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EFFECTIVENESS OF *Echinacea purpurea* EXTRACT ON IMMUNE DEFICIENCY INDUCED BY AZATHIOPRINE IN MALE ALBINO RATS

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Abstract

This study was performed to evaluate the effectiveness of Echinacea purpurea (E.P.) on azathioprine (AZA)-induced immune deficiency in albino rats. Thirty six male albino rats were divided into six equal groups. The first group served as normal control, the second and third groups were treated with two doses of AZA (3 and 5mg/kg/b.w/day IP), respectively for six weeks. The fourth group was treated with 50 mg kg/b.wt/day of Echinacea. The fifth and sixth groups were treated with3 and 5 mg AZAm respectively followed by50 mg E.P. administration. At the end of the experimental period, both doses of AZA revealed a significant reduction in total body and spleen weights, increase in tissue total protein with a significant increase in serum total protein and albumin, a marked decrease in the number of WBCS associated with a decrease in the number of lymphocytes, a significant decrease in serum total anti-oxidant capacity. Also,concentration of immunoglobulins (IgG and IgM) and interleukins (IL4 &IL6) showed a significant increase, while the level of IL10 decreased significantly in splenic tissue. The dose of AZA (5 mg /kg b.wt.) only resulted in a highly significant increase in serum level of T3 and T4. However, treatment with Echinacea purpurea extract had a significant influence on immune deficiency induced by azathioprine. These findings demonstrated that E.P. extract is a promising immunomodulatory agent with a potent therapeutic value in stimulating the immune response.

Keywords: Azathioprine. Cytokines. *Echinacea purpurea*. Immunoglobulin. Rats.

1. Introduction

Azathioprine (AZA) is widely used in the treatment of cancer and inflammatory conditions (Aarbakke et al. 1997), as well as in the therapy of organ transplant patients (Weinshilboum 2001). This drug possesses both cytotoxic and immunosuppressive properties (Ginestal et al. 2019). Upon its administration, it is rapidly converted into several compounds, including the active6-MP and exerts its effects by several mechanisms, including the inhibition of DNA synthesis, purine metabolism, nucleotide synthesis, (Shin et al. 2014).

The immune system is one of our most complex biological systems in the body. It is a network of cells, tissues, and organs that work together to protect the body against attacks by “foreign” invaders in some instances. The organs of the immune system are positioned throughout the body. They are called lymphoid organs. The primary lymphoid tissues are the bone marrow and thymus. The secondary lymphoid tissues are the lymph nodes, spleen, and lymphoid tissues accompanied with mucosa and skin (Charles et al. 2001). Immune responses require coordinated action of leukocytes that travel the body and communicate via direct contact and/or via production as well as receipt of soluble proteins and cytokines. Immune system involves

in the functions of various types of cells including immunostimulants and immunosupperessors (Haque et al. 2013). Immunity needs to be modulated by stimulating the immune response to increase resistance to infection and by suppressing the immune response to treat autoimmune diseases, to prevent rejection of graft and hypersensitivity reactions (Choudhary 2015; Nagoba and Davane 2018).

Several plants and Herbs are being used as immunomodulators (Nagoba and Davane 2018). *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, anti-oxidation, and immunomodulation effects (Chiou et al. 2017; Khattab et al. 2019). *Echinacea purpurea* contains active ingredients of carbohydrate, glycoside, alkaloide, alkylamide, and polyacetylene (Lalone et al. 2007). Echinacea is commonly used for the prevention and treatment of upper respiratory tract infections (URTIs), viral, bacterial, and fungal infections and it is also used as a complementary therapy to support the immune system, (Goldhaber-Fiebert and Emper 1999).

The present study was undertaken to investigate the immunomodulation effects of *Echinacea purpurea* extract against AZA -induced immunosuppression in male albino rats.

2. Material and Methods

Animals

A total of thirty-six male albino rats, about ten- to thirteen-week-old, weighting 180 – 240 gm, were obtained from animal house at the Egyptian Atomic Energy Authority. The animals were housed in cages with standard diet and tap water *ad libitum* and acclimated for one week before the experiment. The experimental protocol was approved by the Research Ethics Committee, serial number 14 A/19 of the National Center for Radiation Research and Technology, the Atomic Energy Authority, Egypt.

Drugs

A commercially available formulation of AZA tablets (50 mg) was used and purchased from ACDIMA Arab Co for Drugs Industries and Medical Appliance, 4 Ard El-Fawwelah ST, Abdin, Daher, Cairo, Egypt. *Echinacea Purpurea* was purchased from Harraz – Agricultural Seeds, Spices and Medical Plants Company, Cairo**,** Egypt.

