EFFECT OF ECHINACEA PURPUREA AND CLOVE (SYZYGIUM AROMATICUM) AQUEOUS EXTRACT ON IMMUNE DEFICIENCY INDUCED BY CISPLATIN IN RATS

Asmaa M. Abd El-Hafez*; Omnia G. Refaat; H.G. El-Masry and Alaa O. Aboraya
Nutrition and Food Science Department, Faculty of Home Economics, Helwan University, Cairo, Egypt
*E-mail-asmaa892006@gmail.com

ABSTRACT

The present study was aimed to investigate the effect of Echinacea purpurea and clove (Syzygium aromaticum) aqueous extract on raising the immune response in rats with immune deficiency diseases. Forty-eight male albino rats were randomly divided into 8 equal groups (n=6). Group 1 was negative group, whereas the other 7 groups injected by cisplatin. Group 2 kept as the immunotoxic control group (+ve group). Groups 3 and 4 received 1 and 2 ml of Echinacea extract, whereas 1 and 2 ml of clove extract were given to groups 5 and 6, respectively. Groups 7 and 8 received 1 and 2 ml of Echinacea and clove extract mixture, respectively, for 8 weeks. Results of body weight, spleen weight, serum immunoglobulin G, immunoglobulin M (IgM), total protein, albumin, globulin, neutrophil, lymphocyte, monocytes and eosinophil significantly decreased. Whereas leukocyte significantly increased by cisplatin in positive control group as compared to the negative control group. On the other hand, administration of Echinacea and clove extracts and their combination attenuated these adverse effects and markedly ameliorated biochemical alterations that caused by cisplatin administration. In conclusion, Echinacea and clove extracts stimulate the immune system of rats with cisplatin-induced suppressed immunity. This study recommended increasing the consumption of the Echinacea and clove in the diet, this may be raising the immune response in patient with immune disorders.

Key Words: Echinacea purpurea, Clove, Cisplatin, Immune System, Rats.

INTRODUCTION

Cisplatin (also known as cisplatinum or cis-diaminedichloroplatinum) is the first Food and Drug Administration, it is a platinum-containing compound which inhibits synthesis of RNA, DNA and protein in cells. Cisplatin is one of the most effective anticancer drugs used for the treatment of various oncologic diseases, including testicular, cervical, ovarian, mammary, head and neck,
esophageal, lung and brain cancers (Dasari and Tchounwou, 2014 and Zhou et al., 2022). Several adverse effects including myelosuppression, hepatotoxicity, nephrotoxicity and immunotoxicity are the result of cisplatin administration (Hasaan et al., 2010). The toxic effects of cisplatin are attributed to several factors, such as peroxidation of the cell membrane, DNA damage, mitochondrial dysfunction, inhibition of protein synthesis, and ability to affect host immune response (Zhu et al., 2017). Immunotoxicity could be the result of direct or indirect action of a chemical on the immune system, causing a suppression or activation of the immune response (Tuneh et al., 2014). Compromised immune response can result in suppression of host resistance to infectious agents as well as tumor cells. Most anticancer drugs are found to suppress hematopoiesis in bone marrow and cause myelosuppression and lymphocytopenia, resulting in reduction or inhibition of lymphocytic responses. Cisplatin has a cytotoxic effect on immune cells when they are rapidly dividing.

Several plants and herbs are being used as immunomodulators (Nagoba and Davane, 2018 ; Zhang et al., 2020). Echinacea is a plant genus within the family of Asteraceae (compositae) and is comprised of 11 taxa of herbaceous and flowering plants (Sharifi-Rad et al., 2018). It is an indigenous medicine of the native American Indians and Europeans with multiple biological activities, such as anti-inflammation, antioxidation and immunomodulation effects (Chiou et al., 2017 and Khattab et al., 2019). Echinacea purpurea contains active ingredients of carbohydrate, glycoside, alkaoleide, alkylamide and polyacetylene (Lalone et al., 2007).

