Diyala Journal for Veterinary sciences
Open Access Journal Published by College of Veterinary Medicine
University of Diyala, Iraq
P-ISSN: 2410-8863
E-ISSN:2958-6178
Vol. 2, NO. 3, September 2024
https://doi.org/10.71375/djvs.2024.02310

Ameliorative Role of *Nigella Sativa* Seeds and Levamisole on the Immune Response of Adult Male Rabbits

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Abstract:
This study aimed to evaluate the potency of *Nigella sativa* seeds ethanol extract and levamisole on the immune regulation in male rabbits with H_2O_2-induced immune suppression. Thirty rabbits were distributed into: control group (6 rabbits): free access to food and water and the H_2O_2 group (24 rabbits): was administered with the ad-libitum supply of drinking water containing 0.5% H_2O_2 (Hydrogen peroxide solution 35% diluted 70 times). After 28 days, stopping H_2O_2. Then, animals of H_2O_2 (24 rabbits) were subdivided into four groups (6 rabbits /group) in separate cages. Group *Nigella sativa* seeds extract (*N.sativa* S.E): was administered orally (by gavages needle) (1.5 g/ Kg B.W.). Group levamisole (LEVA): was given orally (by gavages needle) (every 72 hrs.) (5mg /Kg B.W.); a group of animals received *N.sativa* S.E (1.5 g/ Kg B.W.) + LEVA (5mg /Kg B.W.) for 28 days and the last group cessation of H_2O_2 administration (C.H_2O_2). The immunity response of rabbits indicated that exposure of animals to 0.5% H_2O_2 for 28 days revealed a significant decrease ($P \leq 0.05$) in the percentage of lymphocytes, RBCs, phagocyte activity with thrombocytopenia ,leukocytopenia and increase in neutrophils. Histopathological examination of the spleen in the H_2O_2 group showed a significant reduction and degeneration of lymphoid tissue. Combined use of oral *N.sativa* S.E.+ LEVA showed a clear improvement in various immunosuppressive indices compared to the H_2O_2 group. The study concluded that after stopping H_2O_2 as a source of oxidative stress and using *Nigella sativa* seed extracts, levamisole and together showed increased significance in the experiment.

Keywords: Immune system; Immune stimulant; Levamisole; *Nigella sativa* seeds; Rabbit

Introduction

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E-ISSN:2958-6178
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Levamisole (LEVA) is a broad-spectrum synthetic anthelmintic drug widely used in Veterinary Medicine and critically used as an immunomodulatory agent. It improves weight gain in animals (9,17). Levamisole is a drug used to boost the immune system of patients undergoing long-term treatment for stage III colon cancer by increasing natural killer cells and activating T cells. Also, it can stimulate the formation of antibodies against any foreign body, increasing responses and proliferation of T-cells, improving phagocytosis, induced chemotaxis and raising neutrophil motility and adhesion (2).

Hydrogen peroxide (H_2O_2) is a colourless liquid with a chemical formula similar to water (H_2O). The extra oxygen molecule in hydrogen peroxide represents the main difference between the formulas and makes hydrogen peroxide work as a powerful oxidizing agent (28). Oxygen radicals called reactive oxygen species (ROS) are free radicals which contain hydroxyl radicals, superoxide anions, singlet oxygen and non-radical species such as hydrogen peroxide. ROS are unstable oxygen-containing molecules that easily interact with other molecules in the cell and hinder the immune cells' response to foreign invasions. Several studies have shown that ROS play a crucial role as messengers in normal cell signal transduction and cell cycle (14). The appearance of reactive oxygen species in cells may damage DNA, RNA and proteins and may cause cell death. ROS can be produced in vivo by external factors such as exposure to oxidants, smoke from condensed pollutants or during an irregular diet, from internal causes such as biochemical reactions, lipid peroxidation, inflammation and secondary lesions (24).

In addition, overproduction of free radicals produces a side effect of activating immune cells and various stress states as food and environmental conditions. The immune cell has a higher concentration of cleavable polyunsaturated fatty acid for oxidation by FRs caused by different types of intracellular problems, especially intracellular tissue damage with subsequent depression in immune system function. Increasing the proportion of reactive oxygen related to H_2O_2 will cause the activation of stress-sensitive proteins signalling pathways or inflammatory-activated kinases, similar to JNK (Jun Nuclear Kinase protein) playing a role in the pathological process of the immunosuppressive effect of oxidative stress (13, 31).

