Clinicopathological, hematological and immunological studies on the effect of Nigella sativa-L extract on rabbit hemorrhagic disease vaccinated rabbitries in Damietta Governorate

By

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SUMMARY

Rabbit hemorrhagic disease (RHD) attacked worldwide countries in the last few decades, including Egypt causing severe economic losses where mortality reached 80-100%. Vaccination programs failed to control of disease spread completely.

In this study we tried to assure the immunogenic and protective effects of Nigella sativa and utilizing it in improving immune response in vaccinated and infected rabbits. The experiment was designed to include 40 rabbits, divided into 4 groups each of 10 individuals. The 1st group was kept as normal vaccinated control group, the 2nd group was experimentally infected with identified RHD virus, the 3rd group was vaccinated and Nigella sativa chloroform extract and the 4th group was vaccinated, Nigella sativa treated and experimentally infected with isolated and identified RHD field virus.

Differential leucocytic count studies showed marked lymphopenia and neutropenia in infected group, which were significantly improved by Nigella sativa treatment in groups 3 and 4.

Biochemical studies showed significantly increased liver transferases (AST, ALT and GGT) in experimentally infected group (G2) indicating severe liver damage, these changes returned towards normal levels in Nigella sativa treated groups (G3 and G4) indicating hepatoprotective effect Total proteins and albumin concentrations showed non significant change in group 3, while total protein was significantly increased in group 4 accompanied with significant increase in globulin, also globulin was significantly increased in group 3 accompanied with decreased albumin/globulin ratio (A/G), indicating immunostimulating effect of Nigella sativa.

Immunological studies performed using ELISA test results showed both immunostimulating effect of Nigella sativa extract treated groups and immunosuppressive effect of RHDV experimental infection. Cell mediated immunity, in Nigella sativa treated groups (G3 and G4), was sig-
nificantly increased as showed in increased percentage of rosette shape formation of macrophages engulfing neutral red granules.

**Pathological studies:** Experimentally infected rabbits showed acute signs of the disease 2 days post infection, and on necropsy showed pale liver, congested spleen and kidneys with petichial hemorrhage and accumulation of intra-abdominal blood clots. Histopathological studies showed acute necrotic hepatitis accompanied with disseminated intravascular coagulopathy (DIC), which is a pathognomonic process.

In conclusion, at a practical level it is recommended to use the natural plant extract of Nigella sativa for immunostimulation for better rabbit farming.

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**INTRODUCTION**

Rabbit Hemorrhagic Disease (RHD) caused by rabbit hemorrhagic disease virus, a calicivirus, was first recorded in 1984 when a major epidemic syndrome in domestic rabbits in China. Rabbit calicivirus is a highly contagious virus, transmitted by direct contact with infected rabbits or indirectly by contact with objects contaminated with virus. Morbidity is often near 100% and mortality 60-90%. Infection results in a peracute febrile disease causing hepatic necrosis, enteritis, and lymphoid necrosis followed by massive coagulopathy resulting in hemorrhages in a variety of organs (Xu *et al.* 1986 and Xu and Chan 1988).

RHD damages the liver, intestines, and lymphatic tissue and causes terminal massive blood clots. The incubation period is about 24 to 48 hours. Predominantly, young adult and adult rabbits die suddenly within 6 to 24 hours of the onset of fever with few clinical signs. Fever may be as high as 105 °F (40.5 °C) but often is not detected until rabbits show terminal clinical signs. Most animals appear depressed or reluctant to move in the final hours and may show a variety of neurologic signs, including excitement, incoordination, paddling, and opist-
hotonos (abnormal position of the head due to spasms of the muscles at the top and back of the neck). Some affected rabbits may have a foamy nasal discharge. The death rate for RHD ranges from 50 to 100 percent (Xu and Chan 1988).

Necropsy revealed the marked hypertrophy of the thymus with numerous petechiae, hemorrhagic pneumo-tracheitis as well as hypertrophy and degeneration of the liver. Histopathology mainly showed lesions of necrotic hepatitis. Nigella sativa [NS], or 'black cumin', an annual herb belonging to the family Ranunculaceae, has strong immunomodulatory and interferon-like activity.

