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

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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; T1: was orally administered with N.s.E. (1.5 g/kg, b.w.); T2: was injected with dexamethasone (2 mg/kg, b.w., i.m); T3: was combined treated concomitantly with N.s.E. and dexamethasone; T4: was treated with dexamethasone for 21 days followed by N.s.E. for 21 days. The results of body weight gain revealed significant increase in T1 and significant decrease in T2 among the experimental groups. Submandibular lymph node weights of T1, T2 and T4 were significantly higher than that of C. Kidneys weights in T3 and T4 registered significant increase compared with C. Bone weight in T1 and T3 groups was significantly higher than that of other groups. Liver weight in T1 was significantly higher and in T2 was significantly lower than other groups. Total leucocytes count and lymphocytes, monocytes and eosinophils percentages were significantly decreased in T1, while showed no significant differences in T3, T2 and T4 groups compared with that of control. Phagocytes activity and bone marrow mitotic index were significantly reduced in T2 group, while returned to normal in T1, T3 and T4 groups compared with control. Titer of IgM, IgA, C3, and C4 showed no significant differences among groups, while IgG titer was increased in T3 and T4 and decreased in T2. 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 الواثق لمستخلص بذور الحبة السوداء في ذكور الأرانب المعاملة بالدكساميثازون

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الخلاصة

تم تقييم فعالية المستخلص الكحولي لبذور الحبة السوداء في تحسين ظروف الجهاز المناعي لذكور الأرانب المستخدمة فيها التنبيط المناعي تجريبياً باستخدام مستخلص الككساميثازون. بعد تسجيل الأوزان الإبدائية، تم توزيع 50 أرنبًا نكرًا عشوائياً على خمس مجموعات متساوية (سيطرة وأربع مجموعات) ماء الشرب، وجربت المعلمة الأولى (T1) مستخلص بذور الحبة السوداء (1,5 غ/كم من وزن الجسم)، وحقت الثانية (T2) بقار الككساميثازون (2 مل/كم بالعسل)، وغرونت الثالثة (T3) بالعاملتين معاً، وعملت الرابعة (T4) بقار الككساميثازون لمدة 3 أسابيع أقيمت بذور الحبة السوداء لمدة 3 أسابيع. استمرت التجربة لمدة 6 أسابيع، سجلت بعدها أوزان الجسم النهائية وسجبت نماذج دم لغرض الدراسة المناعية وفحصها. أظهرت نتائج الزيادة الوزارية ارتفاعاً معنويًّا في معدل مجموعة T1 و T2 و T3 معنويًّا في معدل مجموعة T4 والاختلاف معنويًّا في معدل مجموعة T1 و T2 و T3 ومعنويًّا في معدل مجموعة T4 بمقارنة مع السيطرة. سجلت أوزان كلي بمقارنة مع السيطرة. أظهرت نتائج الزيادة معنويًّا مع السيطرة. أظهرت نتائج ارتفاعاً معنويًّا في مجموعتي T1 و T2 في مقابلة مع السطوة. أظهرت نتائج ارتفاعاً معنويًّا في مقابلة مع السطوة. أما وزن الكبد فقد كان الأعلى معنويًّا في مجموعة T2 والألق معنويًّا في مجموعة T4 بمقارنة مع المجموعات الأخرى. كما أظهرت
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 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 immuno modulatory 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., i.m) 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 10⁹/ 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, C₁ and C₄ assessment: according to the manufacturer instructions.

Experimental design

Fifty mature male rabbits were randomly assigned to five equal groups (control and four treatment groups); C₁: (10 male rabbits) were administered drinking water (orally) and injected with normal saline (i.m) daily for 6 wks. T₁: (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. T₂: (10 male rabbits) were administered drinking water (orally) and injected with dexamethasone Sod. Phosphate (2mg/ kg, b.w., i.m) daily for 6 wks. T₃: (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. T₄: (10 male rabbits) were injected dexamethasone Sod. Phosphate...
they were significantly higher (P<0.01) than that of control group. Rabbits administered with N.s.S.E alone (T_1) or with dexamethasone for 6 weeks (T_3) 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 significance 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.

