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Immunomodulatory effect of *Nigella sativa* seed extract in male rabbits treated with dexamethasone

J.A.A. Al-Sa'aidi[1]*, Kh.A. Dawood[1], A.D. Latif[2]

[1] Department of Physiology and Pharmacology, Collage of Veterinary Medicine, Al-Qadisiya University,
[2] Department of Pharmacology and Toxicology, Collage of Medicine, Wasit University, Iraq
*Email: jbr20042002@yahoo.com

Abstract

The potent ameliorating effect of ethanol extract of *N. sativa* seed on the immune system has been assessed in dexamethasone-induced immune-suppressed male rabbits. Fifty mature male rabbits were randomly assigned into five equal groups (control and four treated groups). Animals were daily treated, for 42 days, as follow: C: was orally administered with drinking water; T_1: was orally administered with *N.s.*S.E. (1.5 g/ kg, b.w.); T_2: was injected with dexamethasone (2 mg/ kg, b.w., im); T_3: was combined treated concomitantly with *N.s.*S.E. and dexamethasone; T_4: was treated with dexamethasone for 21 days followed by *N.s.*S.E. for 21 days. The results of body weight gain revealed significant increase in T_1 and significant decrease in T_2 among the experimental groups. Submandibular lymph node weights of T_1, T_2 and T_3 were significantly higher than that of C. Kidneys weights in T_2 and T_3 registered significant increase compared with C. Bone weight in T_1 and T_4 groups was significantly higher than that of other groups. Liver weight in T_2 was significantly higher and in T_4 was significantly lower than other groups. Total leucocytes count and lymphocytes, monocytes and eosinophils percentages were significantly decreased in T_1, while showed no significant differences in T_2, T_3 and T_4 groups compared with that of control. Phagocytes activity and bone marrow mitotic index were significantly reduced in T_2 group, while returned to normal in T_1, T_3 and T_4 groups compared with control. Titers of IgM, IgA, C_3, and C_4 showed no significant differences among groups, while IgG titer was increased in T_1 and T_4 and decreased in T_2. On the basis of the results obtained, it can be concluded that the examined extract showed a certain immunomodulating effect. Of the immunological aspects, cellular immunity was potentially ameliorated in intact and dexamethasone-induced immunosuppressed- male rabbits.

Keywords: *Nigella sativa*; Dexamethasone**;** Immunomodulation**.**
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التأثير المناعي الواقي لمستخلص بذور الحبة السوداء في ذكور الأرانب المعاملة بالدكساميثازون

جبار عباس أحمد الساعدي[١] و خيري عبد الله داود[١] و أحمد ظاهر لطيف[٢]

[١] فرع الفسلجة والأدوية، كلية الطب البيطري، جامعة القادسية، [٢] فرع الأدوية والسموم، كلية الطب، جامعة واسط، العراق

الخلاصة

تم تقييم فعالية المستخلص الكحولي لبذور الحبة السوداء في تحسين وظائف الجهاز المناعي لذكور الأرانب المستحدث فيها التثبيط المناعي تجريبياً باستخدام عقار الدكساميثازون. بعد تسجيل الأوزان الابتدائية، تم توزيع ٥٠ أرنباً ذكراً عشوائياً على خمس مجموعات متساوية (سيطرة وأربع معاملات)، جرعت السطرة (C) ماء الشرب، وجرعت المعاملة الأولى (T_1) مستخلص بذور الحبة السوداء (١,٥ غم/ كغم من وزن الجسم)، وحقنت الثانية (T_2) بعقار الدكساميثازون (٢ ملغم/ كغم بالعضل)، وعوملت الثالثة (T_3) بالمعاملتين معاً، وعوملت الرابعة (T_4) بعقار الدكساميثازون لمدة ٣ أسابيع أعقبت ببذور الحبة السوداء لمدة ٣ أسابيع. استمرت التجربة لمدة ٦ أسابيع، سجلت بعدها أوزان الجسم النهائية وسحبت نماذج دم لغرض الدراسة المناعية وفحوصات الدم. أظهرت نتائج الزيادة الوزنية ارتفاعاً معنوياً في معدل مجموعة T_1 وانخفاضا معنوياً في معدل مجموعة T_2 في حين تقاربت معدلات مجموعتي T_3 و T_4 مع معدل السيطرة. وبينت النتائج ارتفاعاً معنوياً قي وزن الغدة اللمفية تحت الفكية لمجموعات T_1 و T_2 و T_3 بالمقارنة مع السيطرة. سجلت أوزان كلى مجموعتي T_2 و T_3 زيادة معنوية مقارنة مع السيطرة. أظهر وزن عظم الفخذ ارتفاعاً معنوياً في مجموعتي T_1 و T_4 بالمقارنة مع المجاميع الأخرى. أما وزن الكبد فقد كان الأعلى معنوياً في مجموعة T_2 والأقل معنوياً في مجموعة T_4 بالمقارنة مع المجاميع الأخرى. كما أظهرت