The diagnosis of RHD is mainly based on observation of necropsy findings and identification of the virus in the liver by hemagglutination with human type O erythrocytes and ELISA test (Liu et al., 1984).

In Egypt many outbreaks were recorded in several provinces with severe economic losses and mortality rate reached 100% (Ghanem and Ismail, 1991).

**Nigella sativa L.** (Ranunculaceae) is a grassy plant with green to blue flowers and small black seeds, which grows in temperate and cold climate areas. Seeds contain 1.5% volatile oil, while 37.5% Non volatile oil. In addition to this Albumen, Sugar, Organic acids, Glucoside Melanthin, Metarbin. Also thymoquinone, dithymoquinone carvacol and anethole 4-terpinole are found (Worthen et al., 1998; Bruits and Bucar, 2000). It improved the T-cells activity (El-Kadi and Kandil, 1987) and also prevents the decrease in hemoglobin level and leukocytes counts caused by cisplatin (Nair et al., 1990). Thymoquinine has shown antioxidant and immunomodulator effects on macrophage, Th1 and Th2 (Salem, 2005).

**MATERIAL AND METHODS**

1-Experimental design: A total of 40, clinically healthy, Bouscat rabbits with age range about 4 months were divided into 4 groups, each of 10 individuals, under strict hygienic and isolation conditions, as follows:

- **Group 1 (G1):** kept as normal RHD, intramuscularly, vaccinated control group.
- **Group 2 (G2):** kept as non vaccinated group and experimentally infected with isolated RHD field virus (strict isolation measures were taken to avoid spread of the infection).
- **Group 3 (G3):** kept as intramuscularly RHD vaccinated group and intramuscularly injected with Nigella sativa L seed extract.
- **Group 4 (G4):** kept as RHD vac-
vaccinated group and intramuscularly injected with Nigella sativa L seed extract followed by experimental infection with isolated RHD field virus, one week post last vaccination (strict isolation measures were taken to avoid spread of the infection).

2-Vaccination: Sera of all groups of apparently healthy rabbits were checked with hem agglutination inhibition test to assure seronegativity for RHD infection (HI <10) and then groups 1, 3 and 4 were vaccinated with a dose of 0.5 ml injected into the fore back, subcutaneously, with oil adjuvant inactivated RHD virus (2^{11} HAU per dose). The vaccine was produced by LABORATORIOS HIPRA, S. A., SPAIN, under a commercial name CUNIPARVAC-RHD. The 2nd group was kept as non vaccinated experimentally infected group.

3-Nigella sativa L seed extract: 600 g of ground black seed in 1500 ml chloroform were incubated in 25°C for one week, during which vibration was carried out up to 5 times a day. The resultant solution was filtered. In order to obtain a completely dry extract, the resultant extracts were transferred to glass dishes and were left in a 50°C oven for 24 hour. Then, they were left at 40°C until assessment of their antimicrobiological activities. (Mashhadian and Rakhshandeh, 2005).

4-Preparation of samples: From field, RHD suspected to be infected rabbits, of about 4 months age, submitted to Damietta Veterinary research Laboratory for examination, with symptoms of mucoid bloody nasal discharge and mucoid diarrhea and post mortem lesions of congested internal organs with clotted blood in the abdominal cavity.

5-Virus isolation and identification: 20 grams of liver tissue samples were homogenized with phosphate buffer saline solution (pH 7.2) for 15 minuets, then clarified by centrifugation at 4000 x g for 15 minuets, the supernatant was filtered through 0.22 µm filter, antibiotic was added and the supernatant was kept at -70°C.

A- Hemagglutination test: The suspected virus in the supernatant was identified as an antigen of RHD virus by hemagglutination against human erythrocytes type (O) at a titer of 1024 (Chasey et al., 1995).

B- ELISA test: Hyperimmune serum was prepared by vaccination of healthy rabbits with alive RHD vaccine (Cunical, Rhone-Merieux, France) intramuscularly and boosterized twice at 2 weeks intervals. The serum was collected and stored at – 7. The prepared hyperimmune serum of RHDV is
used as positive control. ELISA test procedure was conducted according to Cluet et al. (1995).

