

Efficiency of *Lepidium sativum* Seeds in Modulation the Alterations in Hematological Parameters Induced by Sodium Nitrite in Rats

Enas S. Abdel-Baky

Department of Biological and Geological Sciences, Faculty of Education, Ain Shams University, Cairo, Egypt

ABSTRACT

Background: Sodium nitrite is an inorganic compound with the chemical formula NaNO_2 . It is probably best known as a food additive to prevent botulism.

Aim of the work: The current study was designed to investigate the possible protective effect of aqueous extract of *Lepidium sativum* seeds on hematotoxicity induced by sodium nitrite (NaNO_2) in male albino rats.

Materials and Methods: The experimental animals were divided into four groups. The first group without any treatment and served as control. The second group was given an intraperitoneal injection (IP) of a single dose of sodium nitrite (50 mg/kg body weight) 24 h prior to decapitation. The third group was received orally 300 mg/kg body weight of *Lepidium sativum* seeds extract for four weeks. The fourth group were treated orally with *Lepidium sativum* seeds extract (300 mg/kg body weight) for four weeks, prior to IP injection with a single dose of sodium nitrite (50 mg/kg body weight). Finally, blood samples were collected for hematological parameters estimation including red blood cell count (RBCs), hemoglobin (Hb), packed cell volume (PCV), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), platelet count (PLTs) and total and differential white blood cells (WBCs).

Results: The obtained data revealed that NaNO_2 induced a significant decrease in the percentages of RBCs count, Hb content, PCV, MCH, MCHC, platelets, WBCs, neutrophils, monocytes, eosinophils and basophils count. On the other hand, a noticeable increase in the percentage of lymphocytes was recorded in NaNO_2 treated rats. Whereas, the treatment with *Lepidium sativum* seeds extract alone to rats did not cause a significant alterations in the hematological indices comparing to control group. There was a significant increase in total WBCs and neutrophils counts and a significant decrease in lymphocytes, monocytes, eosinophils and basophils counts. The treatment with *Lepidium sativum* seeds extract before injection with sodium nitrite were significantly ameliorated the changes induced in hematological parameters which caused by sodium nitrite comparing with the control group. Whereas, there were a significant increase in total WBCs count, neutrophils and eosinophils and a significant decrease in lymphocytes, monocytes and basophils.

Conclusion: It could be concluded that the treatment with *Lepidium sativum* seeds extract significantly improved the changes in hematological parameters that caused by sodium nitrite and induced a significant increase of WBCs count may stimulate the animal's immune system activation.

Key words: Hematological parameters, *Lepidium sativum* seeds, Sodium nitrite.

INTRODUCTION

Food additives are substances intentionally added to food and used for various purposes, including preservation, coloring and enhance food ⁽¹⁾. They are products of oxidation of nitrogen by microorganisms in plants, soils and water. Nitrites are used as food additives mainly for production of specific flavor and for preservation of meat products. Several organic nitrites and nitrates have been used clinically but the only inorganic nitrite of therapeutic using is sodium nitrite ⁽²⁾. The nitrites used in the form of salts or free acids

and it is one of the principal preservatives. Previous researchers reported that nitrites are environmental pollutants present in food and water and it is responsible for most of the toxic effects observed with excess nitrate ingestion ⁽³⁾. Sodium nitrite is a white to slightly yellowish inorganic salt, with the chemical formula NaNO_2 . It may interact in the stomach with amines of the foods, producing nitrosamines or free radicals ⁽⁴⁾. These products may induce lipid peroxidation that may increase the harmful hazards to the

different organs of the body ⁽⁵⁾, including liver and kidney ⁽⁶⁾. Also, Sodium nitrite in blood is highly reactive with hemoglobin, so affecting hematopoiesis process. It reduce hemoglobin's ability to transport oxygen a major concern in considering the toxicology of sodium nitrite is the induction of methemoglobinemia, a condition in which there is a reduction in hemoglobin's ability to transport oxygen ⁽⁷⁾.

The garden cress (*Lepidium sativum*) belongs to Brassicaceae family; it is present in Egypt and west Asia. The seeds are used enormously as salad and spice ⁽⁸⁾. It has a wide therapeutic application, so it has been used widely around the world, the previous studies have reported the protective action of the plant against carcinogenic compounds and growth prevention of *Pseudomonas aeruginosa*, a bacteria strain has a strong antibiotic resistance ⁽⁹⁾. The leaves and seedpods have been reported to have various biological activities including antioxidant, anti-inflammatory, hepatoprotective effect against oxidative damage, and therefore, it is used as dietary supplements ⁽¹⁰⁾.

