Ameliorative effect of *Echinacea purpurea* and curcumin on dexamethasone-induced immunosuppressive rabbits

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Abstract

**Objectives**: This study was delineated to investigate the ameliorative effect of *Echinacea purpurea* and curcumin against oxidative stress and immunosuppression induced by dexamethasone in rabbits.

**Methods**: A total of 20 healthy rabbits were used in this study and were randomly classified into 4 groups of 5 each. The 1st group (non-treated) was served as a control, the 2nd group intramuscularly medicated with dexamethasone (2 mg/kg) 3 times at 6 hours interval, the 3rd group medicated with dexamethasone at the same dose then received *E. purpurea* (130 mg/kg) orally day after day for 21 days, and the 4th group medicated with dexamethasone at the same dose then received curcumin (400 mg/kg) orally every day for 21 days. Blood and serum samples were collected after 1, 7, and 14 days post-dosing.

**Results**: Dexamethasone medicated group showed a significant decrease in WBCS, lymphocytes, neutrophils, and monocytes at the 1st, 7th, and 14th day, but *E. purpurea* and curcumin medicated groups showed significant improvement in total and differential leucocytic count. Also, both plants showed a significant increase in lysozymes and nitric oxide against the suppressive effect of dexamethasone. Here, the dexamethasone group showed a significant decrease in serum catalase (CAT) and significant elevation in serum malondialdehyde (MDA) at 1st and 7th-day post-treatment. *E. purpurea* and curcumin groups showed a significant increase in serum CAT and significant depletion in MDA levels. Injection of dexamethasone significantly decreased the serum levels of total protein, albumin, and globulin as compared to the control group. These parameters were significantly increased after treatment with *E. purpurea* and curcumin. Treatment with *E. purpurea* or curcumin ameliorated tissue damages of liver, kidney, and spleen induced by dexamethasone.

**Conclusion**: Treatment with *Echinacea purpurea* or curcumin could ameliorate oxidative stress and immunosuppression induced by dexamethasone in rabbits.

**Keywords**: *Echinacea purpurea*, curcumin, oxidative stress, immunomodulatory, rabbit.

1. Introduction

Rabbit's industry plays an important role in Egypt economic development, as it provides high-quality flesh which is characterized by less fat, easily digestible, and higher nutritional value. Rabbits contribute to fur production and were used for the production of antibodies and antisera using in different research aspects (Abdelhady et al., 2017, Gaber, 2016).

Medicinal plants are extensively used nowadays in the treatment of many diseases (Elgazar et al., 2018; Choi et al., 2011; Selim et al., 2019). Medicinal herbal plants, such as Echinacea, curcumin, garlic, onion, ginger, and astragalus, are used as immunomodulators (Abu Khudir et al., 2019; El-Magd et al., 2017; Sharma et al., 2017). Echinacea purpurea is a medicinal herb that contains phytochemicals such as polysaccharides, flavonoids, caffeic acid derivatives (chicoric acid), essential oils, polyacetylenes, and alkyl amides, with a particular immunostimulatory effect for polysaccharides and chicoric acid (Manayi et al., 2015). Curcumin has strong antioxidant, anti-inflammatory, hepato-renal protective, immunostimulant effect. It can modulate T lymphocytes, neutrophil, B lymphocytes, dendritic cells, and macrophages to regulate the release cytokines and chemokines proinflammatory (He et al., 2015).

This study aimed to investigate the possible effects of Echinacea and curcumin on dexamethasone injected rabbits through the evaluation of hematological, biochemical, immunological parameters, and the associated pathological lesions.

2. Materials and methods

2.1. Materials

Echinacea purpurea was purchased from Arab company for pharmaceuticals and medicinal plants, Egypt, in the form of dry extract capsules (175mg/capsule). Curcumin was purchased from EL-
Gomhoria, Egypt in the form of powder (10g/ bottle). Dexamethasone sodium phosphate ampoules (8mg Dexa/ ampule) was brought from AMRIYA for pharmaceutical industries, Alexandria, Egypt.