6-Experimental infection: Rabbits were inoculated subcutaneously and intranasally each with 0.5 ml of the prepared virus supernatant filtrate, one week post last vaccination (Chasey et al., 1995).

7-Sampling: Whole blood with EDTA anticoagulant, sera, peritoneal exudates and livers were collected for hematological, biochemical, immunological and histopathological studies, respectively, three days post last infection in experimentally infected group and one week post vaccination and Nigella sativa extract, treatment.

8-Hematological studies: Blood smears were prepared and stained with May-Grünwald-Giemsa stain. Differential leucocytic count was done using four field-meander method according to Schalm et al. (1991).

9-Serum biochemistry: All serum samples were examined for gamma glutamyle transferase (GGT) activity according to (Henry, 1974), Aspartate aminotransferase (AST) and Alanine aminotransferase (ALT) were estimated according to Reitman and Frankel (1957). Total protein (g/dl) according to Peter (1968). Serum albumin (g/dl) according to Drupt (1974). Serum globulin (g/dl) was estimated by the difference between total protein and albumin while albumin/globulin ratio (A/G) ratio was calculated mathematically according to Kaneko (1996). Parameters were estimated using standard kits and procedures according to instruction supplied by Bio-merieux, France.

10-Phagocytic cells percentage: The macrophage cell mediated immunity was studied for examining the ability of living macrophage to engulf neutral red particles forming a characteristic rosette of red granules (Nelson, 1969).

A- Reagents: Neutral red The stain was formulated as 1% neutral red (Adwic – Prolabo, France) in normal saline, used for staining and counting macrophage in PE-cells.

B- Procedure: 1- one part of 1 % neutral red stain in normal saline was added to 9 parts of peritoneal exudates cell suspension.

2- The mixture was left for 10 min to 15 min, and then the number of macrophage was counted. The percentage of phagocytic cells was determined depending on the rosette shape using lens 40 (Cohn and Wiener, 1963).

11-Pathological studies:
Rabbits were sacrificed and gross pathological lesions were recorded. Livers were preserved in buffered formalin saline (10%), paraffin wax blocks were proc-
essed, sectioned and stained with Haematoxylin & Eosine stain and examined microscopically (Carlton and McGalvin, 1995).

12-Statistical analysis: Data obtained were statistically analyzed by analysis of variance (ANOVA) using SPSS computer software, version 6.0.

RESULTS

Studied experimentally infected group (G2), differential leucocytic count of examined rabbits revealed lymphocytopenia and neutropenia, (50.7 ± 5.78 and 31.4 ± 2.93 respectively) these decreases were significant when compared with the other experimental groups, G1 (57.1 ± 2.82 and 34.1 ± 2.82), G3 (60.1 ± 4.82 and 37.2 ± 2.81) and G4 (63.2 ± 4.62 and 32.3 ± 3.1). Also infected group showed significant increase in monocytic percentage (15.7 ± 1.92) compared with the other test groups G1 (7.4 ± 0.92), G3 (2.3 ± 0.24) and G4 (2.5 ± 0.12).

Vaccinated Nigella sativa treated group (G3) and vaccinated, Nigella sativa treated and experimentally infected group (G4) both showed significant increase in both lymphocytic and neutrophilic percentages (60.1 ± 4.82 and 37.2 ± 2.81) and (63.2 ± 4.62 and 32.3 ± 3.1), respectively, in comparison with all test groups, this increase, in both groups, was accompanied with significant monocytopenia. (3 ± 0.24 and 2.5 ± 0.12), respectively, in comparison with all other test groups. Eosinophilic count showed non significant change in G2 (1.1 ± 0.09) and G4 (0.9 ± 0.05) compared with normal vaccinated non infected group G1 (1.0 ± 0.02), while G3 showed significant decrease (0.2 ± 0.02), in comparison with all test groups. Basophilic count in G2 and G4 showed significant increase (1.1 ± 0.03 and 1.1 ± 0.07), respectively, compared with G1 and G3 (0.4 ± 0.01 and 0.2 ± 0.07), respectively. All differential leucocytic count percentages were shown in table (1).