Clove, Syzygium aromaticum, is an aromatic medical plant of the family Myrtaceae. It is commonly applied as a natural additive in the food industry, antiseptic against infectious diseases, and local anesthetic in dentistry (Cortés-Rojas et al., 2014). In addition to its antimicrobial, anti-fungal, and anti-viral properties, clove possesses anti-inflammatory properties (Chaieb et al., 2007). Eugenol, eugenyl acetate, carvacrol, tanene, and thymol were detected as major constituents of the clove (Amelia et al., 2017). It has been reported that constituents of clove impart anti-oxidant activities and inhibit lipid peroxidation (Dibazar et al., 2015). Effects by clove or main constituents (like eugenol) on specific immune system components/mechanisms have only recently begun to be examined in detail (Yogalakshmi et al., 2010 ; Bachiega et al., 2012 and Grespan et al., 2012).
Therefore, this study was conducted to study the effect of aqueous extracts of *Echinacea purpurea* and Clove (*Syzygium aromaticum*) on raising the immune response in rats with immune deficiency diseases.

**MATERIALS AND METHODS**

**Materials**

*Echinacea purpurea* and Clove (*Syzygium aromaticum*) were obtained from Agriculture Research Center, Egypt. Cisplatin, casein, cellulose, choline chloride, D-L methionine, vitamin and mineral constituents were purchased from El-Gomhoriya Pharmaceutical Company, Cairo, Egypt. Starch, soy oil, and sucrose were obtained from the Egyptian local market. Forty-eight adult male rats (Sprague Dawley strain), weighing about 180±10 g b.wt. were obtained from the Laboratory Animal Colony, Agricultural Research Center, Giza, Egypt.

**Methods**

1. **Preparation of Echinacea and Clove Aqueous Extract:**
   
   Twenty-five gm of dried Echinacea was submerged in 100 ml of distilled water and allowed to soak overnight, then filtered to obtain a liquid extract. A known concentration of *Echinacea* aqueous extract was given orally by stomach tube.

   The Clove flower buds were dried in the sun and ground to fine powder with the aid of an electric blender. Thereafter, 25 g of the milled clove powder was soaked in 100 ml distilled water, then filtered to prepare an aqueous extract (*Dibazar et al.*, 2015). A known concentration of Clove aqueous extract was given orally by stomach tube.

2. **Induction of Immune Deficiency Diseases:**
   
   Cisplatin-induced immune deficiency diseases in rats. Intraperitoneal injection of male albino rats with cisplatin (3.5 mg/kg) once every 3 days for consecutive 2 weeks (*Wang et al.*, 2013).

3. **Diet Preparation and Experimental Design:**
   
   The basal diet was prepared according to AIN-93M diet (*Reeves et al.*, 1993). Forty-eight male albino rats were randomly divided into 8 equal groups (n=6). Group 1 was negative control group. Whereas the other 7 groups were injected by cisplatin. Group 2 was kept as the immunotoxic control group (+ve group). Groups 3 and 4 received 1 and 2 ml of *Echinacea* extract. Whereas 1 and 2 ml of clove extract were given to groups 5 and 6, respectively. Groups 7 and 8 received 1 and 2 ml of *Echinacea* and clove extract mixture (1:1), respectively, for 8 weeks. During the experiment period, the quantities of diet, which were
consumed and/or waste, were recorded every day. In addition, rat’s weight was recorded weekly to determine body weight gain and feed efficiency ratio according to Chapman et al., (1959).

4. Biochemical Analysis:

At the end of the experimental period (8 weeks), rats were fasted overnight before scarifying and blood samples were collected from each rat and centrifuged at 3000 rpm for 15 min to obtain the serum for biochemical analysis. Levels of leukocytes, neutrophil, lymphocyte, monocytes, eosinophil and basophil were estimated according to Ochei and Kolharkar, (2008). Immunoglobulin M (IgM) and immunoglobulin G (IgG) were measured according to Ziva and Pannall, (1984). Concentration of total protein was determined according to Burtis and Ashwood (1999), albumin and globulin were determined according to Young, (1995).