Nigella sativa is an evergreen herb bearing blue flowers with black seeds, in the diet widely consumed in countries like Pakistan, Gulf countries, Bangladesh Middle-East, India and Iraq. *Nigella sativa* seeds contain many mineral elements (K, Ca, Mg, Na, Fe, Zn, Mn, P and Cu). These minerals are rich sources of fatty acid esters such as (arachidonic acid, palmitic acid, oleic

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acid, linoleic acid, eicosenoic acid, myristoleic acid, and others). The mineral and fatty acids possess antioxidant and free radicals scavenging activity (19). The volatile oil of Nigella sativa contains thymol, carvacrol, thymoquinone, dihydro thymoquinone, t-anatole, ρ-cymene, sabinene, terpinolene, β-pinene, β-myrcene, limonene, alkyl group hexanoate, γ- terpinene, and α-thujene in addition to the compounds responsible for a large part of the pharmacological effects of *Nigella sativa* seed (23). It was used in natural treatment for diseases and injuries like cardiac diseases, cancer, diabetes, dermatology, dental problems, constipation, allergies, and pain (12,32). This study aims to clarify the role of *Nigella sativa* seed extract and LEVA alone /combined in the immune response in male rabbits.

Material and Methods

Experimental Animals

A total of thirty healthy mature adult New Zealand male rabbits (four to six months old, weighing 1400g to 155g) and all animals were bred in an animal house (Kut Technical Institute). During the experiment, All groups of animals were free-supplied with a diet and drinking water. The experiment conditions were maintained for animals at a temperature of 23±2 ºC with a 12-hour light cycle from 7:00 to 19:00. The rabbits were allowed to get used to the laboratory conditions for two weeks before the start of the experiment. Each day at 8 a.m., animals were treated.

Extraction of the medicinal herbs

Dried *Nigella sativa* seeds were purchased from a local herb store in Kut City, Iraq. The seeds were tested and certified by the State Board for Seed Testing and Certification (SBSTC) under the Ministry of Agriculture in Iraq. After cleaning the seeds, an electrical grinder was used to turn them into powder. To prepare the powdered seeds, used 70% ethanol (60-80°C) in a soxhlet extraction apparatus to remove the fat (8).

Experimental design

The rabbits were divided randomly after a period of adaptation into two groups in separate cages and given different treatments as follows: The control group (6 rabbits) with free access to food and water and the H_2O_2 group (24 rabbits) which was administered with the ad-libitum supply of drinking water containing 0.5% H_2O_2 (Hydrogen peroxide solution 35% diluted 70 times) (13). After 28 days, stopping H_2O_2. Then, animals of H_2O_2 (24 rabbits) were subdivided into four groups (6 rabbits /group) in separate cages. Group *Nigella sativa* seeds extract (*N.sativa* S.E): This group was administered orally (by gavages needle) (1.5 g/ Kg B.W.) (3). Group levamisole (LEVA): was given orally (by gavages needle) (every 72 hrs.) (5mg /Kg B.W.); a group of animals received *N.sativa* S.E (1.5 g/ Kg B.W.) in addition to LEVA (5mg /Kg B.W.)

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for 28 days and the last group cessation of H_2O_2 administration ($C.H_2O_2$) without additional treatment.

Collection of sample

Blood samples were collected from the marginal ear vein and transferred to plastic tubes. Two types of tubes were used (tubes without anticoagulants were left at room temperature for 20 minutes to allow the blood to clot, while the other tubes containing 10% EDTA solution were used to determine various haematological parameters. A centrifugation process to obtain blood serum at 0, 28, and 56 days of the experiment for measuring serum parameters. Red blood cell counts and white blood cell counts were measured by using the hemocytometer according to the method in (26).

Differential leukocyte counts (DLC): blood smears are most commonly pigmented with May Grünwald-Giemsa-Wright solutions and analysis of the stained blood smears in which numbers of various types of leukocytes are counted per 100 cells in various fields of a smear (34), active T-lymphocyte and Total T- lymphocyte were tested by Erythrocyte-rosette test. Procedure for E. Rosettes: 0.25 ml of the lymphocyte suspension was mingled with 0.25 ml, of 0.5% SRBC and incubated at 37°C for 5 min. The suspension of mixed cells was spun at 200 g for (5 min) and then incubated in ice for 1-2 hr. The liquid above the sediment was removed, and the sediment was carefully remixed by shaking. A tiny amount of the sediment mixture was placed on a slide, covered by a cover slip, and sealed. Then, 200 lymphocytes were counted, and only the lymphocytes that bound more than three sheep red blood cells (SRBCs) were considered positive (11). To assess the phagocytic activity of blood leukocytes, a mixture of 0.1ml fresh heparinized blood (with 5u of heparin per 1ml of blood) and 0.05ml of 2-hydroxyethyl methacrylate particles (MSHP) was incubated for an hour at 37°C with periodic shaking. The phagocytic activity of leukocytes was determined by calculating the percentage of leukocytes that phagocytized three or more MSHP (18). Besides, serum samples were collected for measuring immunoglobulin G (IgG) and immunoglobulin M (IgM) was determined using the commercial IgG and IgM ELISA kits, purchased from Bethyl Laboratories, USA.