Extraction procedure

According to the method of Al-Manhel and Niamah (2015), 2g of dried *Echinacea purpurea* was freshly prepared, soaked in 40 ml of boiled hot water and left for 24 h. then the extract was filtered using filter paper.

Experimental design

The animals were equally divided into six groups as follows: group (1) normal control rats, group (2) rats received intraperitaneal (i.p.) injection of 3mg AZA /kg/bw/day for 6 weeks, group(3) rats received i.p.injection of 5mg AZA/kg/day for 6 weeks, group (4) rats received *Echinacea* extract by oral gavage 50mg/kg/bw/day for 6weeks, groups (5) and (6) received i.p. 3mg and 5 mg AZA/kg/day, respectively followed by oral administration of 50mg *Echinacea* extract/kg for 6 weeks.

At the end of the experimental period, total body weight was recorded, and then rats were sacrificed by cervical decapitation. Spleens were removed and weighted for each animal. Parts of spleen were fixed with 10% neutral formalin for subsequent histological examination.

Subsection

Paste the methodology (Figure 1). The authors can and should use short subheadings, especially those concerning the reporting guideline items. As needed, use italic, superscript and subscript, but do NOT use boldface. Do NOT insert page or section breaks.

Preparation of spleen Homogenate

One gram spleen from each rat was homogenized in 10 mL ice cold homogenate buffer (0.3 Msucrose and phosphate buffer; pH 7.4) using a Teflon pestle connected to a Braun Homogenizer Motor (25 strokes/min at 1000 rpm). The homogenate was centrifuged at 30,000 x g for 30 min at 4°C to remove cell debris and nuclei. The resulting supernatant was used for biochemical analysis.

Measurement of biochemical parametersin spleen tissue

Total protein was determined by a colorimetric method according to the principle of Gornall et al. (1994) using a biodiagnostic kit supplied by the Egyptian Company of Biotechnology. The levels of immunoglobulins (IgG and IgM) were determined using commercial Elisa kits according to Vos et al. (1979). IL4, IL6 and IL10 were measured according to the methods of Brown and Hural (1997), Hirano (1998) and Rutz et al. (2011), respectively.

Hematological examination

A complete blood picture was counted in whole blood samples by manual method according to Dacie and Lewis (2016).

Determination of biochemical parameters in serum

Serum samples were separated to measure; total protein by the method of Lowry et al. (1951) and serum albumin was measured according to the method of Doumas et al. (1971). Determination of total antioxidant capacity was measured according to the method of Koracevic et al. (2001). Serum total T3 and T4 were estimated using radioimmunoassay technique according to the method of Larsen (1972) and Tietz (1995), respectively.

Histological examination

Specimens from spleen were fixed in 10% neutral buffer formalin. Sections of 5 micron thickness were stained by hematoxylin and eosin (H and E) and examined microscopically according to Bancroft et al. (1997).

Statistical analysis

Data were expressed as mean ±SE. The statistical significance among groups was analyzed using one-way ANOVA followed by Duncan's multiple –range test using SPSS, 20 software. Results were considered significant if the P-value ≤ 0.05.

3. Results

As shown in table 1, the weights of total body and spleen were significantly decreased ($p \leq 0.05$) in the azathioprene groups as compared to the control group. These levels were reversed in the groups treated with Echinacea administration. Echinacea increased these values significantly in groups previously treated with both doses of AZA.

On the other hand, total protein in splenic tissue was significantly increased under the effect of AZA compared to the control. However, Echinacea administration significantly reduced tissue total protein as compared to both doses of AZA. Serum total protein showed a significant increase in the group administered 3 mg AZA ($p \leq 0.05$) compared to the control. Treatment with Echinacea caused a high significant elevation in serum total protein compared to the control. As for albumin level, 5 mg AZA significantly decreased albumin level. Echinacea+ AZA groups significantly decreased albumin level compared to the control.

Table 1. Effect of Echinacea on body weight, spleen weight, tissue total protein, serum total protein, and albumin of immunodeficient albino rats.