Biochemical studies of the experimental groups showed significant increase in total protein in group 4 (7.4 ± 0.62) compared with groups 1, 2 and 3 (6.9 ± 0.92, 6.6 ± 0.41 and 6.8 ± 0.53) respectively. Non significant difference was observed between group 1 and group 3 (6.9 ± 0.92 and 6.8 ± 0.53) respectively, while group 2 showed significant decrease (6.6 ± 0.41) as compared with other test groups. Albumin concentration in group 2 and group 4 showed significant decrease (2.5 ± 0.45 and 2.6 ± 0.61) respectively, as compared with group 1 and 3 (3.7 ± 0.32 and 3.1 ± 0.53). Globulin levels in groups 2, 3 and 4 were significantly increased (4.1 ± 0.62, 3.7 ± 0.72 and 3.7 ± 0.72) respectively, compared with normal vaccinated control.
group (3.2 ± 0.71). Albumin globulin ratio (A/G) was significantly decreased in groups 2, 3 and 4 (0.60 ± 0.06, 0.83 ± 0.02 and 0.54 ± 0.03) respectively, compared with normal vaccinated control group G1 (1.15 ± 0.02).

Liver transferase enzymes Aspartate Aminotransferase, Alanine Aminotransferase and Gamma Glutamyle Transferase (AST, ALT, and GGT) showed, AST level, significant increase in group 2 (98.6 ± 5.61) as compared with groups 1, 3 and 4 (54.0 ± 4.22, 56.2 ± 3.76 and 60.3 ± 3.11) respectively.

Serum level of ALT showed significant increase in group 2 (58.2 ± 2.72) as compared with groups 1, 3 and 4 (45.1 ± 2.71, 46.1 ± 2.13 and 48.2 ± 2.28) respectively.

GGT level showed significant increase in group 2 (9.6 ± 0.84) in comparison with groups 1, 3 and 4 (4.3 ± 0.92, 5.2 ± 0.55 and 5.3 ± 0.61), respectively. All biochemical results were shown in table 2.

ELISA test showed significant increase in group 3 (0.465 ± 0.02) and group 4 (0.412 ± 0.03) compared with groups 1 and 2 (0.432 ± 0.02 and 0.387 ± 0.01), while infected group (G2) showed significant decrease compared with all previously mentioned test groups. Phagocytic activity of peritoneal exudates macrophages showed significant increase in group 3 (38.1 ± 1.21) as compared with groups 1, 2 and 4 (36.8 ± 1.58, 34.1 ± 0.92 and 36.2 ± 1.12) respectively, as shown in table 3.

Pathological studies: Experimentally infected rabbits showed acute signs of the disease 2 days post infection, and on necropsy showed pale liver, congested spleen and kidneys with petichial hemorrhage and accumulation of intra-abdominal blood clots. Histopathological studies showed coagulative necrotic hepatitis Fig. (1), accompanied with disseminated intravascular coagulopathy (DIC), which is a pathognomonic process, as shown in Fig. (2).
Table (1): Differential Leucocytic Count in different groups of rabbits (Mean ± SE)

<table>
<thead>
<tr>
<th></th>
<th>Lymphocytes $10^{-3}$/µl</th>
<th>Monocytes $10^{-3}$/µl</th>
<th>Neutrophils $10^{-3}$/µl</th>
<th>Eosinophils $10^{-3}$/µl</th>
<th>Basophils $10^{-3}$/µl</th>
</tr>
</thead>
<tbody>
<tr>
<td>G1</td>
<td>57.1c ± 2.82</td>
<td>7.4c±0.92</td>
<td>34.1b±2.82</td>
<td>1.0a±0.02</td>
<td>0.4b±0.01</td>
</tr>
<tr>
<td>G2</td>
<td>50.7d ± 5.78</td>
<td>15.7a±1.92</td>
<td>31.4d±2.93</td>
<td>1.1a±0.09</td>
<td>1.1a±0.03</td>
</tr>
<tr>
<td>G3</td>
<td>60.1b ± 4.82</td>
<td>2.3d±0.24</td>
<td>37.2a±2.81</td>
<td>0.2b±0.02</td>
<td>0.2b±0.07</td>
</tr>
<tr>
<td>G4</td>
<td>63.2a ± 4.62</td>
<td>2.5b±0.12</td>
<td>32.3c±3.1</td>
<td>0.9b±0.05</td>
<td>1.1a±0.07</td>
</tr>
</tbody>
</table>

Table (2): Biochemical changes in different groups of rabbits (Mean ± SE).