2.2. Experimental design

This study was performed on twenty healthy white New Zealand rabbits (1.8-2.0kg) obtained from the Faculty of Agriculture, Mansoura University. After one week of adaptation, they were randomly divided into 4 groups. The control (G1) non-medicated, the dexamethasone group (G2) injected intramuscularly with dexamethasone at a dose of 2 mg/kg body weight 3 times at 6-hour intervals (Jeklova et al., 2008), (G3) medicated with dexamethasone at the same dose then supplemented with E. purpurea at dose 130 mg /Kg b. w orally after day (Farid, 2010) for 21 days, and (G4) medicated with dexamethasone at the same dose then supplemented with curcumin at a dose of 400 mg/kg/day for 21 days (Sadjarwo et al., 2011).

2.3. Blood and tissue sampling

Two blood samples were collected from the ear vein at the 1st, 7th, and 14th day of dexamethasone therapy. Blood samples were collected either in EDTA-coated tubes for hematological studies or in plain tubes for serum separation to determine some immunological, oxidative stress, and biochemical parameters.

At the end of the experiments, rabbits were slaughtered and liver, kidney, and spleen specimens from rabbits of each group were carefully examined by the naked eye for the detection of any abnormalities. Small specimen of these organs were taken and immediately fixed in 10% formalin for histopathological examination.

2.4. Hematological and biochemical parameters

The serum lysozymes levels were assayed according to Metcalf and Deibelt (1969) and serum nitric oxide according to Montgomery and Dymock (1961). Catalase was determined according to Aebi and Bergmeyer (1984), while Malondialdehyde was determined according to Kei (1978). Serum total protein was determined according to Doumas and Biggs (1971).

2.5 Histopathological examination

The fixed liver, kidney, and spleen specimens were dehydrated in ascending grades of ethyl alcohol, cleared, embedded in paraffin, sectioned (5 μm), stained by Haematoxylin and Eosin (H&E), and examined under a light microscope.

2.6. Statistical analysis

Data were analyzed using repeated measures ANOVA then one way ANOVA at each time point with post hoc Duncan multiple comparison tests (SPSS for Windows, version 20, USA). The result was considered significant at \( P < 0.05 \).

3. Results and discussion

3.1. Effect of E. purpurea and curcumin on total and differential leucocytic count

As illustrated in Table 1, dexamethasone-treated animals (G2) showed depletion in WBCs count at the 1st, 7th and, 14th day post-treatment as compared to the control group (G1). However, E. purpurea-treated group (G3) and curcumin-medicated group (G4) revealed a significant decrease in WBCs count at the 1st day as compared to G1 but they showed a significant increase in WBCs with a higher count in G4, at the 7th and 14th day as compared to G1 and G2. G2 showed a significant decrease in lymphocyte% at the 1st, 7th and 14th day relative to G1. G3 and G4 showed a significant depletion in lymphocyte% at the 1st day as compared to G1, but they showed a significant increase at the 7th and 14th day as compared to G2. While G3 and G4 showed non-significant changes in lymphocyte% on the 14th day as compared to G1. G2 revealed a significant decrease in neutrophils % on the 1st and 7th day in comparison with G1. G3 and G4 evoked a significant increase in neutrophils % on the 7th day relative to G2. Moreover, there was a significant decrease in monocytes % in G2 at the 1st, 7th, and 14th day post-dosing as compared with G1. On the other side, G2 and G3 showed a significant increase in monocyte % at the 7th and 14th day post-treatment as compared to G2. There were no significant differences in eosinophils and basophils % between groups.

The results of the present study are in agreement with Abu-Akkada (2015) who found that single administration of dexamethasone in rats markedly decrease not only the number of WBCs, but also the % of lymphocytes, monocytes, neutrophil, and eosinophil possibly due to redistribution of blood cells, a variable degree of cell death, and decrease regeneration. Also, Ahmed et al. (2019) reported a similar decrease in WBCs and lymphocytes in rabbits following oral administration of dexamethasone. Similar to our findings, Torkan et al. (2015) reported that the administration of Echinacea to rats evoked a significant increase in WBC, neutrophil, and monocyte counts and phagocyte activity. Similarly, Sharma et al. (2011) reported that supplementation of curcumin to mice increased neutrophil, eosinophil, and lymphocytic count as compared to the control group.

