To assess the anti-anemic potential of seed extract of *Brassica nigra*

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Abstract:

Background: The medical benefits of Brassica nigra have been utilized for centuries, particularly in countries with limited resources. People believe medicinal plants as safe and cost effective however, unaware of their toxic effects. Hematological parameters are essential tool to assess the degree of toxicity on cellular components of blood. Hence, the aim of this study is to evaluate the effect of *Brassica nigra* on hematological parameters in rats using an automated analyzer. **Methodology**: Albino wistar rats were randomly assigned into three groups of six rats each. Group 1 was control, while group 2 and group 3 received 250 mg/kg and 500 mg/kg body weight of extract respectively, orally once daily. **Results**: No significant difference was observed in the WBC count of extract treated groups (P>0.05) at both doses. The group received 250mg/kg group did not show any significant difference (P>0.05) in RBC count, HCT, Hb, MCV, MCH, MCHC, platelet count, as compared to control. Whereas, HCT and Hb were siginificantly increase at 500mg/kg as compared to the control. **Conclusion:** Oral administration of *Brassica nigra* at 500mg/kg increases Hb and HCT; while no significant difference was observed at 250mg/kg on different hematological parameters.

Key words: *Brassica nigra*, Hematocrit, Hemoglobin, RBC count, WBC count

1. Introduction:

Since medicinal herbs play a significant role in both preventing and treating a wide range of ailments, medicinal herbs have been utilised for ages around the world [1]. The use of traditional medicinal herbs has been widespread in developing countries due to the adverse reactions and contraindications of conventional medications. Cultural beliefs, is one of the reason that people prefer traditional remedies which are safe, cost effective and easily available [2].

Brassica nigra belongs to the family Brassicaceae (Cruciferae). The plant is found in different regions of the world like, south Asia, southern Mediterranean region of Europe Canada, Germany, Ethiopia, North America, New Zealand, Ukraine, Myanmar, Russia [3,4]. *Brassica nigra* seeds can be used as a spice. Rheumatism has also been treated with *Brassica nigra* seeds. Arthritis and the common cold are treated with the seed oil. The seed stimulates the production of urine and increases hunger, which helps relieve water retention (edoema). [5]. The seeds have considerable quantity of fatty oil, which can be used as cooking oil. Seeds of the plant can be mixed with honey and have been used as cough suppressant. It can be used to treat respiratory infections. *Brassica nigra* seed extracts can be used in grand mal seizure treatment in mice [6]. The oil extracted from the seeds is very effective as antibacterial [7,8].

The seed principally contains oligosaccharides belonging to the raffinose family; amino acids; fatty acids such as palmitic, stearic, oleic, linoleic, linolenic, eicosenoic and erucic acids; vitamins; minerals (mostly iron); anti-nutritional factors (in particular, enzyme inhibitors); glucosinolates; and a wide variety of phenolic compounds[9]. Various animal studies document its medicinal use as antioxidant and anti-inflammatory [10], anti-microbial [11], thrmbolytic, anthelmentic, anxiolytic, [12] and anti-malarial activity [13].

People perceive that herbal therapies are harmless and unaware or ignorant of their toxic effects on different biological system [14]. Therefore, the data on toxicity of medicinal plants is needed to be explored. Keeping in view the significance of this plant in the treatment of several ailments, the aim of the present study was, to assess the effects of Brassica nigra extract on different hematological parameters in albino wistar rats.

2. Materials and Methods:

2.1 Collection and Identification of Plant:

The seeds were purchased from local market of Karachi, Pakistan. The sample of the seeds specimen was identified by Prof. Dr Mansoor Ahmad, Department of Pharmacognosy, University of Karachi and issued a voucher specimen no 20170302-1.

2.2 Extract Preparation:

The seeds (1000 gram) were grounded into coarse powder using an electrical grinder. The coarse powder was macerated with methanol for 15 days at room temperature. The methanol extract was then filtered, evaporated under reduced pressure in rotary evaporator and stored in refrigerator until needed. The thick gummy extract was reconstituted with 10 % Dimethyl sulfoxide (DMSO) to enhance the solubility of extract in water [15]. All the glass wares used in this study was made of pyrex.