	Control	Azathioprene 3 mg/kg	Azathioprene 5 mg/kg	Echinacea purpurea	Echinacea + Azathioprene 3 mg/kg	Echinacea + Azathioprene 5 mg/kg
B.wt (g)	339.3 ± 10.55^{b}	208.66 ± 7.97^{d}	273.33 ± 17.21^{c}	431.5 ± 20.94^{a}	349.33 ± 10.82^{b}	380.66 ± 19.34^{b}
Spleen wt. (g)	1.11 ± 0.032^{a}	0.546 ± 0.049^{c}	0.766 ± 0.045^{b}	1.054 ± 0.096^{a}	0.855 ± 0.037^{b}	0.79 ± 0.044^{b}
Tissue total protein (mg/g)	83.8 ± 3.24^{d}	152.91 ± 5.39^{b}	197.38 ± 5.04^{a}	89.33 ± 0.68^{d}	122.33 ± 5.82^{c}	110.6 ± 0.68^{c}
Serum Total protein (mg/dl)	9.21 ± 0.31^{b}	10.41 ± 0.41^{a}	8.32 ± 0.18^{b}	11.02 ± 0.38^{a}	8.54 ± 0.39^{b}	8.68 ± 0.35^{b}
Serum Albumin (mg/dl)	3.35 ± 0.06^{a}	3.64 ± 0.17^{a}	2.91 ± 0.14^{b}	3.35 ± 0.12^{a}	2.95 ± 0.65^{b}	2.98 ± 0.06^{b}

Values are expressed as means ± SEM (n=6). Values in the same row with different superscripts are significantly different ($p \leq 0.05$).

The results shown in table 2 revealed non-significant changes in Hb, Hct, MCHC with a significant decrease in RBCs and a significant increase in MCV, MCH in AZA treated groups compared to the control. Echinacea administration led to significant increases ($p \leq 0.05$) in Hb, MCH, MCHC levels and significant decreases in MCV count compared to the control. *Echinacea purpurea* administration did not affect Hct, RBC's levels compared to the control. It is noticed that the two doses of AZA had the same significant effect on Hb, Hct, RBCs, MCV, MCH and MCHC compared to the control.

Table 2. Effect of Echinacea on Hb, Ht, RBC's, MCV, MCH and MCHC of immunodeficient albino rats.

	Control	Azathioprene 3 mg/kg	Azathioprene 5 mg/kg	Echinacea purpurea	Echinacea + Azathioprene 3 mg/kg	Echinacea + Azathioprene 5 mg/kg
Hb (g/dl)	13.87 ± 0.071^{c}	14.1 ± 0.34^{c}	14.34 ± 0.52^{c}	18.04 ± 0.42^{a}	16.71 ± 0.63^{b}	16.04 ± 0.52^{b}
Hct (%)	52.42 ± 0.75^{ab}	53.42 ± 1.21^{ab}	53.57 ± 1.73^{ab}	55.44 ± 1.34^{a}	51.45 ± 2.05^{ab}	49.74 ± 1.26^{b}
RBC's (x10^{12}/l)	5.62 ± 0.09^{b}	5.17 ± 0.09^{c}	5.13 ± 0.18^{c}	6.44 ± 0.13^{a}	5.82 ± 0.18^{b}	5.72 ± 0.19^{b}
MCV (fl)	93.1 ± 1.11^{b}	103.32 ± 1.29^{a}	104.57 ± 1.68^{a}	87.2 ± 0.49^{c}	88.18 ± 1.06^{c}	86.95 ± 0.85^{c}
MCH (pg)	24.92 ± 0.35^{c}	27.3 ± 0.74^{b}	27.94 ± 0.05^{ab}	28.12 ± 0.26^{ab}	28.64 ± 0.26^{a}	28.98 ± 0.04^{ab}
MCHC (%)	26.77 ± 0.35^{b}	26.45 ± 0.9^{b}	26.98 ± 0.47^{b}	32.55 ± 0.25^{a}	32.5 ± 0.19^{a}	32.17 ± 0.30^{a}

Values are expressed as means ± SEM (n=6). Values in the same row with different superscripts are significantly different ($p < 0.05$).

It is clear from table 3 that rats treated with 3 mg AZA/kg only showed highly significant decreases ($p \leq 0.05$) in WBCs, lymphocytes and neutrophils counts as compared to the other dose, and a significant increase in monocytes compared to the control. A dose of 5mg AZA significantly increased ($p \leq 0.05$) neutrophiles count. Eiosinophiles and basophiles did not change significantly in all groups compared to the control. The role of 3 mg AZA + Echinacea was more observed as compared to the greater dose, where it ameliorated WBCs, neutrophiles, lymphocytes and monocytes.

Table 3. Effect of Echinacea on total and differential leucocytic count (WBCs) of immunodeficient albino rats.