5. Statistical Analysis:

Results were expressed as the mean standard error ± SE. Data were statistically analyzed for variance “ANOVA” test at P ≤ (0.05) using SPSS statistical software, version 20 was used for these calculations according to Armitage and Berry, (1987).

RESULTS AND DISCUSSION

The results in Table 1 show that intraperitoneal injection of cisplatin to rats caused a decrease in feed intake and a significant ($P<0.05$) reduction in body weight gain (BWG%), feed efficiency ratio (FER) and spleen relative weight % when compared to the negative control group. Oral administration of Echinacea and Clove extracts and their mixture to rats inflicted with immune deficiency diseases (IDD) caused increasing in feed intake and a significant ($P<0.05$) increases in FER, body weight and spleen weight as compared to the positive control group.

Lin et al., (2018) demonstrated that cisplatin administration resulted in significantly decrease in feed intake, body weight and feed efficiency, as found in the present study. Immunotoxicity may parallel alterations in the weight of lymphoid organs (spleen) (Pearse et al., 2009). The results in the present study were in the same line with El-Sherbiny et al., (2021) that the administration of Echinacea extract stimulated the increase in weight of spleen as well as body weight in rats with immune deficiency. Furthermore, Ali, (2008) reported that Echinacea has a positive effect on body weight gain after 4 weeks of
treatment. Moreover, the obtained results agree with Agbaje et al., (2009) who showed an improvement in body weight by clove in rats.

Table 1: Effect of Echinacea and Clove Extracts on Feed Intake (FI), Efficiency Ratio (FER), Body Weight Gain (BWG), and Spleen Relative Weight (SRW) of Rats with Immune Deficiency Diseases

<table>
<thead>
<tr>
<th>Group</th>
<th>Parameter</th>
<th>FI (g/d/rat)</th>
<th>FER</th>
<th>BWG%</th>
<th>SRW%</th>
</tr>
</thead>
<tbody>
<tr>
<td>1- Control (-ve)</td>
<td>25</td>
<td>0.039±0.001a</td>
<td>28.54±1.04*</td>
<td>0.88±0.05a</td>
<td></td>
</tr>
<tr>
<td>2- Control (+ve)</td>
<td>16</td>
<td>0.029±0.001c</td>
<td>14.06±0.93*</td>
<td>0.42±0.08f</td>
<td></td>
</tr>
<tr>
<td>3- 1ml Echinacea</td>
<td>18</td>
<td>0.036±0.001ab</td>
<td>19.03±0.91b</td>
<td>0.52±0.08e</td>
<td></td>
</tr>
<tr>
<td>4- 2ml Echinacea</td>
<td>21</td>
<td>0.035±0.002ab</td>
<td>21.76±2.22a</td>
<td>0.61±0.11d</td>
<td></td>
</tr>
<tr>
<td>5- 1ml Clove</td>
<td>18</td>
<td>0.036±0.003ab</td>
<td>19.45±1.20a</td>
<td>0.52±0.09e</td>
<td></td>
</tr>
<tr>
<td>6- 2ml Clove</td>
<td>20</td>
<td>0.037±0.002ab</td>
<td>22.07±1.74a</td>
<td>0.64±0.08d</td>
<td></td>
</tr>
<tr>
<td>7- 1ml E:C</td>
<td>22</td>
<td>0.036±0.001ab</td>
<td>23.05±0.77c</td>
<td>0.71±0.15c</td>
<td></td>
</tr>
<tr>
<td>8- 2ml E:C</td>
<td>22</td>
<td>0.036±0.002a</td>
<td>23.59±1.93c</td>
<td>0.77±0.16b</td>
<td></td>
</tr>
</tbody>
</table>

*Mean values are expressed as means ± SE.
*Mean values at the same column with the same superscript letters are not statistically significant at P<0.05.
*E:C=Echinacea: Clove.