<table>
<thead>
<tr>
<th></th>
<th>TP (g/dl)</th>
<th>Alb. (g/dl)</th>
<th>Glob. (g/dl)</th>
<th>A/G ratio</th>
<th>AST (IU/l)</th>
<th>ALT (IU/l)</th>
<th>GGT (IU/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>G1</td>
<td>6.9±0.92b</td>
<td>3.7±0.32a</td>
<td>3.2±0.71d</td>
<td>1.15±0.02a</td>
<td>54.0±4.22d</td>
<td>45.1±2.71d</td>
<td>4.3±0.92c</td>
</tr>
<tr>
<td>G2</td>
<td>6.6±0.41c</td>
<td>2.5±0.45c</td>
<td>4.1±0.62b</td>
<td>0.60±0.06c</td>
<td>98.6±5.61a</td>
<td>58.2±2.72a</td>
<td>9.6±0.84a</td>
</tr>
<tr>
<td>G3</td>
<td>6.8±0.53b</td>
<td>3.1±0.53b</td>
<td>3.7±0.72c</td>
<td>0.83±0.02b</td>
<td>56.2±3.76c</td>
<td>46.1±2.13c</td>
<td>5.2±0.55b</td>
</tr>
<tr>
<td>G4</td>
<td>7.4±0.62a</td>
<td>2.6±0.61c</td>
<td>4.8±0.92a</td>
<td>0.54±0.03d</td>
<td>60.3±3.11b</td>
<td>48.2±2.28b</td>
<td>5.3±0.61b</td>
</tr>
</tbody>
</table>

Each value represented mean of 10 individuals ±SE. Different superscript litters in the same column mean significant difference, while the same litters mean non significance (P < 0.05).
Table (3): Immune parameters in different rabbit experimental groups (Mean ± SE).

<table>
<thead>
<tr>
<th></th>
<th>ELISA (Optical density)</th>
<th>Phagocytic cells (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>G1</td>
<td>0.432±0.02(^b)</td>
<td>36.8 ±1.58(^b)</td>
</tr>
<tr>
<td>G2</td>
<td>0.387± 0.01(^d)</td>
<td>34.1 ±0.92(^c)</td>
</tr>
<tr>
<td>G3</td>
<td>0.465±0.02(^a)</td>
<td>38.1±1.21(^a)</td>
</tr>
<tr>
<td>G4</td>
<td>0.412±0.03(^c)</td>
<td>36.2 ±1.12(^b)</td>
</tr>
</tbody>
</table>

Each value represented mean of 10 individuals ± SE. Different superscript litters in the same column mean significant difference, while the same litters mean non significance (P < 0.05).

Fig.(1): Showing hepatic peripheral lobular coagulative necrosis (H&E. X 300)

Fig.(2): Showing disseminated intravascular Coagulopathy (thrombosis) (H&E. X 300)
DISCUSSION

