The Effect of Echinacea purpurea and Clove (Syzygium aromaticum) Extracts on Raising the Immune Response in Rats with Immune Deficiency Diseases

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Abstract

The present study 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 histopathological and 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.

Keywords: Echinacea purpurea, Clove, Cisplatin, Immune System, Rats.

INTRODUCTION

The immune system plays the most effective role in preventing invasion of pathogens through immune response in order to maintain the physiological balance. However, several factors may affect or even destroy the immune response, and cause immune disorder further, such as malnutrition, application chemotherapy and stressors (Huang et al., 2016). The damaged immune system can lead to inflammatory diseases, autoimmune diseases, and even cancer (McComb et al., 2019). 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. Compromised immune response can result in suppression of host resistance to infectious agents as well as tumor cells (Zandvoort et al., 2001).

Cisplatin (also known as cisplatinum or cisdiamminedichloroplatinum) 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 (Baek et al., 2003). Despite its effective anticancer efficacy, cisplatin exerts many undesirable adverse effects, including myelosuppression, hepatotoxicity, nephrotoxicity and immunotoxicity (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 (Jordan and Carmo-Fonseca, 2000).

Herbs are traditionally used in many therapeutic practices, if not as the main, then as the accompanying therapy in combination with medications, aimed at boosting immunity for prevention. Phytotherapy has repeatedly proven its effectiveness, including its ability to cope with infectious diseases (Zhang et al., 2020). Echinacea purpurea (family: Asteraceae) is an important medicinal plant with various pharmacological properties. E. purpurea was found to exhibit significant antioxidant, anti-inflammatory, and immunoregulatory activities (Lee at al., 2009). Several active constituents, such as flavonoids, caffeic acid derivatives (alkamides), essential oils, and poly acetylenes, were isolated from this plant. Among various phytocomponents, alkamides were found to possess immunoregulatory effects (Matthias et al., 2008). The caffeic acid derivatives, alkamides and polysaccharide fractions were found to show inhibition against in vitro Cu(II)-catalyzed oxidation of human low-density lipoprotein (LDL) that proves the presence of antioxidant property (Dalby Brown et al., 2005).
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 (Chaib et al., 2007). Eugenol, eugenyl acetate, carvacrol, tannene, and thymol were detected as main 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; 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 *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 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 (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 sacrificing 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 Kolharktar, (2008). Immunoglobulin M (IgM) and immunoglobulin G (IgG) were measured according to Ziva and Pannall, (1984). Concentration of total protein was determined according to Bryant and Ashwood (1999), albumin and globulin were determined according to Young, (1995).

5. **Histopathological Examination:**

   Specimens from the spleen were placed in 10% neutral buffered formalin for histopathological examination according to Bancroft and Stevens, (1996). Histopathological examination was done in Veterinary medicine, Cairo University, Egypt.

6. **STATISTICAL ANALYSIS:**

   Results were expressed as the mean standard error ± SE. Data were statistically analyzed for variance “ANOVA” test at $P \leq (0.05)$ 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 showed 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. Immunootoxicity 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 are agreed with Agbaje et al., (2009) who showed an improvement in body weight by clove in rats.

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 administered 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, were confirmed by Nassef et al., (2018) reported that cisplatin injection to rats caused a significant reduction in serum immunoglobulin G (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).

Results illustrated in Table 3, showed 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 extract (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 were 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, in agreement with 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, were 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).

Results in Table 4, showed 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 and 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 cichoricacid and echinacocide that stimulate bone marrow and the reformation of hematopoietic stem cells (Goel et al., 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 leukocytes. Ezz, (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 Kmiec 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.

Histopathological Examination

Microscopically, spleen of rats from group 1 revealed the normal histological structure of white pulp (Photo 1). On the other hand, spleen of rats from group 2 showed lymphocytic necrosis and depletion (Photo 2). Some examined sections from group 3 revealed intravascular permeation of inflammatory cells (Photo 3). Moreover, spleen of rats from group 4 showed no histopathological alterations except extramedullary megakaryocytes in some examined sections (Photo 4). Furthermore, spleen of rats from group 5 exhibited no histopathological alterations (Photo 5). Moreover, slight lymphocytic necrosis and depletions in some follicles was the only histopathological lesion observed in spleen of rats from group 6 (Photo 6). Additionally, spleen of rats from group 7 revealed no histopathological alterations (Photo 7). Furthermore, spleen of rats from group 8 exhibited no histopathological alterations except slight lymphocytic necrosis and depletion in some sections (Photo 8).