Results presented in Table 2, revealed that rats inflicted with immune deficiency diseases by cisplatin had significant (P<0.05) reduction in serum immunoglobulin G (IgG) and immunoglobulin M (IgM) antibodies levels when compared with the negative control (-ve) group. Administration with Echinacea and Clove extracts and their mixture to IDD rats resulted in significant (P<0.05) increases in serum IgG and IgM as compared to the positive control group. It was also observed that rats administrated with 2 ml mixture of Echinacea and Clove extracts recorded the best results for increasing IgG and IgM when compared to the negative control group.

Results in Table 2, are confirmed by Nassef et al., (2018) who reported that cisplatin injection to rats caused a significant reduction in serum immunoglobulin (IgG, IgM). Rehman et al., (1999) Mahmoud et al., (2022), showed that Echinacea administration increased IgG and IgM production in rats with immune deficiency. The effects of immune activation by Echinacea were investigated by measuring total immunoglobulin (IgG, IgM). Mishima et al., (2004) investigated the effects of immune activation by Echinacea by measuring T lymphocyte subsets in the peripheral blood of mice following whole-body irradiation.
and reported that Echinacea activates macrophages to stimulate IFN-gamma production in association with the secondary activation of T lymphocytes, resulting in decreases of IgG and IgM production. Also, the improvement of IgG and IgM may be due to that clove act as additional bonds with immunoglobulin molecules at the Fc receptors, which stimulated the immune response (Ahmed et al., 2013).

Table 2: Effect of Echinacea and Clove Extracts on Serum Immunoglobulin G (IgG) and Immunoglobulin M (IgM) of Rats with Immune Deficiency Diseases

<table>
<thead>
<tr>
<th>Group</th>
<th>Parameter</th>
<th>IgG (g/L)</th>
<th>IgM (g/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1- Control (-ve)</td>
<td>12.00±0.57*</td>
<td>252.33±3.56a</td>
<td></td>
</tr>
<tr>
<td>2- Control (+ve)</td>
<td>5.46±0.88c</td>
<td>127.34±2.04c</td>
<td></td>
</tr>
<tr>
<td>3- 1ml Echinacea</td>
<td>6.90±0.62d</td>
<td>219.66±1.76ed</td>
<td></td>
</tr>
<tr>
<td>4- 2ml Echinacea</td>
<td>8.32±0.18c</td>
<td>231.66±3.35bc</td>
<td></td>
</tr>
<tr>
<td>5- 1ml Clove</td>
<td>6.89±0.16d</td>
<td>212.33±2.84d</td>
<td></td>
</tr>
<tr>
<td>6- 2ml Clove</td>
<td>8.46±0.32c</td>
<td>233.66±4.25bc</td>
<td></td>
</tr>
<tr>
<td>7- 1ml E:C</td>
<td>9.80±0.21b</td>
<td>231.00±2.40bc</td>
<td></td>
</tr>
<tr>
<td>8- 2ml E:C</td>
<td>10.72±0.15b</td>
<td>235.52±1.15b</td>
<td></td>
</tr>
</tbody>
</table>

*Mean values are expressed as means ± SE.
*Mean values at the same column with the same superscript letters are not statistically significant at P<0.05.
*E:C= Echinacea: Clove.

