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Some Biochemical, Hemotological, Immunological, And Bacteriological Studies On *Nigella sativa* Oil In Newzealand Rabbits

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ABSTRACT

The oil of seeds of the spice plant *Nigella sativa* (Ranuncluacea) exhibits bronchodilatory, hypotensive, antibacterial and immunopotentiating activities. The fixed oil of *Nigella sativa* (*N.sat.*) was used in the current study to evaluate the immune status after treatment and vaccination of rabbits with *Pasteurella multocida* vaccine, and some toxicologial and chemical studies. Twenty five Newzealand white rabbits were divided into five equal groups, the 1st, 2nd, 3rd, and 4th groups . All animals were twicely vaccinated with *Pasteurella multocida* (formalized and oil adjuvant) vaccine (21 days, apart), the fixed oil of *Nigella sativa* was orally administered with dose levels of 25, 50 and 100 mg oil / kg.b.wt. to the 2nd, 3rd, and 4th groups, respectively once a day, for 42 days. The 5th group served as the negative normal control (non vaccinated, non – treated) and the 1st group served as the positive control (vaccinated, non treated). Clinical biochemicel, haematological and serological studies were carried out after 21 and 42 days of oil treatment (21 days post 1st and 2nd vaccination). Some Micro (trace) and Macro – elements were determined in the serum of rabbits at 42 days of oil treatment. No significant toxic effects could be detected by oil treatment in rabbits till 3 week, but moderate hepatotoxicity and nephrotoxicity could be detected at 6-weeks of *Nigella sativa* oil treatment. Based on the significant increase of lymphocyte percent of oil – treated rabbits, the protection of mice with rabbits serum against challenge with the virulent strain of *Pasteurella multocida* and the slight increase of the specific antibody titers in *Nigella sativa*. treated rabbits than that of the positive control rabbits, it could be concluded that *Nigella sativa* oil showed moderate immunostimulant activity against pasteurellosis in rabbits.

INTRODUCTION

Nigella sativa plant belong to family Ranunculaceae, order Renals, grade Dialypetalae, subphylum Dicotyledons and phylum Angiospermae *(1)*. All the *Nigella sativa* constituents (Volatile and fixed oils) can be considered as moderately toxic (LD_{50} ranged from 616.6 to 3371 mg/kg. B. wt.), the volatile oil more toxic than the fixed oil due to the presence of thymoquinone compound which may induce minimal neurological dificit *(2)*. On the other side, *Nigella sativa* oils has many beneficial effects made it ready for using in the traditional medicine, that it decreased the lipid peroxidation and liver enzymes and increase the antioxidant defence system activity in carbon tetrachloride poisoning *(3)*. The seeds of *Nigella sativa* (black seeds or black cumin) was used in folk (herbal) medicine in human for treatment and prevention of number of diseases include diarrhoea, asthma and dyslipidaemia. The seeds contain both fixed and volatile oils, proteins and saponin. Much of the biological activity of the seeds may be due the thymoquinone (the major compound in the volatile oil, which also present in the fixed oil) *(4)*.

The acute toxicity of *Nigella sativa* fixed oil is low (oral LD50 in mice is 28.8ml/ kg. B.wt.) suggesting a wide margin of safety for therapeutic doses of the fixed oil *(5)*. The seeds of *N. sativa* have antiinflammatory, analgesic, antipyretic, antimicrobial, antineoplastic antihypertensive *(4)* and hypoglycaemic activities *(2)*.

The immunostimulant activities of *Nigella sativa* were recorded against Brucella melitensis Rev-1 *(6)*, and Eimeria vaccines in rats *(7)*, infectious Bursal disease vaccine in

broilers vaccines *(8)*, Reovirus disease and Newcastled disease vaccine in chickens*(9)*.

No available data concerning the relation between the immune status of rabbits vaccinated with *Pasteurella multocida* and their treatment with *Nigella sativa* oil and this is the target of the current study, beside that some haematological, biochemical, and chemical (serum elements) studies were investigated after *Nigella sativa* fixed oil treatment in rabbits.