The aim of the current work was to investigate the possible protective effect of aqueous extract of *Lepidium sativum* seeds on hematotoxicity induced by sodium nitrite (NaNO₂) in male albino rats.

Materials and methods

Chemicals

Sodium nitrite (NaNO₂) was purchased from sigma Aldrich, St Louis, Mo. It was prepared as a freshly solution by dissolving 5 g in 100 ml saline solution, and was administered intraperitoneally (IP) at 50 mg/kg body weight (1 ml dosing volume) according to **Gluhcheva et al.** ⁽⁷⁾.

Preparation of plant extract (*Lepidium sativum* seeds extract)

Lepidium sativum seeds (known as garden cress) (LS) were obtained from Agricultural Research Center, Egypt. Briefly, the grinded seeds were washed, dried and crushed to powder using electric blender. The freshly prepared suspension of garden cress seeds powder were administered to each rat orally by stomach tube at a dose 300 mg/kg body weight once daily for four weeks.

Experimental animals

Twenty-four male albino rats (*Rattus norvegicus*) weighing 125–150 g were used throughout the experiments. The animals were housed in metal cages in a conditioned room and were provided with a standard laboratory diet and water *ad libitum*. They were purchased from animal house colony of the national research center.

Experimental design

The rats were divided randomly into four groups (n=6). The first group rats received no treatment and served as control; the second group rats received a single dose of sodium nitrite intraperitoneally (50mg/kg body weight) 24h before decapitation; the third group rats received orally garden cress extract (300 mg/kg body weight) for four weeks; The fourth group rats received garden cress extract orally (300 mg/kg body weight) for four weeks and then received a single dose of sodium nitrite intraperitoneally (50 mg/kg body weight) 24h before decapitation.

The rats were starved for 12h overnight prior the collection of blood samples. Rats were anaesthetized with ethyl ether and the blood was collected in sterilized vials by cervical dislocation.

Hematological studies:

A part of blood samples was collected in EDTA vials. Red and white blood cell counts, hemoglobin concentration, mean corpuscular hemoglobin (MCH), mean corpuscular volume (MCV), mean corpuscular hemoglobin concentration (MCHC) and platelets count were performed according to **Dacie and Lewis methods**.

Differential Leucocytes Count

Freshly collected blood samples of 20 µl were spread on clean slides as a thin film. Each smear was left to air dried and fixed with methanol for 2-3 min and then labeled. After that, blood smears were stained with 10% Giemsa's stain (Aldrich), examined under light microscopy and different types of blood leucocytes were counted according to **Schalm**.

Statistical analysis

Data were expressed as means ±S.E. Statistical analysis was performed using one-way analysis of variance (ANOVA) followed by Duncan's multiple range tests.

Results

The data represented in tables (1&2) display the effects of treatment on hematological indices. There were a significant reduction in RBCs count, Hb, PCV, MCV, MCH, MCHC and PLT in the sodium nitrite-treated group comparing to the control group (Table 1). On the other hand, the garden cress treated group shows a normal values in these hematological parameters. Co-administration of sodium nitrite with the garden cress extract were significantly prevented to some extent the changes recorded in sodium nitrite group in these parameters as compared with control group.

Results presented in table (2) show the alterations of the WBCs count and the differential white blood cell counts. In sodium

nitrite treated group, there were a significant decrease in WBCs count and differential white blood cells count, including neutrophils, monocytes, eosinophils and basophils. Whereas, there was a significant increase in lymphocytes count comparing with the control group. The garden cress treated group revealed a significant increase in the count of total WBCs and neutrophils. But, the count of lymphocytes, monocytes, eosinophils and basophils were significantly decreased comparing with the control values. On the other hand, in the co-administration of sodium nitrite and the garden cress extract treated group, the total white blood cells count and neutrophils were significantly increased. Whereas, there were a significant decrease in the lymphocytes and the monocytes count compared to the control group.

Table (1): Effect of orally administrated with garden cress extract on the hematological parameters in rats intoxicated with sodium nitrite.