Results illustrated in Table 3, show that rats injected intraperitoneally with cisplatin had significant decreases in the serum levels of total protein, albumin and globulin when compared with the negative control group. Oral administration of Echinacea and Clove extracts (2 ml) and their mixture to IDD rats significantly normalized (P<0.05) the levels of total protein, albumin and globulin in the serum. These findings are confirmed by Parameshappa et al., (2012) and Khalaf et al., (2019), who observed significant reduction in total protein, globulin and albumin concentration in rats administrated with cisplatin. Results also,are in agreement with that of Sadigh-Eteghad et al., (2011), who showed that treatment with Echinacea (500 mg /4 weeks) ameliorates the alteration in total protein and albumin. Concerning results of clove administration, are in agreement with Abozid and El-Sayed (2013), who found that rats treated with clove extract increased plasma total protein and albumin compared with +ve group due to the polyphenolic compound and flavonoids that present in clove extract (Gulcin et al., 2004).
Table 3: Effect of Echinacea and Clove Extracts on Serum Total Protein, Albumin and Globulin of Rats with Immune Deficiency Diseases

<table>
<thead>
<tr>
<th>Group</th>
<th>Parameter</th>
<th>Total Protein mg/dl</th>
<th>Albumin mg/dl</th>
<th>Globulin mg/dl</th>
</tr>
</thead>
<tbody>
<tr>
<td>1- Control (-ve)</td>
<td>7.82±0.75a</td>
<td>4.85±0.61a</td>
<td>3.57±0.07a</td>
<td></td>
</tr>
<tr>
<td>2- Control (+ve)</td>
<td>3.87±0.89d</td>
<td>2.15±0.16d</td>
<td>1.66±0.12d</td>
<td></td>
</tr>
<tr>
<td>3- 1ml Echinacea</td>
<td>4.55±0.42cd</td>
<td>2.36±0.26cd</td>
<td>1.89±0.12d</td>
<td></td>
</tr>
<tr>
<td>4- 2ml Echinacea</td>
<td>5.10±0.27bc</td>
<td>3.24±0.15b</td>
<td>2.37±0.22c</td>
<td></td>
</tr>
<tr>
<td>5- 1ml Clove</td>
<td>4.42±0.20cd</td>
<td>2.38±0.29cd</td>
<td>1.83±0.21d</td>
<td></td>
</tr>
<tr>
<td>6- 2ml Clove</td>
<td>5.24±0.55bc</td>
<td>3.13±0.16bc</td>
<td>2.82±0.60b</td>
<td></td>
</tr>
<tr>
<td>7- 1ml E:C</td>
<td>5.48±0.25b</td>
<td>3.32±0.83b</td>
<td>3.04±0.17b</td>
<td></td>
</tr>
<tr>
<td>8- 2ml E:C</td>
<td>6.00±0.12b</td>
<td>3.38±0.63b</td>
<td>3.17±0.14b</td>
<td></td>
</tr>
</tbody>
</table>

*Mean values are expressed as means ± SE.
*Mean values at the same column with the same superscript letters are not statistically significant at P<0.05.
*E:C= Echinacea: Clove.

Results in Table 4, show that the positive control group had a significant increase (P < 0.05) in leukocytes and a significant decrease (P < 0.05) in levels of neutrophil, lymphocyte, monocytes and eosinophil as compared to the negative control group. On the other hand, rats that administrated with different levels of Echinacea & Clove extracts and their mixture had significant reduction in leukocytes and a significant elevation in neutrophil, lymphocyte, monocytes and eosinophil as compared to the positive control (+ve) group. The highest improvement was recorded in group that treated with the high level (2 ml 1E:1C) of combination of Echinacea and Clove extract.

In complementary of the present results, Khalaf et al., (2019) found that administration of cisplatin to mature rats resulted in marked immunotoxic effects represented by leukopenia, lymphocytopenia and neutrophilia. On the other hand, Markovic et al., (2011) reported that cisplatin increased the number of leukocytes due to the consequence of infection and inflammation. On the other hand, administration with Echinacea extract significantly reduced leucopenia induced by cisplatin which indicates that the extract could stimulate the haemopoietic system. This may be attributed to the contents of Echinacea as cichoric acid and echinacocide that stimulate bone marrow and the reformation of hematopoietic stem cells (Chopra and Goel 2002). Mishima et al., (2004) reported that administration of Echinacea 360 mg/kg/day for 3weeks increases the number of leukocytes. This elevation is due to ability of polysaccharides and echinacocide to increase the number of