MATERIAL AND METHODS

Twenty five apparently healthy rabbits (950 gm average weight) were used in the current study. All rabbits were kept under observation for two weeks. The rabbits were divided into five equal groups (5 animal per group), the rabbits of the 1st, 2nd , 3rd and 4th groups were twicely vaccinated with *Pasteurella multocida* formalized and oil adjuvant vaccines (21 days apart) respectively. The 2nd, 3rd and 4th groups were orally administered fixed oil of *Nigella sativa* (Pharco-pharmaceatical. Co.), once a day, for forty two days by the following dose levels: 25, 50 and 100 mg / kg.B.wt respectively. The 1st group served as the positive control (vaccinated, non- treated) and the 5th group served as the negative control (non vaccinated, non treated). Blood samples (without and with heparin anticoagulant) were twicely taken from all rabbits at 21 days post 1st and 2nd vaccinations for biochemical, chemical, serological and haematological studies. The blood used for the following haematological examination: Red Blood corpuscles (RBCS) and whites blood corpuscles (WBCs) counts, haemoglobin (Hb) concentration and differential leucocytic (DlC) count after 1st and 2nd vaccination (except the DLC only after 2nd vaccination) according to schalm *(11)*.

The serum samples of rabbits were used for determinations of some serum constituents at 21th and 42th days (post 1st and 2nd vaccinations respectively) as follows: the activites of aspartic amino transferase (AST) and alanine amino transferase (ALT) enzymes *(12)*, and the concentrations of total bilirubin *(13)*, total protein *(14)*, urea *(15)* and creatinine *(16)*. Also, the serological tests the indirect haemagglutination test *(17)* and the challenge test, (Mouse protection test) *(18)*. Some serum micro (trace) elements iron, cupper, zinc and lead and macroelements sodium, potassium and magnesium were determined using atomic absorption spectroscopy *(19)*. The obtained data were statistically evaluated using t -student test *(20)*.

RESULT

The Serum Micro- and Macro-elements

The group of rabbits administered lower dose (25 mg/kg.B.wt) of *Nigella sativa* fixed oil showed significant decrease of zinc, sodium and potassium than that of control rabbits.

The group of rabbits administered the medium dose level of fixed oil of *N. sativa* (50 mg/kg. B.wt.) showed significant increase of iron and significant decrease of zinc, lead and sodium ions than that of control rabbits.

The group of rabbits that orally administered the higher dose level of N. sativa fixed oil (100 mg/ kg. B.wt) showed significant increase of serum Iron and significant decrease in serum copper, zinc, lead and sodium than that of control rabbits. The serum magnesium did not showed any significant changes between groups of rabbits as shown in Table 1.

Table 1. Some Micro- (trace) and Macro-elements in serum of rabbits (ppm) orally administered with *Nigella sativa* fixed oil for 42 days (21 days post 2nd *Posteurella multocida* vaccination)

Groups	Doses (mg/kg b.wt.)	Iron (Fe)	Copper (Cu)	Zinc (Zn)	Lead (Pb)	Sodium (Na)	Potassium (k)	Magensium (Mn)
1	Positive control	4.045 ±0.282	0.285 ±0.045	0.195 ±0.016	0.120 ±0.009	62.92 ±2.040	16.25 ±1.26	3.485 ±0.104
2	25	Ns 3.630 ±0.091	Ns 0.170 ±0.032	*** 0.065 ±0.005	Ns 0.090 ±0.010	*** 35.60 ±1.65	* 10.62 ±0.89	Ns 3.00 ±0.284
3	50	*** 6.690 ±0.213	Ns 0.325 ±0.042	** 0.115 ±0.010	** 0.070 ±0.008	*** 37.89 ±1.65	Ns 12.37 ±1.44	Ns 3.035 ±0.424
4	100	*** 7.915 ±0.241	* 0.150 ±0.015	*** 0.105 ±0.007	** 0.065 ±0.007	*** 43.05 ±2.059	Ns 11.25 ±1.79	Ns 3.800 ±0.340

*N.B.** = Significant at P ≤ 0.05 ** = Highly significant at P ≤ 0.01
*** = Very highly significant at P ≤ 0.001 Ns = Non – significant.
Tabulated - T = 2.306 (≤0.05)* , 3.355 (≤0.01)**, 5.041 (≤0.001)***

Some serum Biochemical constituents of Rabbits after 21 days of *Nigella sativa* oil treatment:

The all studied serum biochemical constituents such as aspartic amino transferase (AST) and alanine amino transferase (ALT) enzyme activities and the concentrations of serum total protein, total bilirubin, and creatinine showed non – significant changes between the five groups of rabbits, (but the significant increase of both serum urea and creatinine by the higher dose of N. sativa oil (100 mg/kg.B.wt), as shown in Table 2.