Histopathological examination

At the end of the experiment, all rabbits were sacrificed by cervical dislocation to collect spleen tissue. All sections of the spleen in all groups were immediately fastened in aqueous Bouin's solution. They were dehydrated in ascending degrees of ethyl alcohol, cleaned with xylene, and soaked in paraffin wax. Afterwards, all sections were prepared in 5-7 µm thickness and stained with Harri's hematoxylin, eosin (H&E), and Crossmon's stains following Gomori's reticulin method according to (4).

Statistical analysis

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The data was analysed by one–way ANOVA analysis of variance, and then a Student's t-test to compare between means. The values of P less than 0.05 are statistically significant. All results were analyzed by statistical analysis using SPSS software version 16.0 by SPSS Inc.

Results and Discussion

Table (1) showed a significant decrease($P \leq 0.05$) in lymphocyte counts and a significant increase($P \leq 0.05$) in neutrophil count in the H_2O_2 group compared with a control group. After cessation of the H_2O_2 group, *N. sativa* S.E. and *N. sativa* S.E.+LEVA groups showed a decrease($P \leq 0.05$) in the neutrophil count but the lowest reduction($P \leq 0.05$) in the LEVA group compared with the H_2O_2 and C.H_2O_2 groups.

Table (1): The effect of 0.5% H_2O_2, *Nigella sativa* seed extract and Levamisole on differential leukocyte counts (DLC), active T- lymphocytes and Total T-lymphocytes in the control group and different treated groups.

Time	Zero day	28 days		56 days				
Groups / Parameters	Control	Control	H_2O_2	Control	*N.sativa* S.E.	LEVA.	*N.sativa* S.E. + LEVA	C.H_2O_2
Lymphocytes (%)	48.9 ±0.9 A a	47.0±0.1 A a	35.0± 0.8 C c	46.5±0.44 A a	45.0 ±0.5 A a	41.9±0.4 A b	46.8 ±0.7 A a	39.1±0.05 C c
Neutrophil (%)	45.3 ±0.2 B b	42.2±0.7 B b	48.0±0.5 A a	44.1±0.4 B b	41.8±0.1 B b	37.8 ±0.3 C c	43.9 ±0.6 A a	40.0 ±0.4 C c
Eosinophil (%)	2.0 ±0.3	1.99±0.1	2.4 ±0.33	2.0±0.7	2.2 ± 0.4	2.1 ±0.3	2.0 ±0.2	2.3±0.20
Monocyte (%)	7.3 ±0.5 B b	6.8±0.55 B b	9.0 ±0.1 A a	7.0±0.8 B b	8.0 ±0.4 B b	8.2 ±0.6 B b	6.52±0.2 B b	8.0±0.88 B b
Basophil (%)	1.2 ±0.23	1.06±1.01	0.9 ±0.17	1.5±0.1	1.0± 0.1	1.0 ±0.5	1.4 ± 0.3	0.9±0.1
Active T-lymphocyte (%)	38.0 ±0.1 A a	40.0. ±01 A a	16.0 ±0.2 C c	39.0±0.06 A a	29.1±0.6 B b	28.7±0.1 B b	37.9 ±0.9 A a	18.5±0.3 C c
Total T-lymphocyte (%)	41.0±1.1 A a	38±0.99 A a	15.0±2.1 B c	40.7±0.3 A a	34.0±1.1 B b	35.0±1.1 B b	40.4±1.1 A a	17.7±0.1 B c

Values are presented as Mean ± SE (number of animals= 6). Capital letters mean that there is a significant difference within the column between the treatment at the level($P \leq 0.05$), and the different small letters mean that there are differences ($P \leq 0.05$)

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E-ISSN:2958-6178
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between the periods. H_2O_2: hydrogen peroxide, N. sativa S.E.: *Nigella sativa* seeds extract, LEVA: Levamisole, C.H_2O_2: Cessation H_2O_2.

Lymphocyte count, active T lymphocytes and total active T lymphocytes were recorded the highest significance($P\leq0.05$) in the (*N. sativa* S.E.+LEVA) group compared to other treated groups. Monocytes recorded a lower count in stimulation groups compared with the H_2O_2 and C.H_2O_2 groups. Eosinophils and basophils appeared no significant differences($P\geq0.05$) between experimental groups. In the C.H_2O_2 group, the values were higher than in the H_2O_2 group, but the results were not statistically significant ($P\geq0.05$).

Data recorded in Table 2 reveals a significant decrease ($P\leq0.05$) in the mean value of total white blood cells and a decreased platelet count in the H_2O_2 group compared to the control group at 28 days. The results of the co-administration of *N. sativa* S.E.+LEVA groups had high significance ($P\leq0.05$) in the total white blood cells and platelets compared with the H_2O_2 group and other treated groups. Within the time, the results of the C.H_2O_2 group observed no significance ($P\geq0.05$) compared with the H_2O_2 group at the end experiment.