<table>
<thead>
<tr>
<th>Weight (organ)</th>
<th>Groups of animals</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>C</td>
</tr>
<tr>
<td>Bone weight (g/100g)</td>
<td>1.35 b</td>
</tr>
<tr>
<td>±0.06</td>
<td>±0.08</td>
</tr>
<tr>
<td>Lymph nodes weight (g/100g)</td>
<td>0.112 c</td>
</tr>
<tr>
<td>±0.002</td>
<td>±0.003</td>
</tr>
<tr>
<td>Spleen weight (g/100g)</td>
<td>±0.001</td>
</tr>
<tr>
<td>±0.003</td>
<td>±0.063</td>
</tr>
<tr>
<td>Kidney weight (g/100g)</td>
<td>0.62 b</td>
</tr>
<tr>
<td>±0.02</td>
<td>±0.01</td>
</tr>
<tr>
<td>Liver weight (g/100g)</td>
<td>3.40 a</td>
</tr>
<tr>
<td>±0.15</td>
<td>±0.11</td>
</tr>
</tbody>
</table>

Values represents M. ± S. E. for 5 rabbits/ group. Different small letters represent significance at 0.01 level.
Table 2: Effects of N.s S.E. and Dexamethasone on total and differential leucocytes count in mature male rabbits.

<table>
<thead>
<tr>
<th>Leucocyte</th>
<th>Groups of animals</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>C</td>
</tr>
<tr>
<td>Total Leucocyte count (× 10⁹/L)</td>
<td>1451 b</td>
</tr>
<tr>
<td>Neutrophils %</td>
<td>±0.67</td>
</tr>
<tr>
<td>Lymphocytes</td>
<td>±0.54</td>
</tr>
<tr>
<td>Monocytes %</td>
<td>7.40 a</td>
</tr>
<tr>
<td>Eosinophils %</td>
<td>±0.42</td>
</tr>
<tr>
<td>Basophils %</td>
<td>±0.25</td>
</tr>
</tbody>
</table>

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 T₂, T₃ and T₄ compared with control and T₁ groups. T₂ recorded the highest percentage among the experimental groups. Lymphocytes in T₁ recorded the highest significant percentage whereas T₂ recorded the lowest percentage. Monocytes in T₂ recorded the lowest significant percentage among the experimental groups. Eosinophils in T₂ group recorded the highest significant percentage among the experimental groups.

**Phagocytes activity (%)**

Phagocytes indices (%) shown in figure (2) revealed that male rabbit of T₂ registered the highest percentage followed by control, T₁, T₃, and T₂, respectively.

**Bone marrow mitotic index (%)**

Male rabbits of T₁ and T₄ groups registered no significant percentage of bone marrow mitotic activity, whereas T₂ and T₃ groups registered lower significant (P<0.01) percentage of mitotic activity compared with that of control (figure 3).

**Erythrocyte Rosette Test**

Findings of active E.R.I. revealed that T₂ group recorded significant decrease (P<0.01) among the experimental groups. On the other hand, total E.R.I. results showed that T₁ male rabbits recorded the highest mean value, followed by T₃ and T₄, and control which showed no significant differences between each other, whereas T₂ 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. rosette formation.

Immunoglobulin titers (mg/dl)

Results of immunoglobulin titers shown in table (3) revealed significant decrease (P<0.01) in IgG titer of T2 and T3 male rabbits compared with T1, whereas that of T1, T4 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 (C3 & C4) 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.

<table>
<thead>
<tr>
<th>Groups</th>
<th>C</th>
<th>T1</th>
<th>T2</th>
<th>T3</th>
<th>T4</th>
</tr>
</thead>
<tbody>
<tr>
<td>IgG (mg/dl)</td>
<td>2829.81 a</td>
<td>3113.77a</td>
<td>1909.41c</td>
<td>2311.53b</td>
<td>3072.66a</td>
</tr>
<tr>
<td>Titer (mg/dl)</td>
<td>±79.45</td>
<td>±92.78</td>
<td>±71.81</td>
<td>±157.69</td>
<td>±124.38</td>
</tr>
<tr>
<td>IgM</td>
<td>7.26</td>
<td>9.68</td>
<td>7.26</td>
<td>4.84</td>
<td>12.1</td>
</tr>
<tr>
<td>Titer (mg/dl)</td>
<td>±3.70</td>
<td>±3.95</td>
<td>±3.70</td>
<td>±3.23</td>
<td>±4.03</td>
</tr>
<tr>
<td>IgA</td>
<td>4.7</td>
<td>18.8</td>
<td>9.4</td>
<td>4.7</td>
<td>14.1</td>
</tr>
<tr>
<td>Titer (mg/dl)</td>
<td>±4.7</td>
<td>±7.68</td>
<td>±6.27</td>
<td>±4.7</td>
<td>±7.18</td>
</tr>
</tbody>
</table>

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 safety 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 stimulated osteoclast generation at a pharmacological concentration but did not affect in the span of human osteoclasts and dose- dependently increased signal for osteoclastogenesis.

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 hepatotoxicity 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 glucocorticoids 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 lead to decrease in the lymphocytes due to immunosuppressive ability of glucocorticoids 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. 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. (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 scavenging 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).

References