Table 2. Some Serum Biochemical Constituents in Rabbits Orally Administered of with *Nigella sativa* fixed oil for 21 day (21 days post 1st vaccination with *Pasteurella multocida* vaccine).

Groups	Doses (mg/kg b.wt.)	Total protein (g/dl)	AST (U/L)	ALT (U/L)	Total Bilirubin (mg/dl)	Urea (mg/dl)	Creatinine (mg/dl)
1	Positive control	6.800 ±0.092	28.500 ±1.523	36.00 ±2.28	0.40 ±0.032	28.700 ±2.884	0.260 ±0.074
2	25	Ns 6.700 ±0.084	Ns 29.00 ±2.059	Ns 37.50 ±2.291	Ns 0.37 ±0.040	Ns 32.42 ±2.727	Ns 0.320 ±0.041
3	50	Ns 6.600 ±0.097	Ns 31.00 ±1.265	Ns 39.00 ±1.789	Ns 0.466 ±0.024	Ns 33.600 ±2.028	Ns 0.700 ±0.071
4	100	Ns 6.900 ±0.111	Ns 28.00 ±2.00	Ns 37.00 ±2.786	Ns 0.37 ±0.017	* 37.700 ±2.216	** 0.70 ±0.086
5	Negative control	Ns 6.500 ±0.127	Ns 28.00 ±1.66	Ns 37.00 ±2.417	Ns 0.300 ±0.058	Ns 28.200 ±2.280	Ns 0.200 ±0.051

*N.B.** = Significant at P ≤ 0.05 ** = Highly significant at P ≤ 0.01
*** = Very highly significant at P ≤ 0.001 Ns = Non – significant.
Tabulated - T = 2.306 (≤0.05)* , 3.355 (≤0.01)**, 5.041 (≤0.001)***

Some serum biochemical constituents of rabbits after 42 days of treatment with *N.sativa* oil

The lower dose (25 mg/kg)of *N. sativa* induced significant decrease of AST enzyme activity and significant increase in serum creatinine concentration.

The medium dose level (50 mg/kg.B.wt.)of *N. sativa* could induce significant increase of ALT enzyme activity and the concentrations of both total bilirubin and creatinine.

The high dose level (100 mg/kg.) of *N. sativa* induced significant increase of ALT enzyme activity and the concentrations of serum total bilirubin, creatinine and urea as shown in Table, 3 .

Table 3: Some serum Biochemical constituents of rabbits orally administered with *Nigella sativa* fixed oil for 42 day (21 days post second *Pasteurella multocida* vaccination).

Groups	Doses (mg/kg b.wt.)	Total protein (g/dl)	AST (U/L)	ALT (U/L)	Total Bilirubin (mg/dl)	Urea (mg/dl)	Greatinine (mg/dl)
1	Positive control	6.800 ±0.091	28.00 ±2.059	22.00 ±2.040	0.320 ±0.026	28.60 ±2.433	0.360 ±0.015
2	25	Ns 6.700 ±0.097	* 18.00 ±2.280	Ns 28.00 ±4.089	Ns 0.370 ±0.022	Ns 32.40 ±2.416	*** 0.700 ±0.058
3	50	Ns 6.68 ±0.185	Ns 28.00 ±2.953	** 34.00 ±2.039	* 0.430 ±0.022	Ns 33.300 ±1.897	*** 0.900 ±0.014
4	100	Ns 7.021 ±0.167	Ns 30.00 ±2.280	** 36.00 ±2.040	** 0.470 ±0.032	* 37.00 ±2.416	*** 0.950 ±0.058
5	Negative control	Ns 6.500 ±0.152	Ns 27.00 ±2.608	Ns 21.00 ±1.265	Ns 0.300 ±0.032	Ns 28.100 ±2.28	** 0.259 ±0.016

*N.B.** = Significant (at P ≤ 0.05) ** = Highly significant (at P ≤ 0.01)
*** = Very highly significant (at P ≤ 0.001) Ns = Non – significant.
Tabulated - T = 2.306 (≤0.05)* , 3.355 (≤0.01)**, 5.041 (≤0.001)***

Haematological study

The studied haematological parameters, RBCs, WBCs and haemoglobin (Hb) concentration of the blood of rabbits did not showed any significant changes at both studied periods (21 or 42 days after 1st and 2nd vaccinations respectively) by the lower dose level geven (25 mg *N. sativa* oil / kg. b.wt.) than that of the positive control rabbits (vaccinated and non treated).

