blood coagulation factor. Because of these health-threatening effects of synthetic antioxidants, it becomes crucial to explore natural antioxidant compounds as safer substitutes. The seeds of *B. persicum* are rich in essential oils, which have a potent antioxidant activity [12]. Based on the antioxidant activity of *B. persicum*, we hypothesize that plant extracts may have antihematotoxic effects via an oxygen species-dependent mechanism.

In the present investigation, our finding of increased ROS level compared to the normal group is in agreement with previous reports. The treatment using plant extracts such as EFT and BFT reduced the ROS level, suggesting that *B. persicum* plant extracts have a strong potential as antioxidants against free radicals. Surprisingly, we found decreased blast cell populations in EFT- and BFT-treated animals compared to the disease control animals. This showed a correlation of free radicals and blast cells; however, this concept may require further study.

Blood is an important index of physiological and pathological status in man and animals, and the parameters usually measured are differential leukocyte (white blood cell) count, RBC count, and platelet counts [20,21]. In the present study, an imbalance in the blood picture was observed under diseased conditions. After the treatment, the action performed by *B. persicum* extracts in the regulation of monocytes, eosinophils, neutrophils, basophils, and RBCs under toxic conditions, showed their potential against toxicity.

In summary, we have shown for the first time that *B. persicum* plant differential extracts possess antitoxic and antioxidative properties against leukemic blood induced hematotoxicity and free radicals, respectively. These findings suggest and emphasize that ethyl acetate and butanol fractions from the seed extract of *B. persicum* could be further characterized as a valuable therapeutic candidate for the treatment of patients with AML.

Conflicts of interest

None.

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