Immuno-totoxicity may parallel alterations in the normal microscopic architecture of lymphoid organs. However, splenic immunosuppression may attribute to the decreased lymphatic cells numbers in the spleen and other immune organs (Pearse et al., 2009 and Monfared et al., 2014). Menezes et al., (2005), indicated that all extensive injuries were repaired with collagen fibers which may lead to the fibrosis as observed here. Crâciun and Pașca, (2014), reported that cisplatin had a significant toxic effect in both central (thymus) and peripheral (spleen) lymphoid organs. On the other hand, Susan, (2006) reported that the Echinacea treatment markedly ameliorated the altered histopathological features in the spleen tissues. This observed effect may be due to the antioxidant properties of various constituents (e.g., polysaccharides and flavonoids) present in the Echinacea extract. As well as, Echinacea is a potent scavenger of a variety of free radicals and a restoration agent in the production of immune cells which were decreased in positive control group. This observed effect may be due to the antioxidant properties of various constituents (e.g., polysaccharides and flavonoids) present in the Echinacea extract. Furthermore, Dwivedi et al., (2011), reported that the eugenol present in clove extract is an effective cytotoxic agent for different type of cancer cells and it is endowed with apoptotic inducing capability.

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.

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>Groups</th>
<th>Parameters</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±1.04a</td>
<td>0.88±0.05a</td>
<td></td>
</tr>
<tr>
<td>2- Control (+ve)</td>
<td>16</td>
<td>0.029±0.001a</td>
<td>14.06±0.93a</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±2.22c</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±1.20c</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±1.74d</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±1.93b</td>
<td>0.77±0.16b</td>
<td></td>
</tr>
</tbody>
</table>
**The Effect of Echinacea purpurea and Clove (Syzygium aromaticum) Extracts on Raising the Immune Response in Rats with Immune Deficiency Diseases**

Section A - Research paper

*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.

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

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

**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>
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<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>1- Control (+ve)</td>
<td></td>
<td>5.30±0.57e</td>
<td>5.41±0.12a</td>
<td>4.83±0.19a</td>
<td>1.84±0.07a</td>
<td>1.37±0.02a</td>
<td>1.50±0.05a</td>
</tr>
<tr>
<td>2- Control (+ve)</td>
<td></td>
<td>12.03±0.24a</td>
<td>0.79±0.02a</td>
<td>1.01±0.05f</td>
<td>0.35±0.02d</td>
<td>0.30±0.03f</td>
<td>0.19±0.01e</td>
</tr>
<tr>
<td>3- 1ml Echinacea</td>
<td></td>
<td>11.22±0.18b</td>
<td>1.52±0.19d</td>
<td>1.87±0.05e</td>
<td>0.49±0.03cd</td>
<td>0.55±0.02de</td>
<td>0.33±0.01de</td>
</tr>
<tr>
<td>4- 2ml Echinacea</td>
<td></td>
<td>8.40±0.15c</td>
<td>2.83±0.09c</td>
<td>2.80±0.09d</td>
<td>0.56±0.01c</td>
<td>0.71±0.01cd</td>
<td>0.47±0.03d</td>
</tr>
<tr>
<td>5- 1ml Clove</td>
<td></td>
<td>10.88±0.34b</td>
<td>1.73±0.09d</td>
<td>1.69±0.09e</td>
<td>0.46±0.01cd</td>
<td>0.49±0.01e</td>
<td>0.29±0.01de</td>
</tr>
<tr>
<td>6- 2ml Clove</td>
<td></td>
<td>7.99±0.26c</td>
<td>2.71±0.14e</td>
<td>2.91±0.03cd</td>
<td>0.63±0.03d</td>
<td>0.80±0.01bc</td>
<td>0.51±0.01d</td>
</tr>
<tr>
<td>7- 1ml E:C</td>
<td></td>
<td>6.73±0.15d</td>
<td>3.03±0.04c</td>
<td>3.10±0.06e</td>
<td>0.58±0.04b</td>
<td>0.79±0.04bc</td>
<td>0.88±0.01c</td>
</tr>
<tr>
<td>8- 2ml E:C</td>
<td></td>
<td>6.33±0.12d</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>
</tr>
</tbody>
</table>
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*Mean values at the same column with the same superscript letters are not statistically significant at P<0.05.
*E:C= Echinacea: Clove.

HISTOPATHOLOGICAL EXAMINATION

Photo 1: Spleen of rat from group 1 showing the normal histological structure of white pulp (H & E X 400).

Photo 2: Spleen of rat from group 2 showing lymphocytic necrosis and depletion (H & E X 400).

Photo 3: Spleen of rat from group 3 showing intravascular permeation of inflammatory cells (H & E X 400).

Photo 4: Spleen of rat from group 4 showing extramedullary megakaryocytes (H & E X 400).

Photo 5: Spleen of rat from group 5 showing no histopathological alterations (H & E X 400).

Photo 6: Spleen of rat from group 6 showing slight lymphocytic necrosis and depletion (H & E X 400).

Photo 7: Spleen of rat from group 7 showing no histopathological alterations (H & E X 400).

Photo 8: Spleen of rat from group 8 showing slight lymphocytic necrosis and depletion (H & E X 400).

REFERENCES


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