Differential leucocytic count in this study showed significant decrease in both lymphocytic and neutrophilic counts in experimentally infected group (G2). This decrease can be explained as an immunosuppressive effect of the virus, which was in accordance with Plassirat et al. (1992) and Guelfi et al. (1993). Nigella sativa L seed chloroform extract showed restoration of accompanied immunosuppression due to infection as was indicated in our results by the increase in both lymphocytes and neutrophils. This result was in agreement with the results obtained by Afifi and Daghash (1999) and Kanter et al., 2005. Nigella sativa extract inhibit eicosanoid generation in leukocytes and membrane lipid peroxidation (El-Dakhakhny et al., 2002). Nigella sativa is rich in fatty acid, (oleic, linoleic and linolenic acid) and carotene (AL-Jassir, 1992). NS acts as antioxidant in cells, and contains eight essential amino acids, which improves the natural immune system activity (Omar et al., 1999). Monocytic count showed significant increase in experimentally infected group (G2) which was considered as a direct response to induced viral infection on the expense of the decrease of both lymphocytes and neutrophils. Also this group (G2) showed significant increase in basophilic count as compared with normal vaccinated group (G1). Nigella sativa treatment resulted in significant decrease in eosinophilic count in both groups 3 and 4, while basophilic count was decreased significantly in both groups 3 and 4, these results were in agreement with Bamosa and Sowayan (2000). The significant increase in lymphocytic count in Nigella sativa treated groups G 3 and G 4 was in accordance with Salem (2005) who attributed this increase to thymoquinine which had shown antioxidant and immunomodulator effects on macrophage, Th1 and Th2.

Biochemical studies of serum proteins parameters showed significant decrease in total proteins in infected group (G2) which was attributed to the significant decrease in albumin which can be referred to liver damage as a result of infection Ghanem and Ismail (1999), while the significant increase in globulin level is explained as a direct immune response to infection. Nigella sativa treated and infected group (G4) showed significant increase in total protein and both albumin and globulin showed improved liver and immune response illustrated in decreased albumin / globulin ratio (A/G), which is attributed to increased globulin as a direct immunomodulating effect of Nigella sa-
tiva and this result was in agreement with El Kady and Kandil (1986); Wang et al. (1996), Salem (2005) and Gali et al. (2006). Studies on liver transferases assured liver damage in experimentally infected group (G2) as indicated by elevated serum levels of AST, ALT and GGT, which returned towards normal levels in Nigella sativa treated groups (G3 and G4), this effect was explained by the liver protective effect of Nigella sativa and its immunostimulation impact, which was in agreement with Nair et al. (1991) and El Naggar and Deib (1992).

ELISA test results showed the immunostimulating effect of Nigella sativa extract on its treated groups (G3 and G4) compared with both normal vaccinated (G1) and experimentally infected (G2) groups, this immunostimulating effect was in accordance with Tizard (1996). The obtained results were in parallel with those obtained in our study in differential leucocytic count where lymphocytosis was detected in Nigella sativa treated groups, while lymphopenia was obvious in experimentally infected group.

Cell mediated immunity which was evaluated by phagocytic activity of macrophages in the peritoneal exudates which showed significant decrease in infected group (G2) owing to cell mediated immunosuppression in this group and showed increased immune response of these cells in samples collected from Nigella sativa treated groups (G3 and G4) these results were in agreement with El kadi et al. (1989) and El Naggar and Deib (1992). The increase in phagocytic activity might be attributed to in vivo activation of macrophages by production of lymphokines by T helper cells after their stimulation (Tizard, 1996). Also antioxidant effect of Nigella sativa (thymoquinone) appears to enhance host defenses against infection by improving phagocytic cells function through protecting them from oxidative damage due to its high proportion of polyunsaturated fatty acids in their cell membrane and also the surrounding tissues from oxidative attack by free radical produced from activated macrophage during phagocytosis (Hughes, 1999). A decrease in nitric oxide (NO) activity in Nigella sativa treated groups may be attributed to high content of this seed with antioxidant compound (thymoquinone) which had an important role in scavenging harmful free radicals. Nigella sativa seeds caused a dose dependent decrease in Nitric oxide production from peritoneal macrophage when activated with lipopolysaccharide (LPS) of E. coli without affecting on cell viability (Victor et al., 2003).
Pathological studies of examined infected rabbits revealed severe internal hemorrhage with marked hemorrhagic pneumotracheitis as well as pale hypertrophied liver. These observations were in accordance with those recorded by Kavaliski, (1998). The main cause of death was attributed to the severe internal hemorrhage (Xu and Chan, 1982). Histopathologically, acute necrotizing hepatitis with slight inflammatory cell infiltration was recorded and was in agreement with results observed by Fuchs and Weissenboeck (1992). Disseminated intravascular coagulopathy (DIC), recorded in this study, agreed with the results obtained by Plassirat et al. (1992).