3.2. Effects of E. purpurea and curcumin on serum lysozyme level

Serum lysozyme level was significantly decreased in G2 at the 1st, 7th, and 14th day as compared to G1 (Table 2). Meanwhile, E. purpurea and curcumin medicated groups showed a significant depletion in serum lysozymes on the 1st day in comparison with G1 and a significant increase in serum lysozymes level, with higher levels in G4, at the 7th and 14th day as compared to G2 (Table 2).

In the present study, dexamethasone resulted in lowering lysozymes serum levels, suggesting a prospective immunosuppressive effect. In accordance with our results, Kulkarni et al. (2016) explained the immune-suppressive effect of dexamethasone by inhibiting the expression of lysozyme and secretory leukocyte peptidase. We found a significant increase in lysozymes following treatment with Echinacea. In agreement, Nazerian et al. (2017) also found elevated serum lysozyme activity in Echinacea treated mice.

3.3. Effects of E. purpurea and curcumin on serum NO level

Serum NO level was significantly decreased in G2 at the 1st, 7th, and 14th day (Table 2). Meanwhile, E. purpurea and curcumin medicated groups showed a significant depletion in serum NO level at the 1st day relative to G1 and a significant elevation in NO level on the 7th and 14th day post-treatment as compared to G2 (Table 2).

The findings of this study are consistent with those of Mondo et al. (2006) and Ong et al. (2009) who found that plasma nitrate/nitrite, a marker of total body NO synthesis, were reduced in rats and mice injected with dexamethasone. In addition, Fouad et al. (2009) also stated that dexamethasone significantly decreased NO production, and iNOS mRNA and protein expression. Similar to our results regarding induction of NO by E. purpurea, Ezzi (2011) and Martin et al. (2012) also found induction of NO production by E. purpurea. Furthermore, Huang et al.
(2013) and Ragab et al. (2014) also observed a significant increase for NO and antioxidant enzymes following curcumin feeding.

3.4. Effects of *E. purpurea* and curcumin on serum CAT and MDA levels

G2 showed a significant decrease in serum catalase (CAT) activities on the 1st and 7th day as compared to G1. On the other hand, G3 showed a significant decrease in serum CAT activity on the 1st day relative to G1, then elicited a significant increase on the 7th and 14th day post-dosing as compared to G2. Meanwhile, G4 showed a significant increase in serum CAT activity on the 7th and 14th day as compared to G2 (Table 3). G2 showed a significant increase in serum MDA levels at the 1st, 7th, and 14th day in comparison to G1 (Table 3). G2 showed a significant increase in serum MDA levels at the 1st, 7th, and 14th day in comparison to G1 (Table 3). While, G3 and G4 showed a significant decrease in serum MDA at the 1st, 7th, and 14th day as compared to G2.

Our data agreed with Fouad et al. (2009) and Hasona (2018) who reported that dexamethasone administration could evoke a significant decrease in CAT activity. We and both Ezz (2011) and Mishima et al., (2004) found a significant increase in activity of CAT in *E. purpurea*-treated animals. Additionally, we and Sankar et al. (2012) found that curcumin could also significantly increase the CAT level in animals. Similar to our findings, El-Sawy et al. (2018) found a significant increase in serum MDA in dexamethasone-treated rats. *E. purpurea* effect may be due to their antioxidant properties and phenolic compounds (Sharma et al.2009). Curcumin effect also mediated via its antioxidant properties, scavenging of free radicals and/or via preventing lipid peroxidation. Curcumin protective effect was also reported against cisplatin-induced testicular damage and oxidative stress in rabbit (Huang et al., 2009 and Alagawany et al., 2015).

3.5. Effects of *E. purpurea* and curcumin on serum biochemical parameters

G2 showed a significant depletion in serum total protein levels on the 1st, 7th, and 14th. Meanwhile, at 1st, 7th, and 14th day, G3 and G4 showed a significant increase in total protein as compared with G2 (Table 4). There was a significant decrease in the serum albumin level in G2 as compared with G1 at the 1st, 7th, and 14th day. While, G3 and G4 showed a significant increase in serum albumin levels at the 1st, 7th, and 14th day as compared to G2. However, G3 and G4 showed non-significant changes in albumin levels as compared to G1 (Table 4). The obtained results evoked a significant decrease in serum globulin levels at the 1st, 7th and 14th in G2 in comparison with G1. On the other hand, G3 and G4 showed a significant increase in serum globulin level at the 7th and 14th day as compared to G2 (Table 4).