	Control	Azathioprene 3 mg/kg	Azathioprene 5 mg/kg	Echinacea purpurea	Echinacea + Azathioprene 3 mg/kg	Echinacea + Azathioprene 5 mg/kg
WBCs ($x10^9$/l)	17.82 ± 0.59^{ab}	11.38 ± 0.58^{d}	14.85 ± 0.35^{c}	18.65 ± 0.36^{a}	17.02 ± 0.26^{b}	14.51 ± 0.38^{c}
Neutrophils	4.53 ± 0.21^{c}	3.97 ± 0.13^{e}	6.42 ± 0.21^{a}	5.16 ± 0.05^{b}	4.41 ± 0.17^{cd}	4.02 ± 0.08^{de}
Lymphocytes	12.91 ± 0.56^{a}	6.68 ± 0.40^{d}	7.93 ± 0.25^{c}	13.16 ± 0.30^{a}	12.16 ± 0.31^{a}	10.11 ± 0.26^{b}
Monocytes	0.20 ± 0.02^{b}	0.45 ± 0.02^{a}	0.25 ± 0.02^{b}	0.13 ± 0.03^{d}	0.22 ± 0.03^{bc}	0.14 ± 0.00^{cd}
Eosinophils	0.10 ± 0.03^{a}	0.18 ± 0.03^{a}	0.17 ± 0.02^{a}	0.11 ± 0.03^{a}	0.11 ± 0.03^{a}	0.13 ± 0.02^{a}
Basophils	0.10 ± 0.03^{a}	0.08 ± 0.02^{a}	0.09 ± 0.03^{a}	0.10 ± 0.03^{a}	0.09 ± 0.03^{a}	0.10 ± 0.02^{a}

Values are expressed as means ± SEM (n=6). Values in the same row with different superscripts are significantly different ($p \leq 0.05$).

Table 4 shows that the levels of TAC were significantly decreased ($p \leq 0.05$) in rats treated with both doses of AZA, whereas IgG and IgM were significantly increased ($p \leq 0.05$) compared to the control. However, administration of Echinacea tends to ameliorate TAC levels under the effect of 5 mg of AZA with a significant decrease in 3 mg of AZA compared to the control. There was no effect of Echinacea administration on IgG level in both groups treated previously with AZA with a highly significant increase compared to the control. IgM level elevated significantly ($p \leq 0.05$) in both groups treated with AZA. Echinacea administration clearly induced reduction in those elevations caused by AZA.

Table 4. Effect of Echinacea on total anti-oxidant capacity (TAC), IgG and IgM of immunodeficient albino rats.

	Control	Azathioprene 3 mg/kg	Azathioprene 5 mg/kg	Echinacea purpurea	Echinacea + Azathioprene 3 mg/kg	Echinacea + Azathioprene 5 mg/kg
TAC (nmol/mg)	29.68 ± 1.51^{b}	13.98 ± 1.01^{d}	10.52 ± 0.586^{e}	35.51 ± 2.03^{a}	23.51 ± 0.71^{c}	31.46 ± 0.36^{b}
IgG (ng/ml)	34.65 ± 0.641^{d}	100.65 ± 4.39^{c}	112.61 ± 1.11^{ab}	35.61 ± 1.98^{d}	102.7 ± 7.14^{bc}	121.46 ± 3.18^{a}
IgM (ng/ml)	45.1 ± 0.80	74.36 ± 2.65^{b}	94.73 ± 0.57^{a}	33.40 ± 0.39^{f}	54.45 ± 2.69^{d}	62.7 ± 0.24^{c}

Values are expressed as means ± SEM (n=6). Values in the same row with different superscripts are significantly different ($p \leq 0.05$).

As regards the interleukins in table 5, IL-4 and IL-6 were significantly increased ($p \leq 0.05$) in rats treated with AZA, whereas IL-10 activity was significant decreased as compared to the control. Echinacea alone significantly decreases IL-4 and significantly increases IL-10. The effect of Echinacea on IL-4, IL-6 and IL-10 levels in rats treated with AZA was clear, where IL-4 and IL-6 were significantly decreased, and IL-10 was significantly increased compared to the AZA groups.

Table 5. Effect of Echinacea on IL-4, IL-6 and IL-10 of immunodeficient albino rats.