| Groups Parameters | Control | Na NO ₂ | % D | Garden cress | %D | Na NO ₂ + Garden Cress | %D | p-value |
|---|-------------------------|--------------------------|--------|--------------------------|-------|-----------------------------------|--------|---------|
| R.B.Cs (x10 ⁶ /mm ³) | 6.72±0.32 ^a | 5.43±0.211 ^b | -19.2 | 6.71±0.1 ^a | -0.15 | 6.57±0.2 ^a | -2.23 | * |
| Hb (g/dl) | 13.3±0.204 ^a | 9.8±0.45 ^b | -26.32 | 13.03±0.15 ^a | -2.03 | 12.5±0.23 ^a | -6.02 | * |
| PCV% | 38.8±0.6 ^a | 31.24±1.65 ^b | -19.5 | 39.82±0.5 ^a | 2.63 | 38.5±0.52 ^a | -0.8 | * |
| MCV (FL) | 57.23±0.4 ^{ab} | 56.94±1.02 ^{ab} | -0.51 | 59.6 ±1.34 ^{ab} | 4.14 | 56.65±0.51 ^{ab} | -1.013 | * |
| MCH (pg) | 18.53±0.22 ^a | 17.92±0.2 ^b | -3.3 | 19.4±0.22 ^c | 4.7 | 17.98±0.12 ^b | -3 | * |
| MCHC (%) | 32.9±0.21 ^a | 31.4±0.24 ^b | -4.6 | 32.7±0.4 ^a | -0.61 | 31.32±0.33 ^b | -4.8 | * |
| PLT (x10 ³ /mm ³) | 597±11.31 ^a | 372.33±24.8 ^b | -37.63 | 571±16.1 ^a | -4.4 | 507±19.03 ^c | -15.1 | * |

Values represent mean ± S.E. of 6 rats

% D: Percentage difference [(Treated value – Control Value) / Control Value] x 100

Different letters indicate significantly different means p-value < 0.05

Same letters indicate non significant changes.

Table (2): Effect of orally administrated with garden cress extract on total leucocytes count and differential leukocytes count in rats intoxicated with sodium nitrite.

| Groups Parameters | Control | Na NO ₂ | % D | Garden cress | %D | Na NO ₂ + Garden cress | %D | p-value |
|----------------------------------|-------------------------|--------------------------|--------|------------------------|--------|--|--------|---------|
| W.B.Cs (× 10 ⁹ /L) | 7.9±0.13 ^a | 6.02±0.23 ^b | -23.8 | 10.5±0.25 ^c | 32.9 | 9.52±0.3 ^d | 20.5 | * |
| Neutrophils (%) | 24.6±1.12 ^a | 1.5±0.2 ^b | -93.9 | 51.33±1.3 ^c | 108.66 | 52.5±1.34 ^c | 113.41 | * |
| Lymphocytes (%) | 63.85±1.01 ^a | 93.23±0.7 ^b | 46.01 | 40.33±1.2 ^c | -36.84 | 37.7±1.7 ^c | -40.96 | * |
| Monocytes (%) | 6.9±0.2 ^a | 4.3±0.6 ^b | -37.7 | 5.1±0.3 ^b | -26.1 | 5.33±0.3 ^b | -22.8 | * |
| Eosinophil (%) | 4.24±0.31 ^a | 0.72±0.2 ^b | -83.02 | 3.2±0.1 ^c | -24.53 | 4.3±0.31 ^a | 1.42 | * |
| Basophils (%) | 0.5±0.015 ^a | 0.23±0.06 ^{abc} | -54 | 0.1±0.04 ^{bc} | -80 | 0.3±0.06 ^{abc} | -40 | * |

Values represent mean ± S.E. of 6 rats

% D: Percentage difference [(Treated value – Control Value) / Control Value] x 100

Different letters indicate significantly different means p-value < 0.05

Same letters indicate non significant changes.

Discussion

Hematological parameters and the examination of the extent of their deterioration is a useful tool for determination of many diseases. This is because the alteration in blood components is related to the physiological conditions of the animals. Hematological investigations are serious because blood is the main transport system of the body and the hematological estimation usually gives important information on the body's response to all forms of injury, such as toxic injury⁽¹¹⁾.

The purpose of this research work was to determine the protective effects of oral administration of garden cress aqueous extract to rats treated with the sodium nitrite on hematological parameters. The varied levels of significance noticed in the hematological parameters evaluated in this study (post-administration) between the control and the test groups shows that there is an obvious connection between the intake of sodium nitrite, the aqueous extract of garden cress and co-administration between them and the degree of hematological effects that observed. The present work showed that the treatment of rats with sodium nitrite caused a highly

significant decrease in the red blood cells, white blood cells and platelets counts. Also, there are a highly significant decrease in hemoglobin concentration, packed cell volume, means corpuscular hemoglobin, mean corpuscular hemoglobin concentration, mean corpuscular volume values as compared to the control group. Otherwise, there is a highly significant elevation in the count of lymphocytes comparing with control group. The outcome of the present investigation shows that, sodium nitrite causes a significant impact on the different hematological parameters compared to the control. These findings agreed with other researchers⁽¹²⁾, that reported a significant decrease in RBCs count, Hb concentration, MCHC value in the group treated with sodium nitrite. Also, a significant decrease in Hb concentration in the rats that received a high dose of sodium nitrite were reported by **Ibrahim et al.**⁽¹³⁾. Furthermore, **Azabet al**⁽¹⁴⁾, recorded that the administration of sodium nitrite showed a significant decrease in the count of platelets and WBCs in the sodium nitrite treated Guinea pigs. Decreased RBCs count is seen in some anemias. Hb and