Consistent with our findings regarding the inhibitory effect of dexamethasone on total proteins, and globulin, Abd Elaazem and Abo-Kora (2015) also found that dexamethasone induced a significant decrease in total protein, gamma globulin, total globulin beside insignificant decrease in albumin. Similar to our results, Hussein et al. (2014) also reported a significant increase in total protein and globulin in rabbits given Echinacea. Moreover, Radwan et al. (2019) reported that oral administration of Echinacea with dexamethasone resulted in a great normalization in the levels of liver’s protein. Similarly, Diab et al. (2014) found that curcumin induced a significant increase in total protein level and ameliorated the gentamicin-induced decrease in serum total protein, albumin, and albumin/globulin ratio.

3.6. Histopathological findings

Histologically, liver sections of G1 showed normal hepatocytes (arrows, Fig.1A, B). G2 revealed degenerative changes of hepatocytes, intralobular fibroblastic proliferation forming bridging fibrosis (arrows, Fig.1C, D). G3 showed normal hepatocytes which radially arranged around the central vein (arrows, Fig.1E, F). G4 revealed normal hepatocytes with very mild degenerative changes (arrows, Fig.1G, H).

Our histopathological findings in the liver (degenerative changes and intralobular fibroblastic proliferation) are in accordance with those reported by Hussain et al. (2014) and Noel (2013) who found a similar histopathological picture in addition to necrosis of hepatocytes and congestion of sinusoids with fatty changes. These changes were ameliorated following treatment with Echinacea or curcumin. In consisent, Rezaie et al. (2013) and Hashem et al. (2019) found that the infected broiler chicks supplemented with *Echinacea purpurea* showed slight degeneration of hepatocytes and slight inflammatory cell infiltration. El-Agamy (2010) and Kyung et al. (2018) also reported that the oral administration of curcumin ameliorated aflatoxin-B1 induced liver damage in rats and counteracts oxidative stress caused by aflatoxin. Also, confirmed that curcumin ameliorates liver cirrhosis by its anti-inflammatory and ant fibrotic effect.

Histopathological examination of kidney tissue of G1 showed normal renal structure (Fig. 2A, B). In contrast, G2 showed congestion in glomeruli and degenerative changes in renal tubular epithelium lining renal tubules and proliferation of mesangial cells (Fig.2C, D). Cross section Kidney tissue of *E. purpurea*-treated group showed mild proliferation of the glomeruli and normal renal tubular epithelium lining renal tubules (Fig.2E, F). Kidney tissue exhibited normal glomeruli and renal tubular epithelium in the curcumin-treated group (Fig.2G, H).

Our results are similar to those reported by Hussein et al. (2014) who found necrosis and fibrosis in epithelial lining renal convoluted tubules with dexamethasone administration in rats. Also, our results agreed with those reported by Hashem et al (2014) who clarified that kidney of infected broiler chicks with *E.coli* and supplemented with Echinacea showed normal glomeruli with mild degeneration in the epithelial lining of renal tubules. Rezaie et al (2013) also reported a protective effect for Echinacea against diethylnitrosamine toxicity in rats with less necrotic cells in proximal tubules. Moreover, Momeni (2017) found that curcumin significantly reversed the adverse effects of sodium arsenite on the kidney.

Cross section from spleen tissue of the G1 showed normal histological structure (Fig. 3A, B). However, G2 showed depletion of lymphoid tissue and normal red pulp (Fig.3C, D). The spleen of *E. purpurea* or curcumin-treated group showed normal lymphoid aggregation and normal red pulp (Fig. 3E-H).

Our results were consistent with Xiping et al. (2010) who found spotty necrosis and depletion of lymphoid tissue in the dexamethasone-treated group and with Hashem et al. (2014) and Tarasub et al. (2009) who proved that pretreatment rats with curcumin before cadmium application prevent changes in the spleen, splenic sinusoids, while cells in red and white pulp did not show deterioration from normal appearance.
Table 1. Effects of *E. purpurea* and curcumin on total and differential leucocytic count of dexamethasone-injected rabbits.