The medium dose (50 mg *N. sativa* oil) could only induced significant decrease of Hb concentration after 42 days (21 days post 2nd, vaccination), and significant increase of WBCs count than that of positive control rabbits at both periods (after 1st and 2nd vaccinations).

At 21th day post 2nd vaccination, only the Hb concentration was significantly decreased, and the count of WBCs was significantly increased by the high dose given (100 mg/ kg.b.w of N. sativa fixed oil) than that of the positive control rabbits, as showed in Table 4.

Table 4. Some haematological parameters of rabbits orally administered *Nigella sativa* fixed oil at 21 and 42 days of treatments (after 21 days post first and second vaccinations).

Groups	Doses (mg/kg b.wt.)	Haemoofobin (Hb) conc. (mg/dl)		Red Blood corpuscles (RBCs) count ($x10^6$)		White Blood corpusdes (WBCs count ($x10^3$)	
		21days post 1st vaccination	21 days post 2nd vaccination	21 days post 1st vaccination	21 days post 2nd vaccination	21 days post 1st vaccination	21days post 2nd vaccination
1	Positive control	9.300 ±0.600	9.300 ±0.632	3.300 ±0.86023	3.440 ±0.244	4.400 ±0.1049	4.600 ±0.1265
2	25	Ns 9.700 ±0.300	Ns 8.100 ±0.468	Ns 3.400 ±0.10198	Ns 3.200 ±0.319	Ns 4.600 ±0.1020	Ns 4.800 ±0.2433
3	50	Ns 9.800 ±0.39	Ns 6.700 ±0.322	Ns 3.400 ±0.116619	Ns 3.500 ±0.305	* 4.800 ±0.1265	*** 6.00 ±0.1649
4	100	Ns 9.100 ±0.63	*** 5.700 ±0.152	Ns 3.200 ±0.63246	Ns 3.100 ±0.204	Ns 4.200 ±0.1020	* 6.100 ±0.2843
5	Negative control	Ns 9.34 ±0.64	Ns 9.4 ±0.316	Ns 3.300 ±0.63246	Ns 3.500 ±0.316	Ns 4.500 ±0.0894	Ns 4.400 ±0.330

N.B. * = Significant at $P \leq 0.05$ ** = Highly significant at $P \leq 0.01$
*** = Very highly significant at $P \leq 0.001$
Ns = Non – significant. Tabulated - T = 2.306 (≤0.05)* , 3.355 (≤0.01)**, 5.041 (≤0.001)***

Differential leucocytic counts

Neutrophil percentages

The all dose levels of *N. sativa* fixed oil induced significant decrease of neutrophil percentage than that of the positive (vaccinated and non treated) control rabbits.

Lymphocyte percentages

Only, the rabbits administered with 25 and 50 mg of *N.sativa* fixed oil / kg. B. wt. could induce significant increase of lymphocyte percentages than that of positive control rabbits.

Monocytes percentages

Only the rabbits administered the medium dose level (50 mg N. sativa oil/ kgb.wt) showed significant decrease of the monocyte percentage than that of the positive control rabbits.

Eosinophils percentages

All dose levels of *N. sativa* oil could not significantly changed the eosinophils percentages than that of positive control rabbits.

Basophils percentages

Only, the rabbits administered the lower dose levels (25 mg N. sativa/ kg.b.wt) significantly showed increased the basophil percentage than that of the positive control rabbits Table 5.

Table 5. The differential leucocytic counts of rabbits orally administered with *Nigella sativa* Fixed Oil for 42 days (21 days post 2nd *Pasteurella multocida* vaccination).