MCHC values are also very important for diagnosis of such anemias. The decreasing of RBCs count and Hb concentration may be due to the toxic effect of sodium nitrite on bone marrow, spleen and liver⁽¹²⁾ or may be due to the direct interaction of sodium nitrite on Hb, where it oxidizes the ferrous ion of the oxyhemoglobin (oxy Hb) molecules to the ferric state forming met Hb, leading to cell destruction⁽¹³⁾. The membrane and cytoplasmic structures are influenced by an oxidant attack⁽¹⁵⁾. The reduction in WBCs count lower the defense mechanism (immune system), that play an important role in attacking and interacting with foreign antigens and initiating a primary immune response. The decrease in WBCs count after treatment with sodium nitrite may be due to the failure of the hematopoietic tissues to produce new WBCs⁽¹⁶⁾. From the result of the differential white cells count that carried out in this study, the observed increase in lymphocytes count in sodium nitrite treated group might be attributed to the interaction between sodium nitrite and gastrointestinal macrophages, which act as a toxic agent. Macrophages cause the activation of the helper T cells and the B lymphocytes, by serving as antigen presenting cell and the antigenic products (polypeptides)⁽¹⁷⁾. They also secrete substances called interleukin-1/cytokines, that stimulate the activation of lymphocytes and increase their count⁽¹⁷⁾. The main function of lymphocyte is the response to antigen (foreign bodies) by the development of cellular immunity and forming antibodies circulating in the blood⁽¹⁸⁾.

Sodium nitrite might be has a toxic effect on the neutrophils and monocytes cells in the blood or it has a serious effect on the bone marrow, causing the reduction of these blood cells production. Neutrophils with monocytes considered as the first line defense against toxic substances, foreign substances and microorganisms⁽¹⁹⁾. This might be indicator to the disturbance of immune status in the treated animals responding to the toxic effect of sodium nitrite. Also, there were a significant decrease in the eosinophils count that attack multicellular parasites and some infections in vertebrates. In addition to, basophiles were affected in the treated animals comparing with the control group. Some previous studies suggested that basophiles could be regulating the behavior of T cells and the secondary response of immunity⁽²⁰⁾.

Herbal medicine can promote health and alleviate illness, due to the fact that plants contain natural substances⁽²¹⁾. They have shown a strong power in modern phytomedicine against various ailments and the complex diseases and make a various assistance to the health care. The present study showed that the intake of garden cress aqueous extract alone to male albino rats did not reveal a significant change in the hematological parameters comparing to control group. These results are similar to that of **Datta et al.**⁽²²⁾, who reported that garden cress treatment did not show a significant change in the hematological indices. Since, the hematological indices that studied in the present investigation revealed the normal range values; it may be assumed that garden cress did not have serious effects on the animals during the experiment which lasted through four weeks. In other words, there was a significant increase in WBCs count, that means the garden cress contains substances have antibiotic effects⁽⁹⁾, that should stimulate the proliferation of circulating white blood cells to protect the body from teratogens. Eosinophils cells demonstrated a significant decrease in the garden cress treated group comparing with the control group. This decrease in the eosinophils count shows the antioxidant activities of garden cress aqueous extract as the free radical agent stimulate production of more eosinophils.

Co-administration of sodium nitrite and garden cress aqueous extract were improved the alterations recorded in hematological parameters significantly that caused by treatment of rats with sodium nitrite only comparing with the control group. It may be due to the garden cress extract stimulate blood formation due to the presence of essential nutrients and better feed intake⁽²³⁾ and due to the availability of important minerals especially Fe, Cu, Mn and Ca in the garden cress powder⁽²⁴⁾. Garden cress also contains flavonoid and polyphenolic compounds that responsible for its strong antioxidant capacity that might prevent lipid peroxidation in the membranes of erythrocytes, leading to increasing membrane resistance to spontaneous hemolysis, decreasing membrane micro-viscosity, maintenance of their integrity and functional activity⁽²⁵⁾. The significant increase in WBCs count refers to the activation of the animal's immune system. So,

it has been suggested that garden cress intake increased WBCs precursors proliferation from pluripotent stem cell in rats.

Conclusion

From the previous results, it could be stated that the intake of sodium nitrite has been demonstrated to alter the hematological parameters in male albino rats. The supplementation with garden cress aqueous extract have demonstrated a strong potential benefits by stimulated of blood parameters that inducing good health and remaining blood factors that necessary for body defense.

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