النتائج ارتفاعاً معنوياً في عدد خلايا الدم الكلي ونسبة الخلايا اللمفية بينما لم تحصل تغيرات معنوية في نسب كل من الخلايا الوحيدة والحمضة في مجموعة T_1 في حين لم يظهر فرق معنوي بينها في مجاميع T_2 و T_3 و T_4 عند المقارنة مع السيطرة. بينت النتائج انخفاض فعالية الخلايا البلعمية والدليل الانقسامي لنخاع العظم معنوياً في مجموعة T_2 التي حقنت بالدكساميثازون بينما أدى مستخلص بذور الحبة السوداء الى اعادة تلك الفعاليات الى طبيعتها في مجاميع T_1 و T_3 و T_4 لتصل مقاربة للسيطرة. لم تتغير مستويات IgM و IgA و C_3 و C_4 بين مجموعات الدراسة بينما ازداد مستوى IgG في مجموعة T_1 و T_4 وانخفض في مجموعة T_2 بالمقارنة مع السيطرة. يستنتج من نتائج الدراسة الحالية أن لبذور الحبة السوداء دوراً في تحسين المناعة وخصوصاً الخلوية منها التي أظهرت تحسناً في ذكور الأرانب السليمة وتلك المعاملة بعقار الدكساميثازون.

Introduction

Adrenocortical steroids used in medicine for their anti-inflammatory and immune suppressive effects such as severe asthma, acquired hemolytic anemia, allergic reactions of all kinds, organ transplant rejection (1,2). Dexamethasone is one of the several glucocorticoids were used experimentally for induce immunosuppression in many cases (3), but are responsible for some of adverse effects that were occur with large doses or prolonged administration which including suppression of the response to infection or injury and reduce function of osteoblast and unwanted side effects on the organ system and metabolic actions (4). Today world is increasingly seeking ways to replace the synthetic drugs with therapeutic power of natural products, the traditional folk medicine had already found the secret of healing in the nature. Medicinal plant have been used for therapeutic purposes since the beginning of civilization (5). *Nigella sativa* (black seed) is one of the most well known plants used in the history of mankind as a medicament or spice. It is an important medicinal herb in many Arabian, Asian, and African countries. It is used as a natural remedy for a variety of illnesses with important role to enhance human immunity (6,7). The seed have combined effects nutritional and medicinal values, by the helping to relieve the current condition, also helps the body build further resistance against future diseases (8). Many studies recorded that black seed can be used as anti tumor, anti-inflammatory, anti-diabetic, anti-oxidant, anti-histaminic, anti-microbial, anti-parasitic, analgesic and immunopotentiating (9,10,11).

Thus, the present study was undertaken to assess the immunomodulatory effect of the crude ethanol extract of *N. sativa* seed in intact and dexamethasone-induces immunosuppressed male rabbits.

Materials and methods

Preparing the Medicinal Herbs and extraction procedure:

Dried seed of *N. sativa* were purchased from the local herbs store in Al-Kut city, Iraq. The seed has been classified by SBSTC (State Board for Seed Testing and Certification, Ministry of Agriculture, Iraq). The seed was completely cleaned and then turned into powder using an electrical grinder. The 1 kg powdered seed was defatted with 70% ethanol (60-80°C) in a Soxhlet extraction apparatus (12). The yields of the extract was found to be 19.20 % w/w.

Medical drug

Dexamethasone sod. phosphate (Biodexasone, Germany) was used (2mg/ kg, b.w., im) for induction of immune- suppression in rabbits (13).

Experimental animals

Male rabbits, weighting 1500- 1600 g, bred in Animal House (College of Vet. Med., Al-Qadisiya Univ., Iraq) were used in the present study. The animals were fed on alfalfa and pellet diet as well as drinking water *ad libitum*. The animals were maintained at 23±2 ºC with lights on for 12 h (700-1900) per day. Before experimentation, rabbits were acclimated for 2 wk, and the experiment began when the rabbits were 90 days old. Each day at 7 am, body weight was recorded and animals were treated.