Groups	Doses (mg/kg b.wt.)	Neutrophils (%)	Lymphocytes (%)	Monocytes (%)	Eosinophils (%)	Basophils (%)
1	Positive control	9.52 ±0.894	71.44 ±2.786	9.52 ±1.72	4.76 ±1.166	4.67 ±1.020
2	25	* 5.56 ±0.63	Ns 74.99 ±1.897	Ns 5.56 ±1.02	Ns 2.78 ±0.569	*** 11.11 ±0.632
3	50	*** 3.385 ±0.434	* 83.125 ±1.463	** 3.385 ±0.466	Ns 4.495 ±0.566	Ns 5.61 ±0.424
4	100	* 6.44 ±0.605	* 80.30 ±1.902	Ns 6.63 ±0.984	Ns 3.315 ±0.494	Ns 3.315 ±0.494
5	Negative control	** 22.08 ±2.33	** 54.55 ±2.608	Ns 10.390 ±1.265	** 10.39 ±0.894	Ns 2.59 ±0.305

*N.B.** = Significant at P ≤ 0.05 ** = Highly significant at P ≤ 0.01
*** = Very highly significant at P ≤ 0.001 Ns = Non – significant.
Tabulated - T = 2.306 (≤0.05)*, 3.355 (≤0.01)**, 5.041 (≤0.001)***

The specific antibody titers against both capsular – A and capsular – D antigens of *Pasteurvella multocida* vaccine

There was a slight increase of the specific antibody titers against both the capsular A and the capsular D -antigens of the *Pasteurella multocida* vaccine by all dose levels of the *N. sativa* oil, but this increase was non – significant, Tables, 6 and 7 .

Table 6. The specific antibody titers against capsular - A. antigen of *Pasteurella multocida*) of rabbits twicely vaccinated with *Pasteurella multocida* vaccines and orally administered with fixed oil of *Nigella sativa* for 21 and 42 days.

Groups	Doses (mg/kg b.wt.)	Titers prevaccinations		Titers at 21 days post 1st vaccinations		Titers at 21 days post 2nd vaccinations (42 days)	
		Titers	Log_{10}- values of reciprocalititers	Range	Log_{10} – yalues of reciprocal titers	Range	Log_{10}– values of the reciprocal titers
1	Positive control	¼	0.482 ±0.108	1/844	2.926 ±0.307	1/1372	3.137 ±0.190
2	25	¼	Ns 0.482 +0.066	1/867	Ns 2.938 ±0.307	1/1576	Ns 3.198 ±0.307
3	50	¼	Ns 0.542 ±0.132	1/972	Ns 2.990 ±0.191	1/1872	Ns 3.272 ±0.269
4	100	¼	Ns 0.482 +0.066	1/1117	Ns 3.048 +0.307	1/2560	Ns 3.408 +0.307
5	Negative control	¼	Ns 0.482 ±0.108	¼	*** 0.482 +0.066	¼	*** 0.421 ±0.066

*N.B.** = Significant (at P ≤ 0.05) ** = Highly significant (at P ≤ 0.01) *** = Very highly significant (at P ≤ 0.001)
Ns = Non – significant. Tabulated - T = 2.306 (≤0.05)*, 3.355 (≤0.01)**, 5.041 (≤0.001)***

Table 7. The specific antibody titers (against capsular- D, antigen) of *Pasteurella multocida* in rabbits twicely vaccinated with *Pasteurella multocida* vaccines and orally Administered with fixed oil of *Nigella sativa* for 21 and 42 days.

Groups	Doses (mg/kg b.wt.)	Prevaccinaton Titers		Titers at 21 days post 1st vaccinations		Titers at 21 days post 2nd vaccinations (42 days)	
		Titer	Log_{10}-values of reciprocaltiters	Titer range	Log_{10} – values of reciprocal titers	Titer	Log_{10} – values of the reciprocal titers
1	Positive control	¼	0.602 ±0.085	1/685	2.836 ±0.190	1/1372	3.068 ±0.269
2	25	¼	Ns 0.602 ±0.120	1/827	Ns 2.918 ±0.269	1/1576	Ns 3.198 ±0.307
3	50	¼	Ns 0.542 ±0.101	1/987	Ns 2.994 ±0.307	1/1872	Ns 3.228 ±0.190
4	100	¼	Ns 0.542 ±0.101	1/1119	Ns 3.049 ±0.190	1/2560	Ns 3.258 ±0.269
5	Negative control	¼	Ns 0.602 ±0.085	¼	*** 0.542 ±0.101	¼	*** 0.482 ±0.108

*N.B.** = Significant at P ≤ 0.05 ** = Highly significant at P ≤ 0.01
*** = Very highly significant (at P ≤ 0.001) Ns = Non – significant.
Tabulated - T = 2.306 (≤0.05)* , 3.355 (≤0.01)**, 5.041 (≤0.001)***

Mouse Protection Test (Challenge Test)

The injected serum of all vaccinated rabbits that treated with *N. sativa* oil or the positive control (vaccinated, non – treated) could totally (100%) protected the mice injected with alive virulent strain of *Pasteurella multocida* bacteria. In contrast, the serum of the negative (normal) control rabbits could not absolutely (0.00%) protect the mice challenged with the virulent *Pasteurella multocida* as recorded in Table 8.