	Control	Azathioprene 3 mg/kg	Azathioprene 5 mg/kg	Echinacea purpurea	Echinacea + Azathioprene 3 mg/kg	Echinacea + Azathioprene 5 mg/kg
IL-4 (Pg/mg)	20.78 ± 0.87^{e}	75.31 ± 1.08^{b}	107.68 ± 2.0^{a}	13.9 ± 0.07^{f}	54.75 ± 0.52^{c}	42.46 ± 2.44^{d}
IL-6 (Pg/mg)	28.3 ± 0.55^{e}	120.1 ± 0.40^{b}	135.3 ± 2.79^{a}	32.21 ± 0.44^{e}	83.48 ± 0.63^{c}	56.65 ± 2.77^{d}
IL-10 (Pg/mg)	121.66 ± 1.68^{b}	83.1 ± 7.64^{c}	67.15 ± 0.44^{d}	137.85 ± 0.51^{a}	117.51 ± 2.61^{b}	124.81 ± 2.16^{b}

Values are expressed as means ± SEM (n=6). Values in the same row with different superscripts are significantly different ($p \leq 0.05$).

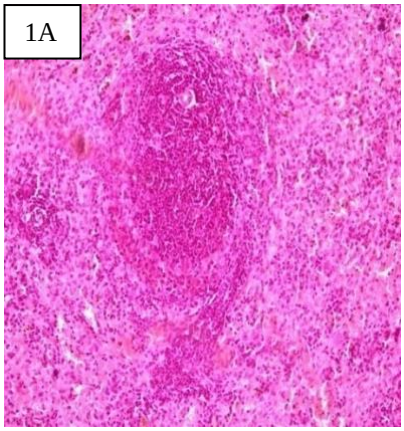
The effect of 5 mg AZA on total thyroid hormone levels is highly remarkable as recorded in table 6. Under the effect of 5 mg AZA, T3 and T4 levels were significantly increased ($p \leq 0.05$), with a non-significant change by 3 mg AZA, compared to the control. Administration of Echinacea alone and Echinacea + 3 mg AZA had no effect on T3 and T4 levels. While Echinacea administration significantly decreased T3 and T4 levels in 5 mg AZA group, their levels remained elevated as compared to the control rats.

Table 6. Effect of Echinacea on thyroid hormones of immunodeficient albino rats.

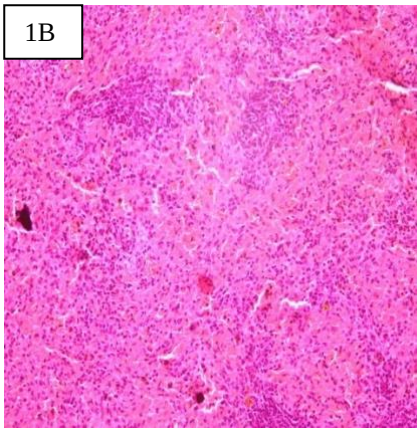
	Control	Azathioprene 3 mg/kg	Azathioprene 5 mg/kg	Echinacea purpurea	Echinacea + Azathioprene 3 mg/kg	Echinacea + Azathioprene 5 mg/kg
T3 (ng/dl)	41.5 ± 0.56^{c}	44.5 ± 0.22^{c}	179.66 ± 4.93^{a}	36.50 ± 0.22^{c}	45.66 ± 1.28^{c}	125.16 ± 11.52^{b}
T4 (ug/dl)	2.31 ± 0.018^{c}	2.70 ± 0.025^{c}	11.33 ± 0.420^{a}	2.20 ± 0.017^{c}	2.46 ± 0.067^{c}	8.26 ± 0.523^{b}

Values are expressed as means ± SEM (n=6). Values in the same row with different superscripts are significantly different ($p \leq 0.05$).

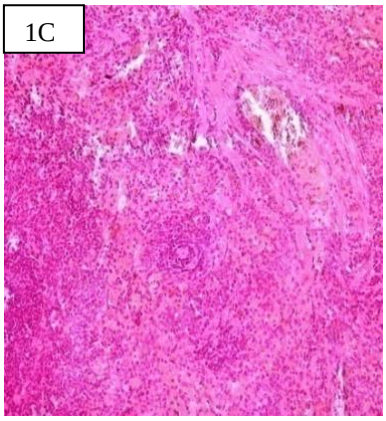
The results of histological examination, as shown in figure 1, showed a generation of the white pulp with atrophied lymphoid follicles in 3 mg AZA group and congestion in the interstitial blood vessel with thick wall together with atrophy of the lymphoid follicles in group 5 mg AZA group. The E.P. treatment markedly ameliorated the altered histopathological features in the spleen tissues. Atrophic changes related to depletion of lymphoid cells, particularly T cell subsets, are induced in spleen after treatment with AZA.