Methods

Total leucocyte count (x 10^9/ L): according to (14). Differential Leucocytes Count (%): according to (15). Phagocytes activity: according to (16). Bone Marrow Cellularity (Mitotic Index): according to (17). Erythrocytes Rosette Test: according to (18,19). IgG, IgM, IgA, C_3 and C_4 assessment: according to the manufacturer instructions.

Experimental design

Fifty mature male rabbits were randomly assigned to five equal groups (control and four treatment groups); C: (10 male rabbits) were administered drinking water (orally) and injected with normal saline (i.m.) daily for 6 wks. T_1: (10 male rabbits) were administered *N.s.*S.E (1.5g/kg,b.w., orally) (20), and injected with normal saline (i.m.) daily for 6 wks. T_2: (10 male rabbits) were administered drinking water (orally) and injected with dexamethasone Sod. Phosphate (2mg/ kg, b.w., i.m.) daily for 6 wks (13). T_3: (10 male rabbits) were administered *N.s.*S.E (1.5g/kg,b.w., orally) and injected concomitantly with dexamethasone Sod. Phosphate (2mg/kg,b.w., i.m.) daily for 6 wks. T_4: (10 male rabbits) were injected dexamethasone Sod. Phosphate

(2mg/kg,b.w., i.m.) daily for 3 wks and then administered *N.s.*S.E (1.5g/kg,b.w., orally) daily for further 3 wks.

At the end of the experimental period, final body weights were recorded and blood samples were obtained from marginal ear vein for hematological and immunological assays. Then male rabbits were sacrificed. Selected organs (lymph node, spleen, kidney, liver, and femoral bone) were removed and weighted. Femoral bones were used to study bone marrow mitotic activity.

Statistical analysis

All data were analyzed using one way analysis of variance; ANOVA-I and LSD for comparison between the experimental groups. level of 0.05 was considered for significance.

Results

Body weight Gain (g.)

Figure (1) revealed that T_1 male rabbits gained the highest body weight, while T2, T3 and T4 registered the lowest gain compared with that of control.

Organ weights (g./100g,b.w.)

Table (1) show the results of organ weights. Liver of T_2 male rabbits registered the significantly highest weight compared with T_3 and T_4, whereas T_4 registered the lowest weight ($P<0.01$) compared with that of other three groups which showed no significant difference between them. Kidneys in T_2 and T_3 groups registered significantly ($P<0.01$) higher weight than C and T_1 groups. Spleen weight showed no significant differences throughout experimental groups. Lymph node weight of T_1 and T_2 revealed no significant differences between each other, but they were significantly higher ($P<0.01$) than that of control group. Rabbits administered with *N.s.*SE alone (T_1) or with dexamethasone for 6 weeks (T_4) have been recorded the highest ($P<0.01$) bone weight, whereas those treated with dexamethasone (T_2) recorded the lowest mean value in comparison with that of control.

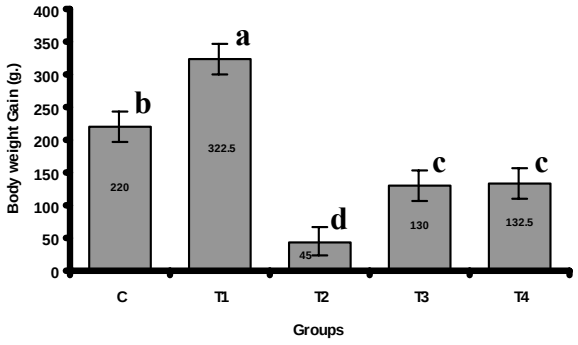

Fig. 1: Effect of *N.s.*S.E. and dexamethasone administration on B. Wt. gain (g.) in mature male rabbits. Values represents M. ± S. E. for 5 rabbits/ group. Different small letters represent significancy at 0.01 level.

Total Leucocytes Counts (× 10^9/ L)

Total leucocytes count shown in table (2) revealed that T_1 and T_3 registered the highest significant ($P<0.01$) mean values and T_2 male rabbits recorded the lowest significant values in comparison with control and T_4 which showed no significant difference when compared with each other.

Table 1: Effects of the *N.s.*S.E. and Dexamethasone on the organs weight in mature male rabbits.