Table 8. Mouse protection test (challenge test) on serum of rabbits orally administered *Nigella sativa* fixed oil for 42 days and twicelly vaccinated with *Pasteruella multocida* vaccine (by 0.1 ml of bactera soln of 100 MID50 / mice)

Groups	Doses (mg/kg b.wt.)	Number of mice	Died / survived	Total survived number	Protection percentage (%)	Lesion scores
1	Positive control	4	0/4	4	100%	-
2	25	4	0/4	4	100%	-
3	50	4	0/4	4	100%	-
4	100	4	0/4	4	100%	-
5	Negative control	4	4/0	0	0.00%	+++

N.B.- = No lesion scores. + = Mild lesion scores.
++ = Moderate lesion scores +++ = Severe lesion scores.

DISCUSSION

The current study on *Nigella sativa* (*N.sat*iva) fixed oil (the main product of its seeds was done in Egypt. The cheapness of seeds or its oil of promote many researchers for using it in human and animal medicine for treatment and prevention of many diseases. The N. sativa seeds or its oils (fixed and volatile) been used as antibacterial, antifungal, anthelmentic, antioxidant. Hypoglcaemic, antiasthmatic, choleretic, galactopoietic (Lactagouge), analgesic, antiinflammatory, antispasmodic, antihypertensive and immunostimulant activities *(21)*. Many of these works achieved mostly in human, so we try to find a role of *N. sativa* in animal health, especially its effect on immune status after vaccination of rabbits against pasteurellosis (the most dangerous disease in rabbit production), beside that some toxicolagical and haematological, studies and some blood elements were determnied for evaluation its use in animals.

Concerning the effect of *N. sativa* oil on the immune status against *Pasteurella multocida* vaccination, the present study revealed that the *N. sativa* treated rabbits showed significant increase of lymphocyte percent than that of the positive control rabbits especially when given at dose of 50 and 100 mg /kg. b.wt, oppositely, the neutrophil and monocyte percentages were significantly decreased by the used doses. The gamma globulins (including the specific antibodies were synthesized in the plasma cells which maturated from B-lymphocytes in the spleen, bone marrow and lymph nodes *(22)*, and 20% of the circultating lymphocyte population are B-lymphocytes *(23)*. Corresponding to the increased lymphocyte in *N. sativa* treated rabbits, there was a slight increase of the specific antibody titers against both capsular - A and B antigens of *Pasteurella multocida* as indicated by the indirect haemagglutination test, but this slight increase of the specific antibodies, beside the significant increase of lymphocytes and the significant increase of total leucocyte populations (especially at dose of 50 and 100 mg *N. sativa* oil/ kg. b.wt), could keeping the life of mice that challenged with the virulent strain of *Pasteurella multocida* as indicated by the mouse protection test (challenge test). As the specific antibody increase was non – significant, the resulted immunity may be essentially a result of cellular type of immunity with the help of humoral one such as recorded in chickens *(8 , 23)*.

Concerning the toxicological and biochemical effects of *N. sativa*, fixed oil there were no significant altrations of serum biochemical constituents that determined after 21 days of oil treatment by all doses (except the increase of both urea and creatinine), but after 42 days post treatment there was a significant increase in ALT enzyme activity and the total bilirubin and creatinine concentrations especially at doses of 50 and 100 mg / kg.b.wt which indicating the presence of certain degree of liver and kidney dysfunction. ALT enzyme levels increased essentially with hepatic disease *(25)*. Bilirubin is the bile pigment in which haemoglobin from the damaged red blood corpuscles (RBCs) metabolized through a series of biochemical reactions *(26)*, so that the singnificant increase of bilirubin in *N. sativa* treated rabbits may be attributed to the RBCs hemolysis and this may explain the significant decrease of haemoglobin content of rabbit's RBCs after treatment with N. sativa oil. *N. sativa* fixed oil may induce hepatotoxicty as indicated by increased ALT and bilirubin after 6 weeks of treatment *(27)*. Also, the increased serum creatinine in treated rabbits by 50 and 100 mg kg after 6 weeks of oil treatment is indicative of presence of a certain degree of kidney dysfunction *(28)* . The increased serum creatinine usually does not occur until renal function is substantially impaired, and its levels also not affected by dietary protein in contrast to serum urea, and this nephrotoxicity need further histophathological confirmation.