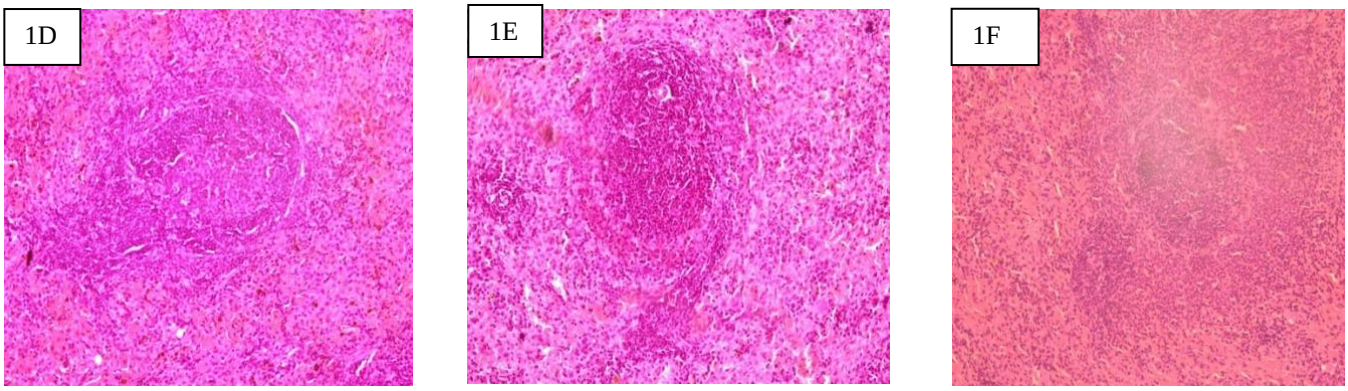
Spleen from control Group showing normal parenchyma; normal white pulp and red pulp together with healthy lymphoid follicles (H&E X 200).



Spleen from Group (3mg AZA) showing degeneration of the white pulp with atrophied lymphoid follicles (arrows) (H&E X 200).



Spleen from Group (5mgAZA) showing congestion in the interstitial blood vessel with thick wall (arrowhead) together with atrophy of the lymphoid follicles (arrow) (H&E X 200).



Spleen from Echinasea Group showing normal parenchyma; normal white pulp and red pulp together with healthy lymphoid follicles (H&E X 200).

Spleen from Group (3mg AZA + Ech) showing normal parenchyma; normal white pulp and red pulp together with healthy lymphoid follicles (H&E X 200).

Spleen from Group (5mg AZA + Ech) showing improved lymphoid follicles (arrow) with normal white pulp and red pulp, (H&E X 200).

Figure 1. Spleen histological examination. A – control Group; B – Group (3mg AZA); C – Group (5mgAZA); D – Echinasea Group; E – Group (3mg AZA + Ech); F – Group (5mg AZA + Ech).

4. Discussion

In the present study, the role of *Echinacea* as an immunomodulator against immunosuppression and toxicity induced by AZA was evaluated. Azathioprine possesses both cytotoxic and immunosuppressive properties. Cytotoxicity is believed to result from the incorporation of thiopurine metabolites into cellular nucleic acids, while the immunosuppressive effects are secondary to inhibition of de novo purine ribonucleotide synthesis and interconversion (Van Scoik et al. 1985). This drug produces free radicals in the tissues, which is one of the most important toxicity factors in organs (Lee et al. 2001). *Echinacea* exerts its pharmacological action via the modulation of nonspecific innate immune parameters such as macrophage phagocytosis and pro-inflammatory cytokine production (Barrett 2003).

In this study, the animals treated with AZA had marked decreases in their body weights. These findings agree with earlier studies, which revealed that weight loss is associated with the AZA use. Onanuga et al. (2014) administrated AZA (10 mg/kg and 20 mg/kg) to male rats for 21 days, and Ajayi et al. (2018) administrated AZA (10 mg/ kg) for four weeks and reported that AZA is an immunosuppressive agent, and the problem of cumulative toxicity is a long-range concern. That is not manifested until damage is extensive. These considerations have arisen because of its widespread use and the fact that it could account for the decrease in the body weight of animals administered AZA. Azathioprine reduced spleen weight, which agrees with the data of Matsumoto et al. (1990) and De Waal et al. (1995), who reported that the decrease in spleen weights observed in AZA-treated rats is a result of the atrophic changes detected on histopathological examination. De Waal et al. (1995) recorded that spleen was significantly reduced in weight with 0.5, 12.5 and 25 mg AZA/kg /b.wt/rats for 28 days. It was shown that the administration of Echinacea extracts stimulated the increase in weight of spleen as well as body weight in the AZA treated rats. This result agrees with the result of Ali (2008), who reported that *Echinacea* has a positive effect on body weight gain after 4 weeks of treatment.