2.3 Animals handling and dietary protocol:

Adult male wistar albino rats (initially weighing between 180-210 gm) were selected for the present study. Animals were purchased from animal house of Agha Khan University Karachi, Pakistan. All animals were acclimatized one week before commencement of experiment and kept under controlled room temperature at 23 ± 2°C in 12 h light/dark cycles with free access to food and water. The experiment was performed in accordance with National Institute of Health (NIH) guidelines for the care and use of Laboratory Animals (National Research Council, 1996), after the approval from Ethical Review Committee (ERC) of Ziauddin University Karachi, Pakistan.

2.4 Grouping of animals and extract administration:

The animals were divided into three groups of six rats in each group, as follows.

Group 1: Received DMSO 10% as placebo (10 ml/kg body weight)

Group 2: Received 250 mg/kg body weight of extract

Group 3: Received 500 mg/kg body weight of extract

All the animals were weighed before starting experiment. The methanol crude extract was administered according to their body weight once daily per oral by gastric intubation for 28 days.

2.5 Collection of blood sample:

On 29th day, all the animals were reweighed, starved for 24 hours and sacrificed under chloroform anesthesia. Blood samples approximately 2.5ml were collected by cardiac puncturing into vacutainers containing EDTA, inverted about 6 times to mix the sample with EDTA [16]. The vacutainers stored in refrigerator until analysis was done, the hematological parameters were analyzed within 24 hours after collecting blood samples.

2.6 Analysis of blood parameters:

Blood samples were analyzed by using automated analyzer sysmax (KX-21), accurately programmed for the analysis of red blood cell (RBC) count, hemoglobin (Hb), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), platelet count, and total white blood cell (TWBC) count.

2.7 Statistical analysis:

Statistical analysis was carried out by using SPSS (Statistical Package for Social Sciences) version 20. Data is presented as mean and standard deviation / standard error of mean. The difference amongst three groups; control, low dose (250mg) and high dose (500mg) of *Brassica nigra* extract was measured by using one way analysis of variance (ANOVA) and where data violated the assumptions of normality, Kruskal Wallis H test (non-parametric test) was used, followed by pairwise comparison post hoc Tukey-test. A p-value of <0.05 was taken to indicate the statistical significance.

3. Results:

The effects of oral administration of *Brassica nigra* on RBC indices in the control, low dose (LD) and high dose (HD) groups are presented in table 1 and table 2.

3.1. Effect of *Brassica nigra* extract at 250 mg/kg on hematological parameters after treatment for 28 days

The Effect of *Brassica nigra* extract at 250 mg/kg on hematological parameters after treatment for 28 days is shown in table 1. The herbal extract of *Brassica nigra* at low dose (250 mg/kg) was administered orally for 28 days to estimate the effects on hematological indices. The hematological parameters did not significantly alter as compared to control as shown in table 1.

Table 1. Effect of *Brassica nigra* extract at 250 mg/kg on hematological parameters after treatment for 28 days

Parameters Mean ± SD	Control	Low dose (BN 250 mg/kg)	P value	95% Confidence Interval	
				Lower	Upper
WBC($x10^3$/µl)	7.0 ± 1.1	7.8 ± 1.2	0.2	-5.4	9.1
RBC($x10^6$/µl)	6.5 ± 0.8	6.9 ± 0.3	0.4	-3.9	10.9
Hb(g/dl)	12.7 ± 0.3	13.3 ± 0.4	0.1	-1.5	9.4
HCT (%)	39.3 ± 1.0	42.6 ± 1.6	0.06	-0.2	10.5
MCV (fl)	61.1 ± 0.8	61.2 ± 1.9	0.9	-6.8	7.4
MCH (pg)	19.6 ± 1	19.1 ± 1.06	0.5	-10.5	4.9
MCHC(g/dl)	32.5 ± 0.5	31.2 ± 0.9	0.08	-12.8	0.7
PLT ($x10^3$/µl)	781 ± 93	889.5 ± 105.8	0.4	-3.3	9.5

*p-value<0.05 was considered significant, Data are expressed as Mean ± SD, n=6, total white blood cell (WBC) count, red blood cell (RBC) count, hemoglobin (Hb), Hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), platelet count (PLT), BN = *Brassica nigra*

3.2. Effect of *Brassica nigra* extract at 500 mg/kg on hematological parameters after treatment for 28 days

The herbal extract *Brassica nigra* was administered in high dose (500 mg/kg) orally for 28 days, to evaluate the effects on hematological indices (Table 2). The hematological indices hemoglobin (Hb) and hematocrit (HCT) were significantly increased (p=0.02, p=0.001) respectively, as compared to the control. However, other blood indices did not significantly alterd as compared to control as shown in table 2.