<table>
<thead>
<tr>
<th>Variable</th>
<th>G1</th>
<th>G2</th>
<th>G3</th>
<th>G4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total leucocytic count (10^3 /μl)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1st day</td>
<td>12.2±0.1</td>
<td>10.5±1.1</td>
<td>10.9±0.3</td>
<td>10.2±0.2</td>
</tr>
<tr>
<td>7th day</td>
<td>12.5±0.1</td>
<td>10.4±0.1</td>
<td>11.6±0.1</td>
<td>10.9±0.2</td>
</tr>
<tr>
<td>14th day</td>
<td>11.3±2.0</td>
<td>10.7±0.1</td>
<td>11.9±0.1</td>
<td>12.4±0.2</td>
</tr>
<tr>
<td>Lymphocytes %</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>1st day</td>
<td>58.4±0.1</td>
<td>45.3±0.06</td>
<td>43.6±1.8</td>
<td>46.6±1.8</td>
</tr>
<tr>
<td>7th day</td>
<td>59.9±0.2</td>
<td>46.3±0.04</td>
<td>52.9±0.7</td>
<td>51.9±0.1</td>
</tr>
<tr>
<td>14th day</td>
<td>61.3±3.3</td>
<td>48.7±2.0</td>
<td>59.2±0.7</td>
<td>57.9±0.9</td>
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<tr>
<td>Neutrophils %</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>1st day</td>
<td>37.4±0.2</td>
<td>31.2±0.4</td>
<td>33.4±0.2</td>
<td>32.8±0.07</td>
</tr>
<tr>
<td>7th day</td>
<td>38.1±0.3</td>
<td>32.3±0.1b</td>
<td>36.9±0.8</td>
<td>37.8±0.2</td>
</tr>
<tr>
<td>14th day</td>
<td>38.9±2.0</td>
<td>34.1±2.9</td>
<td>37.9±5.0</td>
<td>38.9±0.4</td>
</tr>
<tr>
<td>Monocytes %</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1st day</td>
<td>3.2±0.2</td>
<td>2.2±0.3</td>
<td>2.8±0.3</td>
<td>2.5±0.2</td>
</tr>
<tr>
<td>7th day</td>
<td>3.6±0.06</td>
<td>1.7±0.05</td>
<td>2.9±0.02</td>
<td>2.8±0.01</td>
</tr>
<tr>
<td>14th day</td>
<td>3.4±0.1</td>
<td>1.8±0.03</td>
<td>3.3±0.01</td>
<td>3.1±0.02</td>
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<tr>
<td>Eosinophils %</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>1st day</td>
<td>0.08±0.03</td>
<td>0.00±0.00</td>
<td>0.00±0.00</td>
<td>0.02±0.01</td>
</tr>
<tr>
<td>7th day</td>
<td>0.4±0.02</td>
<td>0.00±0.00</td>
<td>0.06±0.04</td>
<td>0.00±0.00</td>
</tr>
<tr>
<td>14th day</td>
<td>0.03±0.02</td>
<td>0.02±0.01</td>
<td>0.03±0.02</td>
<td>0.01±0.01</td>
</tr>
<tr>
<td>Basophils %</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1st day</td>
<td>0.06±0.04</td>
<td>0.00±0.00</td>
<td>0.00±0.00</td>
<td>0.02±0.02</td>
</tr>
<tr>
<td>7th day</td>
<td>0.4±0.02</td>
<td>0.00±0.00</td>
<td>0.04±0.01</td>
<td>0.03±0.02</td>
</tr>
<tr>
<td>14th day</td>
<td>0.06±0.01</td>
<td>0.01±0.08</td>
<td>0.1±0.01</td>
<td>0.01±0.03</td>
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Means within the same row carrying different superscript letters are significantly different at *P*<0.05. Data were presented as mean ± SE.

Table 2. Effects of *E. purpurea* and curcumin on lysozymes and nitric oxide of dexamethasone-injected rabbits.