Weight (organ)	Groups of animals				
	C	T_1	T_2	T_3	T_4
Bone weight (g/100g)	1.35 b ±0.06	1.92 a ±0.08	1.05 c ±0.11	1.23 bc ±0.04	1.69 a ±0.04
Lymph nodes weight (g/100g)	0.112 c ±0.002	0.122 b ±0.003	0.124 ab ±0.003	0.13 a ±0.004	0.117 bc ±0.003
Spleen weight (g/100g)	0.050 ±0.001	0.063 ±0.003	0.060 ±0.003	0.061 ±0.003	0.053 ±0.003
Kidney weight (g/100g)	0.62 b ±0.02	0.61 b ±0.01	0.81 a ±0.02	0.76 a ±0.03	0.66 b ±0.03
Liver weight (g/100g)	3.40 a ±0.15	3.24 bc ±0.11	4.08 a ±0.10	3.47 b ±0.21	2.92 c ±0.06

Values represents M. ± S. E. for 5 rabbits/ group, Different small letters represent significancy at 0.01 level.

Table 2: Effects of *N.s.*S.E. and Dexamethasone on total and differential leucocytes count in mature male rabbits.

Leucocyte	Groups of animals				
	C	T_1	T_2	T_3	T_4
Total Leucocyte count ($\times 10^9$/ L)	1451 b ±44.38	1946 a ±81.43	1038 c ±80.41	1971.4 a ±89.09	1535 b ±45.34
Neutrophils %	47.1 c ±0.67	45.6 c ±1.09	67.3 a ±0.8	53.9 b ±1.56	51.6 b ±1.48
Lymphocytes	41.7 b ±0.54	45.1 a ±0.91	27.9 d ±1.00	37.0 c ±0.80	39.4 bc ±0.56
Monocytes %	7.40 a ±0.62	7.3 a ±0.87	3.5 b ±0.52	7.2 a ±0.88	6.0 ab ±0.97
Eosinophils %	3.0 a ±0.42	1.7 ab ±0.42	0.9 b ±0.23	1.6 ab ±0.52	2.5 a ±0.50
Basophils %	0.8 ±0.25	0.3 ±0.21	0.3 ±0.15	0.3 ±0.15	0.5 ±0.17

Values represents M. ± S. E. for 5 rabbits/ group, Different small letters represent significancy at 0.01 level.

Differential Leucocytes Counts

Results clarified in table (2) showed significant increase ($P<0.01$) in neutrophils percentage of T_2, T_3 and T_4 compared with control and T_1 groups. T_2 recorded the highest percentage among the experimental groups. Lymphocytes in T_1 recorded the highest significant percentage whereas T_2 recorded the lowest percentage. Monocytes in T_2 recorded the lowest significant percentage among the experimental groups. Eosinophils in T_2 group recorded the highest significant percentage among the experimental groups.

Phagocytes activity (%)

Phagocytes indices (%) shown in figure (2) revealed that male rabbit of T_4 registered the highs percentage followed by control, T_1, T_3 and T_2, respectively.

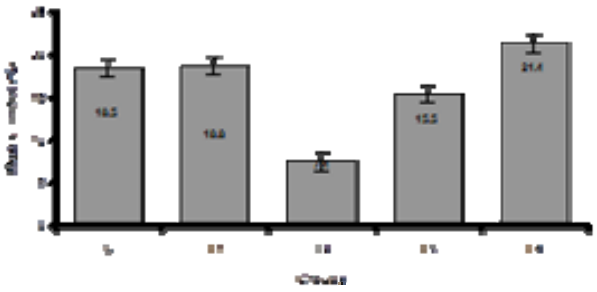

Fig. 2: Effect of *N.s.*S.E. and dexamethasone on Phagocytic activity in mature male rabbits. Values represents M. ± S. E. for 5 rabbits/ group. Different small letters represent significancy at 0.01 level.

Bone marrow mitotic index (%)

Male rabbits of T_1 and T_4 groups registered no significant percentage of bone marrow mitotic activity, whereas T_2 and T_3 groups registered lower significant ($P<0.01$) percentage of mitotic activity compared with that of control (figure 3).

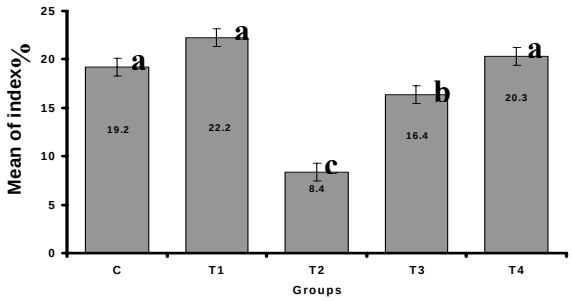

Fig. 4: Effect of *N.s.*S.E. and dexamethasone on bone marrow mitotic index in mature male rabbits. Values represents M. ± S. E. for 5 rabbits/ group. Different small letters represent significancy at 0.01 level.