Table (2): The Effect of 0.5% H_2O_2, *Nigella sativa* seed extract and Levamisole on total Leukocytes and platelets in control and different treated groups.

Time	pretreatment	After 28 Day		After 56 Day				
Groups / Parameters	Control	Control	H $_2O_2$	Control	*N.sativa* S.E.	LEVA	*N.sativa* S.E. + LEVA	C.H_2O_2
Total WBC (x10^9cell/L)	9.006 ±0.120 A a	8.907 ± 0.22 A a	3.806 ±0.220 C c	8.003 ±0.997 A a	5.206± 0.200 B b	4.812± 0.203 B b	8.773±0.210 A a	4.00 ±0.1 C c
Platelets (x10^6cell/L)	521.0 ±14.0 A a	488.5 ±88.7 A a	107.0 ± 18.0 D d	459.3 ±26.1 A a	398.0 ± 16.0 B b	270.0 ± 60.0 C c	500.0 ± 20.0 A a	120.4 ± 2.80 D d

Values are presented as Mean ± SE (number of animals 6). Capital letters mean that there is a significant difference within the column between the treatment at the level($P\leq0.05$), and the different small letters mean that there are differences ($P\leq0.05$) between the periods. H2O2: hydrogen peroxide, N. sativa S.E.: Nigella sativa seeds extract, LEVA: Levamisole, C.H2O2: Cessation H2O2.

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Concerning the ratio of N/L in Table 3, the results showed a significant increase (P≤0.05) in the H_2O_2 group while a decrease in the L/M ratio compared to the control group at 28 days. After stimulation groups with the *N. sativa* S.E., LEVA and co-administration *N. sativa* S.E.+LEVA without any suppressed material, the values of L/M ratio showed elevation in the *N.sativa* S.E. and LEVA groups and the highest significance(P≤0.05) was recorded in the *N. sativa* S.E.+ LEVA group while decreased the N/L ratio in the *N. sativa* S.E., LEVA and *N.sativa* S.E. with the LEVA together groups compared to the H_2O_2 and C.H_2O_2 groups. Table 3 also showed that the values of the L/M ratio and N/L ratio had no significance(P≥0.05) in the C.H_2O_2 group compared to the H_2O_2 group at 56 days.

Table 4 presented the results of immunoglobulin's IgG, IgM and phagocytic activity that showed a significant decrease (P≤0.05) in the H2O2 group compared to the rabbits in control at 28 days. The results of the administration of N. sativa S.E. and LEVA together showed high elevation significance (P≤0.05) in IgG, IgM and phagocytic activity compared to other stimulation groups and the C.H2O2 group. Finally, the results of the C.H2O2 group failed to return to natural values at the end of 28 days of the experiment.

Table 3. The Effect of 0.5% H_2O_2, Nigella sativa seeds and Levamisole on Monocyte-to-lymphocyte (L/M%)and Neutrophil-to-lymphocyte percentage(N/L%) in control and different treated groups

Time	Pretreatment	After 28 Day		After 56 Day				
Groups / Parameters	Control	Control	H_2O_2	Control	*N. sativa* S.E.	LEVA	*N. sativa* S.E.+ LEVA	C.H_2O_2
L/M %	0.92 ± 0.2 B b	0.88 ±0.66 B b	1.37 ± 0.6 C c	0.99 ±0.1 A a	0.92 ± 0.2 B b	0.88 ± 0.75 B b	0.94 ± 0.9 A B b	1.21 ± 55 C c
N/L %	6.70 ± 1.8 A a	7.01 ±0.5 A a	3.88 ± 8.0 C c	6.90 ±0.08 A a	5.48 ± 1.25 B b	5.32 ± 0.7 B b	7.17 ± 3.2 A a	4.12 ± 2.5 C c

Values are presented as Mean ± SE (number of animals 6). Capital letters mean that there is a significant difference within the column between the treatment at the level(P≤0.05), and the different small letters mean that there are differences (P≤0.05)

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between the periods. H_2O_2: hydrogen peroxide, N. sativa S.E.: Nigella sativa seeds extract, LEVA: Levamisole, C.H_2O_2: Cessation H_2O_2.