"Egypt J. of Appl. Sci., 38 (3-4) 2023"
leukocytes. **Doha et al., (2011)** showed that *Echinacea* is involved in the modulation of immune response. Various phytoconstituents present in *Echinacea*, such as caffeic acid derivatives, alkamides, flavonoids, essential oils and polyacetylenes, are known to activate the non-specific cellular and humoral by increasing the production and activation of leukocytes, lymphocytes, monocytes and cytokines (**Kim et al., 2002**). These components also modulate the immune response by macrophage phagocytosis, pro-inflammatory cytokine production, activation of NK cell activity, enhancement of B cell response, increased T cell proliferation and elevated production of T cell cytokines (**Chen et al., 2005**; **Thygesen et al., 2007** and **Khalaf et al., 2019**). Hence, it is plausible that *Echinacea* extract may confer immunoprotection through multifactorial immunomodulatory effects which could be achieved through chemical synergy of various bioactive constituents. On the other hand, **Dibazar et al., (2015)** and **Kniec et al., (2017)**, revealed that clove extract containing eugenol, β-caryophyllene, caryophyllene oxide and α-humulene can increase the proliferation of activity of lymphocytes, lymphoblasts and reactive oxygen intermediate secretion of macrophages. Infected mice will increase IFN-γ levels because there are immunogens that activate the immune system.

**Table 4: Effect of Echinacea and Clove Extracts on Leukocytes, Neutrophil, Lymphocyte, Monocytes, Eosinophil, and Basophil Count of Rats with Immune Deficiency Diseases**

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>1- Control (-ve)</td>
<td>5.30±0.05a</td>
<td>5.41±0.12b</td>
<td>4.83±0.19c</td>
<td>1.64±0.07c</td>
<td>1.37±0.02a</td>
<td>1.50±0.05a</td>
<td></td>
</tr>
<tr>
<td>2- Control (+ve)</td>
<td>12.03±0.24d</td>
<td>0.79±0.02e</td>
<td>1.01±0.05c</td>
<td>0.35±0.02c</td>
<td>0.30±0.03f</td>
<td>0.19±0.01e</td>
<td></td>
</tr>
<tr>
<td>3- 1ml Echinacea</td>
<td>11.22±0.18c</td>
<td>1.52±0.19a</td>
<td>1.87±0.05c</td>
<td>0.49±0.03b</td>
<td>0.55±0.02de</td>
<td>0.33±0.01de</td>
<td></td>
</tr>
<tr>
<td>4- 2ml Echinacea</td>
<td>8.40±0.15a</td>
<td>2.83±0.09c</td>
<td>2.80±0.09de</td>
<td>0.56±0.01d</td>
<td>0.71±0.01cd</td>
<td>0.47±0.03d</td>
<td></td>
</tr>
<tr>
<td>5- 1ml Clove</td>
<td>10.88±0.34a</td>
<td>1.73±0.09a</td>
<td>1.69±0.09c</td>
<td>0.46±0.01a</td>
<td>0.49±0.01e</td>
<td>0.29±0.01de</td>
<td></td>
</tr>
<tr>
<td>6- 2ml Clove</td>
<td>7.99±0.26cd</td>
<td>2.71±0.14c</td>
<td>2.91±0.03cd</td>
<td>0.63±0.03b</td>
<td>0.80±0.01bc</td>
<td>0.51±0.01d</td>
<td></td>
</tr>
<tr>
<td>7- 1ml E:C</td>
<td>6.73±0.15d</td>
<td>3.03±0.04c</td>
<td>3.10±0.06c</td>
<td>0.58±0.04b</td>
<td>0.79±0.04bc</td>
<td>0.88±0.01c</td>
<td></td>
</tr>
<tr>
<td>8- 2ml E:C</td>
<td>6.33±0.12c</td>
<td>4.02±0.10b</td>
<td>4.18±0.10b</td>
<td>0.94±0.03b</td>
<td>0.94±0.01b</td>
<td>1.16±0.09b</td>
<td></td>
</tr>
</tbody>
</table>

*Mean values are expressed as means ± SE.
*Mean values at the same column with the same superscript letters are not statistically significant at P<0.05.
*E:C= Echinacea: Clove.