The current results found that the total protein in the spleen tissues was significantly increased under the effect of AZA in two doses (3 mg and 5 mg AZA); the increase in splenic total protein may be due to hype atrophy in white pulp in spleen tissues (Papet 2003). The decrease of serum proteins and albumin in 5 mg AZA group could be due to AZA and its metabolites-related disruption in mechanisms such as DNA replication, transcription, translation and finally protein synthesis that are involved in synthesis of these proteins. (Hassankhani 2017).

However, Echinacea treated groups (3 and 5 mg /kg AZA + Echinacea 50 mg/kg) showed significantly reduced tissue total protein and reached near to control group. Our result agrees 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**.**

The results revealed that animals treated with AZA at both doses 3 and 5 mg/kg b.wt exhibited a significant reduction in WBCs count and lymphocyte. These results are similar to those of the previous study

by Matsumoto et al. (1990), who found that hematological examination revealed a marked decrease in the number of WBCs, which was associated with a decrease in the number of lymphocytes in rats administered AZA with doses of 0, 2.5, 12.5 and 25.0 mg/kg/day for 28 days. Also, the current results coincide with those of Ahmed et al. (2014), who reported that administration of AZA (50 mg/kg b.wt.) induced a significant decrease in RBCs count, differential count, and this because administration of AZA may cause serious side effects. However, AZA is haemotoxic causing a significant bone marrow depression, which may be manifested as leucopenia. This result agrees with result of Molyneux et al. (2008), who used a dose–response study with AZA gavaged orally 40–120 mg/kg daily for 10 days.

On the other hand, the effect of AZA on monocytes appears to be reversible, because 24-48 hours after treatment is stopped (depending on whether 3 or 200 mg/kg AZA is given), the number of monocytes starts to increase again (Andreas et al. 1975). Administration with Echinacea extract significantly reduced leucopenia induced by AZA which indicates that the extract could stimulate the haemopoetic 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. The current results indicate that the suppressive effect of E.P. on leukopenia is due to an increase in blood antioxidant activity. Irrespective of the dose, this finding also agrees with Osama et al. (2015) and Khalaf et al. (2019), who reported that E.P. treatment markedly ameliorated the altered hematological features against the negative impact of cyclophosphamide. Ezz (2011) showed that E.P. is involved in the modulation of immune response. In the present study, total antioxidant capacity (TAC) was significantly reduced in the AZA groups when compared to the control group. This reduced antioxidant production may be due to increase in oxygen metabolite that causes a decrease in the activity of the antioxidant defense system (El-Beshbishyal et al. 2010). In this study, the oral administration of Echinacea increased TAC activity in peripheral blood because of antioxidants such as echinacocide and caffeine acid in E.P., which eliminate superoxide (O_2^-) by a free radical scavenging effect (Mishima et al. 2004; Aarland et al. 2017).

In this study, increasing the number of immunoglobulins in spleen tissue was recorded as a result from the administration of AZA. This result disagrees with that of Manikandaselvi et al. (2015), who reported that administration of AZA at a dose of 10 mg/kg/21 day reduced the immunoglobulin G level in serum. In addition, Levy (1972) reported that gamma globulin synthesis is significantly reduced by AZA. Different results may be because the side effect of AZA is believed to be the causative factor for inducing the liver cirrhosis (De Boer et al. 2005).

Treatment with Echinacea extracts increased IgG and decreased the production of IgM. This result agrees with that of Rehman et al. (1999), who showed that Echinacea administration for six weeks increased IgG production in rats. The effects of immune activation by E.P. were investigated by measuring total immunoglobulin (IgG, IgM). Mishima et al. (2004) investigated the effects of immune activation by E.P. by measuring T lymphocyte subsets in the peripheral blood of mice following whole-body irradiation and reported that E.P. activates macrophages to stimulate IFN-gamma production in association with the secondary activation of T lymphocytes, resulting in decreases of IgG and IgM production. One of the most typical examples of multifunctional cytokines is IL-6 it regulates immune responses, hematopoiesis, and acute phase reactions, indicating that it plays a central role in host defense mechanism. Among many cytokines, IL-6 is the first one (Kishimoto 1989) and produced by Th2. IL-6 promotes the activation of B cells and facilitates humoral immunity together with IL-4 and IL-10 and improves the super sensitive reaction together with IL-4 (Vazquez et al. 2015). Because IL-6 is a terminal differentiation factor for B lymphocytes, it can enhance body's Ig production. IgM is an immunoglobulin with largest molecular weight existing in circulation, and it is believed to be the main immune response in the primary immune response (Suzuki and Tomasi 1979). It was observed that the increase in the level of IL6 is associated with the increase the level of immunoglobulin IgG and IgM and the decrease of its level in the Echinacea treated groups (3mg AZA+EP) and (5 mg AZA+EP). This result agrees with that of Wang et al. (2017), who suggested that E.P. might have a positive effect on the immune system.