Table 4. Effect of 0.5% H_2O_2, *Nigella sativa* seeds extract and Levamisole on Immunoglobulins (IgG, IgM) and phagocytic activity percentage in control and different treated groups

Time	Pretreatment	After 28 Day		After 56 Day				
Groups / Parameters	Control	Control	H_2O_2	Control	*N. sativa* S.E.	LEVA.	*N. sativa* S.E.+ LEVA	C.H2O2
IgG (mg/dl)	424.4 ±1.47 A a	487.5 ±1.66 A a	148.6 ±1.14 C c	473.2 ±0.22 A a	376.8 ± 2.33 B b	390.6 ±2.48 B a	399.6 ±2.15 A b	150.9 ± 2.5 C c
IgM (mg/dl)	28.00 ±0.19 B a	27.2 ±0.45 A a	10.20 ±0.33 C c	28.5 ±0.4 A b	25.9 ± 0.8 B b	26.60 ± 0.21 B b	33.10 ± 0.84 A a	15.1 0.9± C c
Phagocytic activity %	40.8 ± 0.22 A a	41.2 ±0.1 A a	20.00± 0.32 C c	39.9 ±0.7 A a	32.55 ± 0.41 B b	35.2 ± 0.12 B b	43.4 ± 0.51 A a	27.2 5.5± C c

Values are presented as Mean ± SE (number of animals 6). Capital letters mean that there is a significant difference within the column between the treatment at the level($P \leq 0.05$), and the different small letters mean that there are differences ($P \leq 0.05$) between the periods. H_2O_2: hydrogen peroxide, N. sativa S.E.: Nigella sativa seeds extract, LEVA: Levamisole, C.H_2O_2: Cessation H_2O_2.

The microscopic examination of the spleen tissue in control rabbits showed natural architecture. The lymphatic nodules(splenic follicles) in white pulp were big, intact with eccentric follicular arterioles and contained small and deep basophilic lymphocyte aggregation (Figure A). Many changes appeared in the histopathology of the spleen in the H_2O_2 group, including a progressive lack of white pulp with a relative increment in the red pulp and a loss of difference between white and red pulp. In (Figure B) reticulocytes in the red pulp increased in number and size, and the red pulp was segmented with the volume of hematopoietic cells in the H_2O_2 group increased. Lymph nodules scattered with a decrease in the number of lymphocytes contained enlarged nuclei with loss of chromatin (Figure B). Rabbits treated with LEVA showed minimal histological changes and a clear improvement in the splenic tissue (Figure C). There was a clear difference between the white and red pulp in the group of N.sativa S. E. -treated rabbits (Figure D) and a group of (N. sativa S.E., LEVA) –treated rabbits (Figure E) white pulp had naturalistic cytokine concentration. In contrast, red pulp appears to increase

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P-ISSN: 2410-8863
E-ISSN:2958-6178
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in reticulocytes and macrophages. Part of the spleen sections showed a natural structure for the white and red pulps, similar to the typical control section.

Nigella sativa seeds have strong antioxidant and anti-inflammatory effects that help protect the cells from damage by free radicals (12,19). Levamisole (LEVA) has a sufficient immunostimulant effect on animals exposed to cefotaxime that causes immunosuppression (2,17). Recently many studies applied to search for the potential ameliorative role of natural remedies to minimize immunosuppression which happens after exposure to inhibition external factors or the use of some drugs. According to that, this study was conducted to investigate the potential role of *Nigella sativa* seed extract and levamisole in improving the protective effect of the immune system against H_2O_2-induced oxidative damage in rabbits. The current research demonstrated that daily oral administration of 0.5% H_2O_2 in drinking water for 28 days to adult male rabbits caused immunosuppressant effects manifested by leukopenia, lymphocytopenia, thrombocytopenia, significant decrease in total and active T-lymphocyte percentage, reduction in phagocytic activity, L/M ratio with serum IgG and IgM concentrations and significant elevation in N/L ratio. In the current study, the oral administration of the *Nigella sativa* S.E. and LEVA. concurrently with H_2O_2 exerted significant potent ameliorative against H_2O_2-induced oxidative damage in all endpoints examined. The haematological findings of the current study were in agreement with the research of (13,22,28). After the H_2O_2 oral administration technique, excess ROS-induced lipid peroxidation transformed the immune cells become more susceptible to oxidative damage than other cell types with higher sensitivity to apoptosis and damage to the cell membrane, where receptors for IL-Ks, hormones, and IgG are located, resulting in worse immune response (13).

The spleen is a central site for lymphocyte production, maturation, and storage, and T lymphocytes are key components of adaptive immunity (6). Therefore, histopathological examination of the spleens supported this conjecture by the treated rabbits, revealing obvious degeneration and depletion of lymphoid tissue. The study indicated white pulp hypertrophy and draining of lymphocytes in the red pulp in agreement with other researchers who have noted a decrease in extramedullary hematopoiesis in red pulp with a bump-apoptotic body in the lymphoid follicle (1,30). The results also show the rise of pro-inflammatory cytokines with T cell lymphopenia which can lead to an increase (N/L) ratio and decrease (L/M). The (N/L) neutrophil-to-lymphocyte and (L/M) lymphocyte-to-monocyte percentages are two biomarkers that are utilized to detect systemic inflammation (5, 27).