Erythrocyte Rosette Test

Findings of active E.R.I. revealed that T_2 group recorded significant decrease ($P<0.01$) among the experimental groups. On the other hand, total E.R.I. results showed that T_1 male rabbits recorded the highest mean value, followed by T_3 and T_4 and control which showed no significant differences between each other, whereas T_2 group male rabbits recorded the lowest mean value (figure 6).

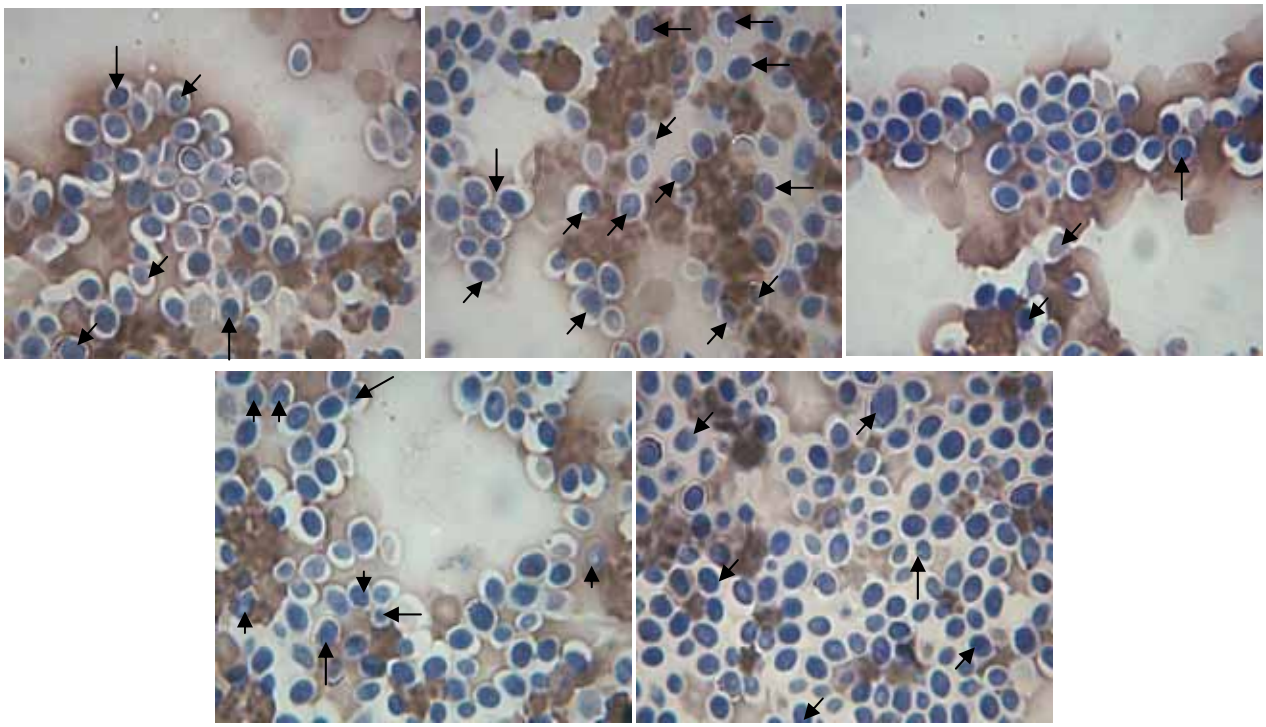

Figure 3: Effect of *N.s.*S.E. and dexamethasone on phagocytes activity in mature male rabbits. Pointed with arrows show that monocytes (blue color) and phagocytes contain yeast (pink-red color).