<table>
<thead>
<tr>
<th>Group</th>
<th>Lysozymes (mg/dl)</th>
<th>NO (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1st day</td>
<td>7th day</td>
</tr>
<tr>
<td>G1 (control)</td>
<td>32.3±1.8a</td>
<td>31.4±1.7b</td>
</tr>
<tr>
<td>G2 (Dex)</td>
<td>27.6±1.4b</td>
<td>26.9±1.3c</td>
</tr>
<tr>
<td>G3 (Dex + E. purpurea)</td>
<td>28.1±1.6b</td>
<td>36.8±1.5b</td>
</tr>
<tr>
<td>G4 (Dex + curcumin)</td>
<td>26.5±0.9b</td>
<td>41.5±1.9a</td>
</tr>
</tbody>
</table>

Table 3. Effects of *E. purpurea* and curcumin on serum CAT activity and MDA level of dexamethasone-injected rabbits.

<table>
<thead>
<tr>
<th>Group</th>
<th>CAT (U/dl)</th>
<th>MDA (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1st day</td>
<td>7th day</td>
</tr>
<tr>
<td>G1 (control)</td>
<td>4.4±0.18a</td>
<td>4.2±0.12b</td>
</tr>
<tr>
<td>G2 (Dex)</td>
<td>3.3±0.03b</td>
<td>2.9±0.02c</td>
</tr>
<tr>
<td>G3 (Dex + E. purpurea)</td>
<td>3.2±0.08b</td>
<td>5.9±0.23c</td>
</tr>
<tr>
<td>G4 (Dex + curcumin)</td>
<td>3.9±0.04b</td>
<td>4.9±0.5a</td>
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Table 4. Effects of *E. purpurea* and curcumin on serum total protein, albumin, and globulin level of dexamethasone-injected rabbits.

<table>
<thead>
<tr>
<th>Group</th>
<th>Total protein (gm/dl)</th>
<th>Albumin (gm/dl)</th>
<th>Globulin (gm/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1st day</td>
<td>7th day</td>
<td>14th day</td>
</tr>
<tr>
<td>G1</td>
<td>6.9±0.2a</td>
<td>6.5±0.4b</td>
<td>6.9±0.2b</td>
</tr>
<tr>
<td>G2</td>
<td>5.9±0.5a</td>
<td>5.7±0.4c</td>
<td>5.6±0.42c</td>
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<tr>
<td>G3</td>
<td>6.3±0.1b</td>
<td>7.4±0.3a</td>
<td>7.8±0.1a</td>
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<tr>
<td>G4</td>
<td>6.5±0.3b</td>
<td>6.9±0.2ab</td>
<td>7.1±0.8a</td>
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</table>
Figure 1. Microscopic picture of liver sections of control group (A and B), Dexamethasone-treated group (C and D), Echinacea-treated group (E and F), and curcumin-treated group (G and H) (H&E, 100x in all image except F which is 400x).

Figure 2. Microscopic picture of kidney sections in control group (A and B), Dexamethasone-treated group (C and D) which showed congestion in glomeruli (arrow) and degenerative changes in renal tubular epithelium and proliferation mesangial cells, Echinacea-treated group (E and F) which showed mild proliferation of glomeruli (arrow) and normal renal tubular epithelium, and curcumin-treated group (G and H) which showed normal glomeruli (arrow) and normal tubular epithelium, HE, 400x.

Figure 3. Microscopic picture of spleen sections showed normal lymphoid tissue in control group (A and B), depletion of lymphoid tissue (arrow) and normal red pulp in dexamethasone treated group (C&D), normal lymphoid aggregation (arrow) and normal red pulp in Echinacea-treated group (E&F), and normal lymphoid aggregation (arrow) and normal red pulp in curcumin treated group (G&H), HE, 100x.
4. Conclusion

Administration of *E. purpurea* or curcumin can reduce the adverse effects of dexamethasone in rabbits. This ameliorative potential could be mediated at least in part through the restoration of biochemical, histological, hematological, and immunological parameters altered by dexamethasone. Therefore, further clinical trials are required to verify this protective/therapeutic role for *E. purpurea* and curcumin.

Conflict of interest statement

The authors declare no conflict of interest in the current research work.

Animal ethics committee permission

The current research work is permitted to be executed according to standards of animal research committee in the Faculty of Veterinary Medicine, Mansoura University.

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