The highly reactive hydroxyl radical formed by H_2O_2 can oxidize apoproteins and other plasma proteins, resulting in the inhibition of immunoglobulins including IgG and IgM (16). Oxidatively modified proteins on the immune cell

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membrane, due to the increased formation of nitrotyrosine and protein carbonyl, may generate neoantigens and trigger an autoimmune response by stimulating T cells (33). The protein becomes hypersensitive to proteolysis which leads to a decrease in plasma protein, IgG and IgM (27). The current study found a significant decrease in phagocytic activity of the cells in rabbits treated with H_2O_2, resulting in an inhibitory effect upon neutrophil function which undergone priming of activation like phagocytosis, nitric oxide release or due to stress that changes neutrophil function (3). The addition of levamisole may increase guanidine monophosphate (GMP) levels in monocytes and neutrophils, as well as the activity of the hexose monophosphate shunt which stimulates phagocytosis and increases chemotaxis. Also, levamisole increases the production of alpha and beta interferon as well as the secretion of cytokines by macrophages (interleukins 1 and 6) that are essential for the activity of T lymphocytes and also cause an increase in the proliferation of monocytes and lymphocytes in the bone marrow (20,29).

Animals that received *Nigella sative* S.E. plus levamisole showed a great significant protective effect against the hemotoxic and immunotoxic effects of H_2O_2. *Nigella sativa* oil is a highly antioxidant plant compound which contains two major components, nigellone and thymoquinone (TQ)that help protect cells from damage caused by unstable molecules called free radicals. One of the active components of the *Nigella sativa* seed is TQ. The effectiveness of TQ immunotherapy is attributed to its anti-toxin and anti-inflammatory properties. TQ affect cell proliferation, DNA synthesis and the ability to scavenge free radicals therefore, it's classified as an anti-toxin and anti-inflammatory compound (23). The intake of *N. sativa* seeds in any form develops the antioxidant defence capacity in the body. Several studies indicate that exposure cells to *N. sativa* seed extract lead to protection against apoptosis, lipid peroxidation and increased antioxidant enzymes (22). The potential of *N.sativa* seeds extract to reduce the harmful effects of H_2O_2 may explain the activity proteins of *N. sativa S.E.* to increase the production of IL-3 and IL-1 by lymphocytes, as they are processed when cultured with or without an allogeneic cell (29, 32). The results of another study confirmed the increase in the proportion of macrophages, monocytes, T cells and their interleukin-secreting activity with a significant decrease in neutrophils when using volatile oil from *N. sativa* seeds extract (23).

The present study demonstrated that the rabbits treated with *N. sativa* seeds extract increased the phagocytic activity due to the role of *Nigella sativa* in stimulating immune cells (7). Many studies showed vitamins E and C as active components of *Nigella sativa* and necessary antioxidants for

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P-ISSN: 2410-8863
E-ISSN:2958-6178
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maintaining optimal immune function through stimulating interleukin-2 production, T-lymphocyte proliferation and platelet aggregation. Finally, some studies concluded that the deficiency in these antioxidant vitamins occurred due to oxidative stress, leading to decreased immune function (25).

Within weeks of stopping oral H_2O_2, we noticed a slight improvement in all immunological parameters and histological sections, gradually restoring normal immune function. Removing the source of free radicals eliminates the effects of oxidative stress that causes activation of inflammatory genes, lipid peroxidation, cell death and immunosuppression, proinflammatory agents, inflammatory biomarkers decline, and circulatory function improvement (21, 24).

Conclusion

Using *Nigella sativa* seed extracts and levamisole together showed increased significance in the experiment after stopping H_2O_2 as a source of oxidative stress. Therefore, it is beneficial to implement these therapeutic strategies for patients suffering from oxidative stress, as it can provide better value to their treatment. Therefore, it is beneficial to implement these therapeutic strategies for patients suffering from oxidative stress, as it can provide better value to their treatment.

Acknowledgements

The authors thank Kut Technical Institute, Middle Technical University for their support and the provision of necessary facilities.

Conflict of interest

The author states that they do not have any conflicts of interest related to the subject matter being discussed.

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E-ISSN:2958-6178
Vol. 2, NO. 3, September 2024