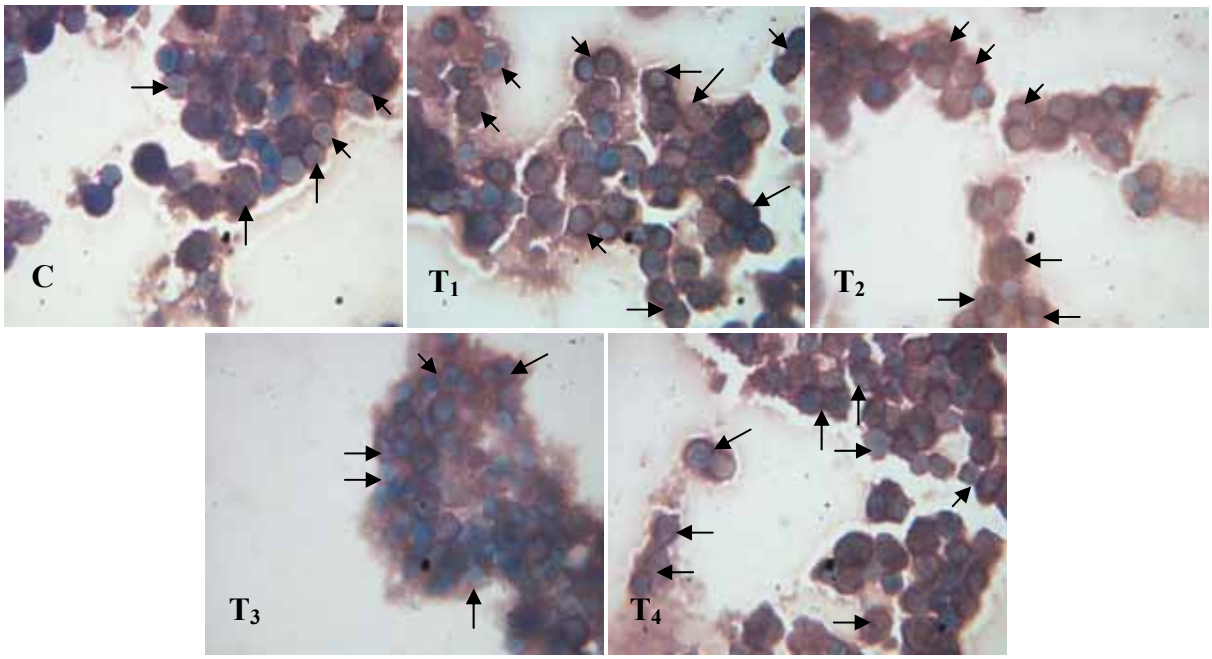

Figure 5: Effect of *N.s.*S.E. and dexamethasone on Bone marrow mitotic index activity in mature male rabbits (pointed with arrows).

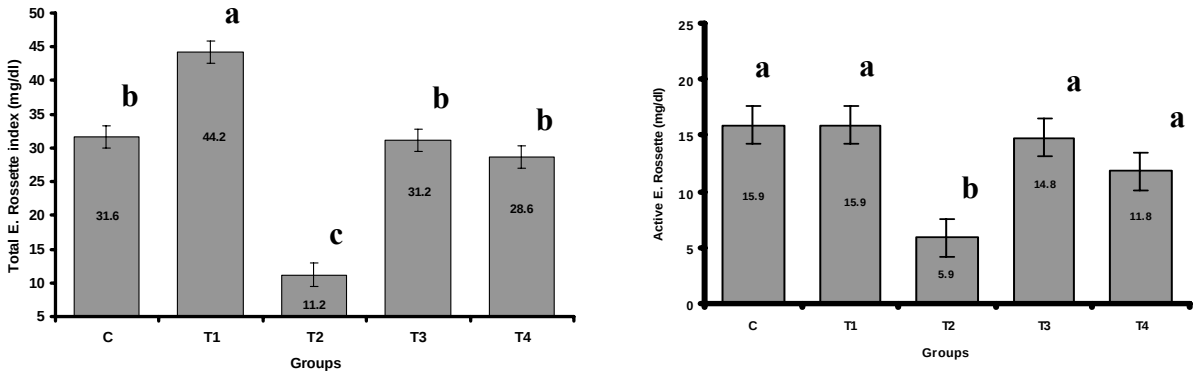

Figure 6: Effect of *N.s.*S.E. and dexamethasone on active and total E.R.I in mature male rabbits. Values represents M. ± S. E. for 5 rabbits/ group. Different small letters represent significancy at 0.01 level.