**CONCLUSION**

The findings of this study demonstrated that Echinacea and clove extract is a promising immunomodulatory agent with a potent therapeutic value in stimulating the suppressed immune response.
REFERENCES


Tumeh, P.; C. Harview; J. Yearley; I. Shintaku; E. Taylor; L. Robert; B. Chmielowski; M. Spasic; G. Henry; V. Ciobanu; A. West; M. Carmona; C. Kivork; E. Seja; G. Cherry; A. Gutierrez; T. Grogan; C. Mateus; G. Tomasic; J. Glaspy; R. Emerson; H. Robins; R. Pierce; D. Elashoff; C. Robert and A. Ribas (2014): PD-1 blockade induces responses by inhibiting adaptive immune resistance. Nature, 515: 568-571.


Zhou, Z.; Y. Zhao; S. Chen; G. Cui; W. Fu; S. Li; X. Lin and H. Hu (2022): Cisplatin promotes the efficacy of immune checkpoint


Egypt. J. of Appl. Sci., 38 (3-4) 2023

Tأثير المستخلص المائي للإكينشيا والقرنفل على نقص المناعة المحدث بواسطة السبيستاتين في الفئران

أسماء محمود عبدالحافظ، أمنية جلال رفعت، هاني جابر المصري، آلاء أسامة أبو ريه

قسم التغذية وعلوم الأطعمة - كلية الاقتصاد المنزلي - جامعة حموان

هدف الدراسة الحالية إلى معرفة تأثير المستخلص المائي للإكينشيا والقرنفل في رفع الاستجابة المناعية في الفئران المصابة بأمراض نقص المناعة. تم تقسيم 48 من ذكور الذئاب البيضاء بشكل عشوائي إلى 8 مجموعات متساوية (N = 6). المجموعة الأولى كانت الضابطة السلبية بينما تم حقن المجموعات السبع الأخرى بواسطة السبيستاتين. احتفظت المجموعة الثانية كمجموعة ضابطة موجبة للدراسة المناعية. تمت寸 المجموعتان 3، 4 مستخلص الإكينشيا بجرعة 1 و 2 مل، في حين أُعطيت المجموعتان 5 و 6 على التوالي 1، 2 مل من مستخلص القرنفل. تمت寸 المجموعتان 7، 8 خليط من مستخلص الإكينشيا والقرنفل بجرعة 1 و 2 مل، على التوالي، لمدة 8 أسابيع. تشير نتائج وزن الجسم ووزن الطحال والبروتين الكلي والإجهاض المناعي (IgM, IgG) إلى أن مستخلص القرنفل ومستخلص الإكينشيا قد خففت من آثار مستخلص الإكينشيا والقرنفل وخلايا الدم البيض بشكل ملحوظ بواسطة السبيستاتين في المجموعة الضابطة البدائية مقارة بالمجموعة الضابطة السلبية. من ناحية أخرى، فإن إعطاء مستخلص القرنفل ومستخلص الإكينشيا وخلايا الدم البيض بدقة وقائية قد خففت من آثار هذه الآثار الضارة وخففت بشكل ملحوظ التغيرات الكيميائية الحيوية التي تسببها حقن السبيستاتين. في الختام، يظهر مستخلص الإكينشيا والقرنفل الجهاز المناعي للقرفاء التي تعاني من نفس المناعة المحدث بواسطة السبيستاتين. أوصيت هذه الدراسة بإعادة استهلاك الإكينشيا والقرنفل في النظام الغذائي، فقد يؤدي هذا إلى رفع الاستجابة المناعية لدى المريض المصاب بإضطرابات مناعية.