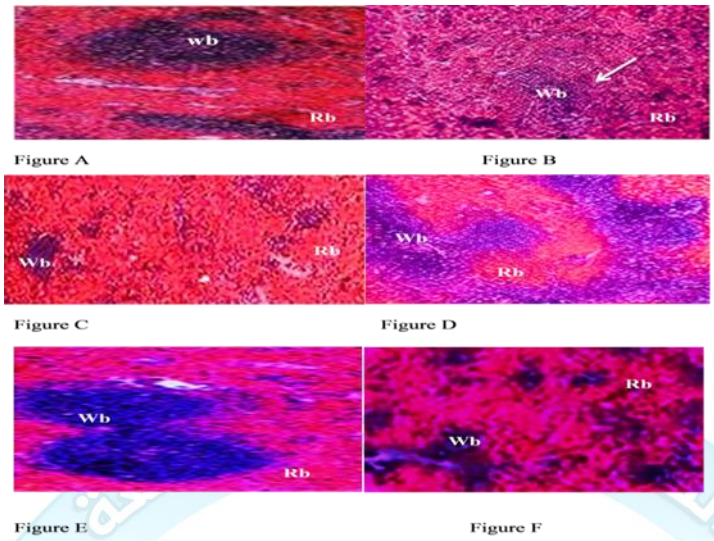

Figure A Figure B

Figure C Figure D

Figure E Figure F

The microscopy of spleen sections of rabbits in different groups (H&E stain X40) (Wb: white bulb, Rb: red bulb) A: Control group showing: Normal spleen architecture. B: The H_2O_2 group displays scattered lymphatic nodules with reduced cellularity. C: LEVA group, D *Nigella sativa*. S.E. group, E: *Nigella sativa*. S.E. + LEVA group that presents a fairly standard white and red pulps structure, group F: The C. H_2O_2 group showed a fairly slight improvement for white and red bulbs.

References

1. Abdelaziz TE, Borai EB, Kamal KH, Hanem EG. Contribution of garlic for improving the cytoprotective effect of mesna against cyclophosphamide toxicity in rats. GSC Biological and Pharmaceutical Sciences. 2019;7(3).
2. Aich N, Ahmed N, Paul A. Issues of antibiotic resistance in the aquaculture industry and its way forward. Int. J. Curr. Microbiol. Appl. Sci. 2018;7(08):26-41.
3. Al-Saaidi JA, Dawood KA, Latif AD. Immunomodulatory effect of Nigella sative seed extract in male rabbits treated with dexamethasone. Iraqi Journal of Veterinary Sciences. 2012 Sep 1;26:141-9.
4. Bancroft JD, Gamble M, editors. Theory and practice of histological techniques. Elsevier Health Sciences; 2008.
5. Berg RE, Forman J. The role of CD8 T cells in innate immunity and in antigen non-specific protection. Current opinion in immunology. 2006 Jun 1;18(3):338-43.
6. Chen Y, Han S, Wang Y, Li D, Zhao X, Zhu Q, Yin H. Oxidative stress and apoptotic changes in broiler chicken splenocytes exposed to T-2 toxin. BioMed research international. 2019 Nov 25;2019.
7. Hamad ZM. Protective effect Ethanol extract of Nigella

Diyala Journal for Veterinary sciences
Open Access Journal Published by College of Veterinary Medicine
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P-ISSN: 2410-8863
E-ISSN:2958-6178
Vol. 2, NO. 3, September 2024

sative. L on hepatic damage induced by naphthalene in male rats. Journal of Al-Qadisiya for Pure Science. 2012;17(2):1-0.

8. Harborne, J.. Textbook of phytochemical Methods. AGuide Modern Techniques of plant Analysis 2nd. (eds.). London. (1984) pp:195- 198
9. Ibrahim HM, Mohammed-Geba K, Tawfic AA, El-Magd MA. Camel milk exosomes modulate cyclophosphamide-induced oxidative stress and immuno-toxicity in rats. Food & function. 2019;10(11):7523-32.
10. Johnstone A, Thorpe R. Immunochemistry In Practice. Blackwell Scientific Publications. Inc., Boston, Massachusetts. 1982:1-332.
11. Jondal M, Holm G, Wigzell H. Surface markers on human T and B lymphocytes: I. A large population of lymphocytes forming nonimmune rosettes with sheep red blood cells. The Journal of experimental medicine. 1972 Aug 1;136(2):207-15.
12. Khan S, Ali M, Albratty MM, Najmi AY, Azeem U, Khan SA, Rather MA. Nigella sative: From chemistry to medicine. InBlack Seeds (Nigella Sative) 2022 Jan 1 (pp. 29-62). Elsevier.
13. Khudair KK. Hydrogen peroxide effects on immune responses (Cellular and Humeral) immunity of adult male rabbits. Iraqi J. Biotech. 2008;7(2):226-38.
14. Kim EK, Jang M, Song MJ, Kim D, Kim Y, Jang HH. Redox-mediated mechanism of chemoresistance in cancer cells. Antioxidants. 2019 Oct 10;8(10):471.
15. Mehkri S, Chandrasagar K, Ashok G, Bopanna K. Evaluation of in vitro phagocytic property of macrophages in the presence of Thymopure™(Nigella sative) oil. International Journal of Biology Research. 2021;6(2):18-21.
16. Melov S. Mitochondrial oxidative stress: physiologic consequences and potential for a role in ageing. Annals of the New York Academy of Sciences. 2000 Jun;908(1):219-25.
17. Mohamed EH, Baiomy AA, Ibrahim ZS, Soliman MM. Modulatory effects of levamisole and garlic oil on the immune response of Wistar rats: Biochemical, immunohistochemical, molecular and immunological study. Molecular medicine reports. 2016 Sep 1;14(3):2755-63.
18. Mojžišová J. CHANGES OF THE IMMUNOLOGICAL AND HAEMATOLOGICAL

Diyala Journal for Veterinary sciences
Open Access Journal Published by College of Veterinary Medicine
University of Diyala, Iraq
P-ISSN: 2410-8863
E-ISSN:2958-6178
Vol. 2, NO. 3, September 2024

PARAMETERS IN RABBITS AFTER THE APPLICATION OF BENDIOCARBAMATE. FOLIA. 2005;49(1):32-5.