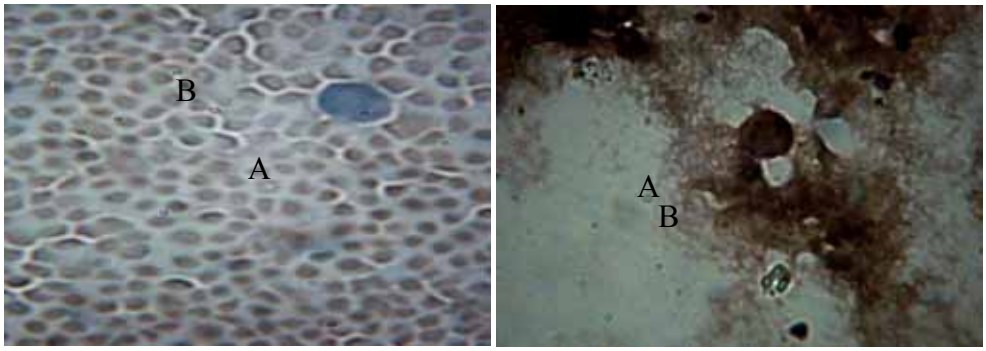

Figure 7: Effect of *N.s.*S.E. and dexamethasone on active and total E.R.I in mature male rabbits. Left (active E. R. test, Trypan blue stain). Right (total E. R. test Giemsa stain). (A) represent monocyte cell (Rabbit) and (B) represent Sheep RBC. Pointed arrow represent *E. rossette* formation.

Immunoglobulin titers (mg/dl)

Results of immunoglobulin titers shown in table (3) revealed significant decrease ($P<0.01$) in IgG titer of T_2 and T_3 male rabbits compared with T_4, whereas that of T_1, T_4 male rabbits recorded no significant differences compared with that of control. On the other hand, IgM and IgA titers recorded no significant differences between groups of the experiment.

Complements titers (mg/dl)

Complement titers (C_3 & C_4) recorded no significant differences ($P>0.01$) among the experimental groups when compared with each other (table 4).

Table 3: Effects of the *N.s.*S.E. and dexamethasone on Immunoglobulin titers (IgG, IgM, IgA) in mature male rabbits.

Groups	C	T_1	T_2	T_3	T_4
IgG	2829.81 a	3113.77a	1909.41c	2311.53b	3072.66a
Titer (mg/dl)	±79.45	±92.78	±71.81	±157.69	±124.38
IgM	7.26	9.68	7.26	4.84	12.1
Titer (mg/dl)	±3.70	±3.95	± 3.70	±3.23	±4.03
IgA	4.7	18.8	9.4	4.7	14.1
Titer (mg/dl)	±4.7	±7.68	±6.27	±4.7	±7.18

Values represents M. ± S. E. for 5 rabbits/ group. Different small letters represent significancy at 0.01 level.

Table 4: Effects of *N.s.*S.E. and dexamethasone on the complements titer (C_3 & C_4) in mature male rabbits.

Groups	C	T_1	T_2	T_3	T_4
C_3	307.68	320.04	304.85	309.24	322.42
Titer (mg/dl)	±6.23	±7.48	±9.43	±8.85	±4.77
C_4	93.24	85.14	78.9	86.82	82.85
Titer (mg/dl)	±3.85	±5.77	±3.99	±4.95	±6.16

Values represents M. ± S. E. for 5 rabbits/ group. Different small letters represent significancy at 0.01 level.

Discussion

Rabbits have been used in the present study as experimental model for mammals, because rabbits are often used as animal model for experimental purposes in human and veterinary researches, such as immunosuppression, as indicated by (3) and (11). Also the present study was conducted by using dexamethasone sodium phosphate as immunosuppressant drug, which may affect cellular and humoral immunity (14), to examine the role of *Nigella sativa* seed extract in ameliorating the adverse effects of the drug. Regarding the safety of *N. s.*, its seeds extract did not produce any adverse effect in the present study, rather it provided potent role. The safety of *N. s.* has been mentioned by other research (21).

Because dexamethasone may indirectly affect the endocrine response (22) through its effect on the satiety center of the hypothalamus as a result of high level of glucose in the glucose sensitive neurons (23), body weight gain was affected in dexamethasone treated groups. On the other hand, dexamethasone may alter the carbohydrate metabolism which may lead to increase glucose utilization (glyconeogenesis) with glucose urea and it's effect in the protein metabolism (anabolism) (conversion of amino acid to protein) was decrease but the catabolism continues or even faster (1). Our results revealed improvement of body weight gain in male rabbits administrated with *N.s.*S.E. with or without dexamethasone. These findings may attributed to the nutritional value of *N.s.* seed, particularly the seed is rich in essential and non essential amino acids, fatty acids and other building blocks for the body such as carbohydrates, protein, fat, vitamins and minerals (24,25,26). It has been shown that effect of *N.s.* seed, as nutritional and medicinal value, may attributed to improving digestion and providing quick energy and increasing body tone (8,27).