The present study revealed a significant decrease of serum zinc, copper, lead , sodium and potassium, and significant increase of serum iron, but magnesium did not showed any significant changes. Such results recorded in rat treated with *N. sativa* oil as it has been observed a significant decrease in zinc, potassium and sodium which decreases significantly, but iron increase were also *(21)*. The difference in some serum element concentrations between the two studies perhaps due to the species differences between rats and rabbits or due the differences in mineral contents of the feed taken by each species.

Based on the current study, it could be concluded that the dose levels between 25-100 mg *N. sativa* oil/ kg. b.wt for 3 weeks could be safe from the toxicological point of view, which showed also moderate immunostimulant effect. Also, the immunostimulant effect could be detected after 6 weeks of oil treatment (based on the significant lymphocyte increase, the slight increase of the specific antibody titers and the positive result of the challenge test, but a moderate hepatotoxicity and nephrotoxicity could be detected (especially at a doses of the 50 and 100 mg dose levels).

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الملخص العربى
بعض الدراسات البيوكيميائية والهيماتولوجية والمناعية والبكتريولوجية على زيت الحبة السوداء فى الأرانب النيوزيلاندى

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لزيت الحبة السوداء تأثيرات مختلفة: موسع للشعب الهوائية، مخفض لضغط الدم، مضاد للبكتريا ومنشط للمناعة، وفى هذه الدراسة تم استخدام الزيت الثابت لبذور الحبة السوداء (الكمون الأسود أو حبة البركة) لدراسة تأثيراتها على المناعة فى الأرانب المحصنة ضد ميكروب الباستيريللا ملتوسيدا مع بعض الدراسات السمية والكيميائية الأخرى، ولهذا الغرض تم تقسيم خمسة وعشرون من الأرانب النيوزيلاندية إلى خمسة مجموعات متساوية، وتم تحصين المجموعات: الأولى والثانية والثالثة والرابعة مرتين بلقاح فورمالينى وزيتى على التوالى (٢١ يوم فاصل بينهم) وتم إعطاء أرانب المجموعات الثانية والثالثة والرابعة زيت الحبة السوداء بجرعات ٢٥ ، ٥٠ ، ١٠٠ مجم/كجم وزن حى مرة فى اليوم لمدة ستة أسابيع، أما المجموعة الأولى فاستخدمت كضابط إيجابى (محصن غير معالج بالزيت) والمجموعة الخامسة كضابط سلبى للتجربة (غير محصن وغير معالج بالزيت).

وتم عمل دراسات بيوكيميائية وهيماتولوجية وسيرولوجية ومناعية بعد ٣ ، ٦ أسابيع من العلاج (٢١ يوم بعد التحصين الأول والثانى)، وتم تعيين بعض عناصر السيرم بعد ٦ أسابيع من العلاج.

أوضحت نتائج الدراسة عدم وجود تأثيرات سمية معنوية بعد ٣ أسابيع من العلاج بزيت الحبة السوداء، ولكن بعد ٦ أسابيع من العلاج فتم اكتشاف وجود خلل وظيفى متوسط بكل من الكبد والكلى، وبناء على الزيادة المعنوية فى نسبة الخلايا الليمفاوية وعلى وقاية الجرذان المحقونة بسيرم الأرانب (المعالجة بزيت الحبة السوداء والمحصنة بلقاح ضد ميكروب الباستيريللا ملتوسيدا) وعلى الزيادة البسيطة فى الأجسام المضادة ضد الميكروب فإنه يمكن استنتاج أن لزيت الحبة السوداء الثابت تأثيراً متوسطاً منبهاً للمناعة ضد ميكروب الباستيريللا ملتوسيدا فى الأرانب.