In conclusion, at a practical level it is recommended to use the natural plant extract of Nigella sativa for immunostimulation for better rabbit farming.

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**الملخص العربي**

في السنوات الأخيرة وفي العديد من الدول ومنها مصر انتشر مرض النزف الفيروسي الأردني مسببًا خسائر اقتصادية شديدة وصلت إلى (80 - 100%) ولم تنجح برامج التحصين في التحكم في انتشار المرض بصورة كافية. في هذه الدراسة قمنا بمحاولة التأكيد على تأثير حبة الكرة الوقائي والمنشط للمناعة و استغلال هذا التأثير في تحسين الاستجابة المناعية للأرانب المحصنة أو التي تم عدويها تجريبيًا.

تم تصميم التجربة لتحتوي على عدد (40) أرنبًا تم تقسيمها إلى أربعة مجموعات تحتوي كل منها على عشرة أفراد تم تحسين المجموعة الأولى منها بلقاح المرض الفيروسي النزفي والاحتفاظ بها كمجموعة ضابطة.
أما المجموعه الثانيه فتم عداها بفيروس المرض، كما تم تحسين المجموعه الثالثه وحدها بمستخلص حببة البركة الكلوروفيومي، أما المجموعه الرابعة فتم تحسينها وتعاملها بمستخلص حببة البركة ثم عداها بفيروس المرض المعزول من العدوى المثلية بعد التعرف عليها.

أظهر العد النوعي لكرات الدم البيضاء في المجموعه التي تم عداها بدون تحسين نقصا معنويا في العدادات والخلايا الليمفاوية والتي أظهرت تحسنها معنويون في المجموعات الثالثة والرابعة التي تم حفظها بمستخلص حببة البركة الكلوروفيومي.

كما أظهرت الدراسات الكيميائيه الحيوية زيادة معنويون في انزيمات الكبد (AST, ALT, GGT) مما يدل على الأصابع الشديدة في الكبد والتي مثلت أن عادات المستويات العادية في المجموعين الثالث، والمجموعه الرابعة والتي تم معالجتها بمستخلص حببة البركة معقدة التأثير الواقي لهذا المستخلص بالنسبة للكبد.

أظهر تركز البروتينات الكلي والزلال عدم التغير في المجموعه الثالثة مع زيادة معنويون في تركيز الجلوبيولين. بينما أظهر الزلال تراجعا معنويون في المجموعه الرابعة بزيادة معنويون لتركيز الجلوبيولين والبروتينات الكلي. أما المجموعه الثالثة فأظهرت زيادة معنويون في الجلوبيولين مصوحون بنقص نسبه الزلال إلى الجلوبيولين مشرقة إلى التأثير المتشبث للعدوى المثلية حببة البركة.

وأوضح الدراسات المناهية باستخدام أسلوب الأليزات التأثير المتشابه للعدوى السائبة لحبة البركة للمجموعات التي عولمت بالمستخلص؛ كما أظهرت التأثير المتشابه للعدوى في المجموعات النازفة الأرجوني.

أظهرت الدراسات الخلوية، باستعمال صبغة الأحمر المعدل، زيادة معنويون في المجموعين الثالث، والرابع، المعالمين بمستخلص حببة البركة كما هو واضح من زيادة النسبة المعنوية لخلايا الماكروفاج التي وصلت محتوى النصائح الذي أظهرت الدراسات الببتولوجية للعدوى التاجية أعراض الأصابع الحادة بعد العدوي بيومين وآوثر الفحص التشريحي شحوب الكبد مع احتقان المجلن والكليتين مع وجود نزيف نازيف بهما وجود تجمعات دموية مرتفعة في فرغ الطين، وأظهر الفحص الميكروسكوبى التهاب الكلد التتكزى مع وجود تجلط الدم داخل الأوعية الدموية بصورة واضحة.

ويستطيع من هذا البحث النصيحه باستخدام مستخلص نبات حبة البركة الكلوروفيومي مصححا لتحسين الأعراض لتفادي التأثير السلبي والخصائص الاقتصادية للعدوى بهذا المرض.

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