Table 2. Effect of Brassica nigra extract at 500 mg/kg on hematological parameters after treatment for 28 days

Parameters Mean ± SD	Control	High dose (BN 500 mg/kg)	P value	95% Confidence Interval	
				Lower	Upper
WBC(x10^3/µl)	7.0 ± 1.1	10 ± 3.6	0.1	-2.1	12.4
RBC(x10^6/µl)	6.5 ± 0.8	7 ± 0.1	0.3	-3.6	11.1
Hb(g/dl)	12.7 ± 0.3	13.8 ± 0.4	0.02*	3.5	14.5
HCT (%)	39.3 ± 1.0	45.5 ± 2.8	0.001*	3.9	14.7
MCV (fl)	61.1 ± 0.8	62.6 ± 2.4	0.2	-2.3	12.0
MCH (pg)	19.6 ± 1	19.6 ± 0.8	0.9	-8.4	7.0
MCHC(g/dl)	32.5 ± 0.5	31.3 ± 0.1	0.1	-12.2	1.3
PLT (x10^3/µl)	781 ± 93	869.5 ± 118.6	0.3	-3.2	11.5

*p-value<0.05 was considered significant, Data are expressed as Mean ± SD, n=6, total white blood cell (WBC) count, red blood cell (RBC) count, hemoglobin (Hb), Hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), platelet count (PLT), BN = *Brassica nigra*

Discussion:

With the vital role of preserving homeostasis, blood is a basic circulatory tissue made up of red blood cells (RBCs), white blood cells (WBCs), and platelets contained in a fluid known as plasma. Every blood cell have a vital role in our body for instance, WBCs enhance immune system and are capable to fight against infections, RBCs carry hemoglobin that are mainly responsible for transportation of oxygen and carbon dioxide as well as valuable in the

diagnosis of anemia; while platelets have a major role in the formation of clot. Hematological studies are essential in the diagnosis of several diseases as well as it could be a helpful tool in early sign of toxicity to cellular components of blood in response to certain natural or chemical agents[17].

Despite of its multiple uses of *Brassica nigra* in different ailments, the data regarding its effect on hematological parameters is meager. Hence, this study was conducted to investigate the effect of oral administration of *Brassica nigra* extract on hematological parameters in rats.

Our study showed that the WBC count was not significantly altered ($P > 0.05$) at 250mg/kg and 500 mg/kg as compared to the control group although an increase count of WBC was observed at 500mg/kg (table 2). The normal WBC count in extract treated groups disclosed that *Brassica nigra* does not have any significant effect on white blood cells or leukocytes.

The results of our study indicate that red blood cells indices including, RBC count, MCV and MCH were not significantly altered ($P > 0.05$) at 250 mg/kg(Table 1). Significant alteration was observed in HCT and Hb values at 500mg/kg; however these parameters remain unaltered at 250mg/kg (Table 1). The MCHC values at both doses were not significantly altered in comparison with control. A decreased MCHC specify hypochromia, in early sign of iron deficit and could be observed with any of the disease that can lead to microcytosis[18].

It has been suggested that the raised RDW together with decreased MCHC and normal / variable MCV can be helpful to differentiate the different types of anemia [19]. In our study, HCT and Hb were significantly increased at high as previously discussed (table 1); our results seems to be similar with the results of previous study in which Asaad et al, evaluate the protective role of aqueous extract of *Brassica nigra* in cadmium induced toxicity. In previous study all blood parameters specifically HCT and Hb were restored to normal. In conclusion we conclude that the *Brassica nigra* has hematinic effect [20].

The results of the current study showed that the high dose of *Brassica nigra* extract does not have any significant effect ($p>0.05$) on platelet count as compared to control and low dose groups (table 2). At low dose no significant difference was observed ($p>0.05$) on platelet count.

Platelet indices altogether could provide better understanding of platelet disorders [21].In several studies,the inverse relationship was found between platelet count and other platelet indices i-e MPV, PDW and P-LCR[22-24]. However, this is not in agreement with our results; since in current study platelet count was not significantly changed in extract treated groups.

Conclusion:

Significant difference was observed at high dose of *Brassica nigra* extract on different hematological parameters specifically on HCT and Hb, which shows that it has hematinic or anti-anemic effect; whereas low dose of extract does not significantly affect the values of RBC indices, WBC count and platelet count. Based on these facts, we can conclude that the low and high dose of *Brassica nigra* does not have any toxic effects on several blood parameters.

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