19. Mukhtar H, Qureshi AS, Anwar F, Mumtaz MW, Marcu M. Nigella sative L. seed and seed oil: Potential sources of high-value components for the development of functional foods and nutraceuticals/pharmaceuticals. Journal of Essential Oil Research. 2019 May 4;31(3):171-83.
20. Pekmezci D, Cakiroglu D. Investigation of immunomodulatory effects of levamisole and vitamin E on immunity and some blood parameters in newborn Jersey calves. Veterinary research communications. 2009 Oct;33:711-21.
21. Pipe AL, Papadakis S, Reid RD. The role of smoking cessation in the prevention of coronary artery disease. Current atherosclerosis reports. 2010 Mar;12:145-50.
22. Qiao L, Yu J, Dent P, Farrell G. NF-κB protects rat ARL-6 hepatocellular carcinoma cells against hydrogen peroxide-induced apoptosis. Cancer biology & therapy. 2005 Nov 1;4(11):1195-202.
23. Qureshi AS, Rehan S, Enbergs H. Nigella sative seed extract affects granulocyte phagocytosis and lymphocyte proliferation in goats. Pakistan Veterinary Journal. 2017 Jan 1;37:411-4.
24. Sakellariou GK, Jackson MJ, Vasilaki A. Redefining the major contributors to superoxide production in contracting skeletal muscle. The role of NAD (P) H oxidases. Free radical research. 2014 Jan 1;48(1):12-29.
25. Schaffer S, Müller WE, Eckert GP. Tocotrienols: constitutional effects in aging and disease. The Journal of nutrition. 2005 Feb 1;135(2):151-4.
26. Schalm OW, Jain NE, Caroll EJ. Veterinary haematology. 3rd ed. Philadelphia: Lea and Febiger; 1975.
27. Stojkovic Lalosevic M, Pavlovic Markovic A, Stankovic S, Stojkovic M, Dimitrijevic I, Radoman Vujacic I, Lalic D, Milovanovic T, Dumic I, Krivokapic Z. Combined diagnostic efficacy of neutrophil-to-lymphocyte ratio (NLR), platelet-to-lymphocyte ratio (PLR), and mean platelet volume (MPV) as biomarkers of systemic inflammation in the diagnosis of colorectal cancer. Disease markers. 2019 Oct;2019.
28. Su LJ, Zhang JH, Gomez H, Murugan R, Hong X, Xu D,

Diyala Journal for Veterinary sciences
Open Access Journal Published by College of Veterinary Medicine
University of Diyala, Iraq
P-ISSN: 2410-8863
E-ISSN:2958-6178
Vol. 2, NO. 3, September 2024

Jiang F, Peng ZY. Reactive oxygen species-induced lipid peroxidation in apoptosis, autophagy, and ferroptosis. Oxidative medicine and cellular longevity. 2019 Oct 13;2019.

29. Sun A, Wang JT, Chia JS, Chiang CP. Levamisole can modulate the serum tumour necrosis factor- α level in patients with recurrent aphthous ulcerations. Journal of Oral Pathology & Medicine. 2006 Feb;35(2):111-6.
30. Sun EW, Shi YF. Apoptosis: the quiet death silences the immune system. Pharmacology & therapeutics. 2001 Nov 1;92(2-3):135-45.
31. Taalab YM, Ibrahim N, Maher A, Hassan M, Mohamed W, Moustafa AA, Salama M, Johar D, Bernstein L. Mechanisms of disordered neurodegenerative function: concepts and facts about the different roles of the protein kinase RNA-like endoplasmic reticulum kinase (PERK). Reviews in the Neurosciences. 2018 Jun 27;29(4):387-415.
32. Thakur S, Kaurav H, Chaudhary G. Nigella sativa (Kalonji): A black seed of miracle. International Journal of Research and Review. 2021;8(4):342-57.
33. Wang G, Wang J, Ma H, Khan MF. Increased nitration and carbonylation of proteins in MRL+/+ mice exposed to trichloroethene: potential role of protein oxidation in autoimmunity. Toxicol Appl Pharmacol. 2009;237:188–195.
34. Witeska M, Kondera E, Ługowska K, Bojarski B. Hematological methods in fish–Not only for beginners. Aquaculture. 2022 Jan 30;547:737498.