Cytokines are glycosylated proteins that are involved in cell-cell communications and are often categorized as "pro-inflammatory" owing to their ability to induce inflammation and to anti-inflammatory

cytokines. The anti-inflammatory IL-10 is well recognized as important negative regulators of pro-inflammatory gene expression in mononuclear phagocytes.IL-10 is an immunosuppressive glycoprotein of 19–21 kDa secreted by Th2 cells, certain B cells, and activated macrophages. It is now clear that IL-10 primarily acts on activated macrophages to suppress their secretion of IL-1, IL-12, TNF-α, and reactive oxygen radicals (Isaacs 1995). In this investigation, AZA depletes the immune T cells and reduces cytokine release in various immune cells. A marked decrease in splenic IL-10 content may be due to the decline in circulating immune cells after AZA treatment. This finding agrees with that of Kim et al. (2018), who found that a significant increase in splenic IL-10 was observed with Echinacea administration, which suggested the potent immunomodulatory of Echinacea. Echinacea extracts ameliorate the depletion of anti-inflammatory interleukin 10 level in AZA groups to the normal. This coincides with the data of Gertsch et al. (2004), who revealed that the addition of Echinacea extract in human peripheral mononuclear cell cultures increased IL-10 expression. Raduner et al. (2006) concluded that the addition of Echinacea alkylamide to human peripheral whole blood cell cultures increased the expression of IL-10. This result is consistent with Zhai et al. (2007), who reported that administration of Echinacea-treated mice demonstrated a highly significant production of IL-10. It is worth mentioning that Echinacea may play an important role in the modulation of the immune system, either independently or synergistically (Dobrange et al. 2019).

The results of histological examination may support the negative effect of AZA on spleen tissue may be due to AZA. Functional polymorphisms of several enzymes involved in the metabolism of thiopurines especially AZA have been linked with toxicity (Schmaier 2008). The harmful effect of AZA on spleen tissues especially vascular degeneration may be because AZA inhibits the regeneration of cells by interrupting with DNA synthesis (Ali 2018). Also, atrophic changes related to depletion of lymphoid cells, particularly T cell subsets, are induced in spleen after treatment with AZA (Yoon et al. 2010). The E.P. treatment markedly ameliorated the altered histopathological features in the spleen tissues. The current result showed that E.P. is a potent scavenger of a variety of free radicals and a restoration agent in the production of immune cells which were decreased by AZA (Susan 2006).

It has been known for decades that the neuroendocrine system can both directly and indirectly influence the developmental and functional activity of the immune system. In contrast, far less is known about the extent to which the immune system collaborates in the regulation of endocrine activity. This is particularly true for immune-endocrine interactions of the hypothalamus-pituitary-thyroid axis. However, thyroid stimulating hormone (TSH) can be produced by many types of extra-pituitary cells-including T cells, B cells, bone marrow hematopoietic cells, intestinal epithelial cells, and lymphocytes (Klein 2006). The occurrence of hyperthyroidism in recipients receiving immunosuppressive therapy is extremely rare (Suher et al. 2004).

The present study revealed that AZA administration differently affects the thyroid hormone levels. There was no difference between 3 mg AZA group as compared to the control group, while there was a significant increase in the levels of T_3 and T_4 in 5ml AZA group. The difference in the two results may be attributed to the difference in the dose concentration. The toxicity of this drug is owing to producing free radicals in the body, which is one of the most toxicity factors. Administration of Echinacea ameliorated the increasing level of T_3 and T_4 in 5 mg AZA+ 50 mg EP group. This may be attributed to the compound of EP as polyphenol and a class of specific antioxidant known as caffeoyl derivatives, which has a protective role against side effect induced by toxic substances (Tsai et al. 2012).

5. Conclusions

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

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