Rabbits treated with dexamethasone recorded significant increase in the liver, kidney and lymph node weight. This result was in agreement with (3,28), whom demonstrated the ability of dexamethasone to increase accumulation and degranulation of leukocytes inflammatory focus. This increment may be due to proliferative lesion and hyperplasia in these organs, or due to migration of eosinophils, basophils, monocytes and lymphocytes from circulation to lymphoid tissues. Many researchers demonstrated that chronic administration of corticosteroid may cause bone fragility and osteoporosis (reduction of bone protein matrix) (29,30). Kondo *et al* (31) attributed the decrement of bone weight primarily to the hypofunction and apoptosis, and secondarily to acceleration of bone resorption and stimulation of osteoclastogenesis. Sivagurunathan *et al*. (30) showed that dexamethasone sitmulated osteoclast generation at a pharmacological concentration but did not affect in the span of human osteoclasts and dose- dependently increased signal for osteoclasteogenesis.

*N.s.*S.E. treated groups showed protective action reflected by improvement of organ weights. This result was in agreement with (5,9,14,32) whom reported the potential effects of *N.s.*S.E. against hepatic and renal damage induced by drugs (Like anticancer) and some toxin (Like carbon tetrachloride), as well as its activity in the prevention of liver fibrosis and cirrhosis in rabbits (33). The possible mode of action may be due to its main constituents thymoquinone, nigellone and d-limonene through their antioxidant and anti-inflammatory activities, coupled with enhancement of detoxification processes (34). Ali and Blunden (35) and Naji *et al* (36) attributed the protective activity of *N.s.*S against nephrotoxicity and hepatotoxicty induced by either disease or chemicals to the effect of thymoquinone via antioxidant mechanism.

Male rabbits treated with dexamethasone showed significant decrease in total Leukocytes and percentages of lymphocytes, monocytes and eosinophils with significant increase of neutrophils. This result was in agreement with the study of (14,37) whom reported the inhibitory effect of glucocorticoides in the number and activity of T-lymphocytes and in agreement with (38,39) whom determined the redistribution of lymphocytes labelled with fluorescent isothiocyanate. Jeklova *et al* (3) detected peripheral blood neutrophilia and lymphopenia together with eosinopenia, monocytopenia and basopenia in rabbits after administration of dexamethasone.

These effects may results from the oxidative damage that affect the biological structures. The toxicity- induced pathophysiology of several disease, has been reported to be due to the shift in the balance of the pro-oxidant (Free radicals) and the antioxidant (scavenging) mediators, where pro-oxidant conditions dominate either due to increase generation of free radicals caused by excessive oxidative

stress, or due to the poor scavenging capability in the body (40) that may be lead to decrease in the lymphocytes due to immunosuppressive ability of glucocorticoides in the reduction of T- lymphocyte proliferation via mechanisms that are at least partially the result of inhibition of the T-cell growth factor IL_2 and blocking a cell cycle progression (41).

The activity of the extract to reduce the adverse effects of dexamethasone may attributed to the ability of the *N.s.*S. proteins to enhance the production of IL-3 and IL-1 by lymphocytes, as it has been proved when cultured with or without allogenic cell (40). Haq *et al* (42) reported increase in the macrophage, monocytes and T-cells percentage and its activity to secrete interleukin with significant decrease in the neutrophils when used volatile oil of *N.s.*S. (43) reported that the effects of *N.s.* is due to its biochemical, immunological and pharmacological actions as anti-inflammatory and immunopotentiating through its effects on the DNA synthesis, cell proliferation and the ability of scaveringing superoxide radicals. Corder *et al.,* (44) and Musa *et al.,* (45) showed that exposure of black seed to cell pretreated with cortisol show evidence of protection against the progressive apoptosis, so can play therapeutic roles in reducing anti-inflammatory and anti-oxidant effects with enhancement of detoxification process. Whereas Salem (40) suggested that immune-enhancing effect of *N.s.* on cell-mediated immunity due to its ability to reduce the inflammatory mediators.

Present study reported significant decrease in phagocytic activity of the cells in rabbits treated with dexamethasone which may due to its inhibitory effect upon neutrophil function, particularly those have undergone priming of activation like phagocytosis or nitric oxide release (46) or due to stress that alters neutrophil function (3). Male rabbits treated with *N.s.*S.E. reported increment in the phagocytic activity due to the role of *N.s.* in stimulating the immune cells and increase the activity of immune potential (5). Antioxidant and anti-inflammatory activity of the *N. s.* may protect the phagocytic cell (40,47) or by the effect of *N.s.* on the DNA synthesis during cell proliferation and the ability of scavenging the super oxide radicals (45).

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