Immunostimulant effects of *Echinacea purpurea* extract on cyclophosphamide induced immunosuppression in rats

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A B S T R A C T

The present study was carried out on a total number of 90 male Albino rats (180-200 gm body weight). Rats were randomly allocated into 6 groups (15 rats/group). Group (1): control negative. Group (2): (cyclophosphamide control group) treated with 50 mg / k.g B.w intramuscular. Group (3): *E.coli*-infected rats at 18th day. Group (4): *E.coli* + cyclophosphamide group. Group (5): *E.p* + cyclophosphamide + *E.coli* group. Group (6) control *Echinacea purprea (E.P.)*: gavaged with *E.p* 130 mg/k.g B.w all over the experimental period. Results showed that TLC and granulocytes percent in *E. p* treated and *E. p* control groups revealed significant increase when compared with *E. coli* + cyclophosphamide-treated group and control group; respectively. Our results revealed non-significant changes in monocytes and lymphocytes counts in *Echinacea* treated and *E. p.* control groups when compared with *E. coli* + cyclophosphamide treated and control groups respectively. Regarding the results of cytokines, the levels of IL-1β in the serum of *E. p* treated group were significantly increased when compared with *E.coli* + cyclophosphamide-treated group at the 4th and 14th days after injection of *E.coli* and third dose cyclophosphamide. Regarding the results of IL-10 in *Echinacea* treated group, the levels of IL-10 were significantly increased when compared with *E. coli* + cyclophosphamide treated group at the 4th day after injection of *E.coli* and third dose cyclophosphamide. In assessing the effect of *E. purpurea* on immunoglobulins, the levels of IgM in *Echinacea* treated group, increased significantly when compared with *E. coli* + cyclophosphamide treated group at the 4th and 14th days after injection of *E. coli* and third dose cyclophosphamide. The data of IgG levels obtained in our study revealed significant decrease in the levels of IgG in *E. p* treated group when compared with *E. coli* + cyclophoaphamide group. Therefore, is concluded that *E. purpurea* have immunostimulation effect in E. coli+ cyclophosphamide injected rats.

Keywords: *E. purpurea*, immunostimulation effect, cyclophosphamide, E. coli

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1. INTRODUCTION

Plants play an important role in the medical practices of many peoples. There is a growing interest in medicinal botanicals as part of complementary medicine. In particular, many botanicals are sold today as dietary supplements. We focus particularly on *Echinacea purpurea*. There are very few data to support such use and even less information about drug toxicity or interactions. In addition, the immunomodulatory activities of these botanicals and the mechanisms by which these botanicals might modulate the immune system are not well known. The risk of accumulation of chemotherapeutic residues in meat and their potential negative impact on human health, consumer pressure in respect to food safety, and the risk of potential cross-resistance of microorganisms to the antimicrobials used to treat humans or animals gave rise to Regulation (EC) No 1831/2003 of the European Parliament and Council which provides for a ban on the prophylactic use of coccidiostats that will go into effect in 2013. In addition to the advantages previously mentioned, herbal extracts do not carry the risk of contaminating meat with harmful substances. To date, the emergence of resistance to herbal products among coccidia species has not been reported (Arczewska-Włosek and Świątkiewicz 2012). In modern herbal medicine, Echinacea is most commonly employed for treating upper respiratory infections, particularly viral infections such as colds (rhinoviruses) and influenza (Caruso and Gwaltney 2005). However, data are in conflict regarding its efficacy, with some studies supporting effectiveness (Goel et al. 2005) and

others discounting it (Barrett et al. 2002; Turner et al. 2005). At least three different species of Echinacea are sold under that name, yet the literature is often reviewed without regard to what particular species was used. Moreover, differences arising from different extraction procedures, solvents, and plant parts used are ignored, and little distinction is made between data obtained with purified polysaccharides and those obtained with crude extracts (Borchers et al. 2000). *E.purpurea* has become one of the most popular medicinal botanicals in Europe and the United States For medicinal purposes, besides *E.purpurea* and *E.angustifolia*, a third species, *E.pallida*, is commonly used. What the American consumer calls Echinacea can be any one of the three above-mentioned species or a combination of two or even of all three of them (Hobbs et al. 1994). *E.p* and other Echinacea extracts were selective in their antibacterial activities; (ii) different organisms showed significant differences in their patterns of sensitivity; (iii) there were no correlations between chemical composition of extracts, in terms of known marker compounds, and their corresponding antibacterial activities; (iv) different preparations of Echinacea showed markedly different effects on bacteria, indicating that *E.p* has distinct mechanisms of action against each bacterium; (v) in general *E.p* can reverse the stimulation of proinflammatory cytokines regardless of the inducing bacterium or virus (Hudson 2012).

Therefore, this study will aim to evaluate the prophylactic, hematological, immunostimulatory and anti -inflammatory effects of *Echinacia purpurea* on experimentally immunosuppressed and *E.coli*-infected rats

2. MATERIALS AND METHODS

2.1. Experimental animals:

The present study was carried out on a total number of 90 male Albino rats (180-200 gm body weight). They were obtained from laboratory animal center, faculty of veterinary medicine, Benha University. They were housed for two weeks in the same environmental and nutritional conditions similar to those under which the experiment would be performed for accommodation. Rats were randomly allocated into 6 groups (15 rats/group) and housed in separate cages. Each group of rats was provided by suitable feeder and water.

2.2. Echinacea purprea.

E. purprea was obtained from Arab Company for Pharmaceuticals and Medicinal plants (MEPACO-MEDFOOD) - Enshas - Sharkia – Egypt.

2.3. Bacteria

Esherichia coli bacteria obtained from the Department of Microbiology and Immunity, Faculty of Veterinary Medicine, Benha University, Egypt. Type of *E. coli* bacteria was O125 (pathogenic) and isolated from hens. *E. coli* broth saline was prepared by suspension of *E.coli* culture in broth at concentration of 1×10^9 CFU, by using turbidimetric method according to Lee, (2009).

2.4. Cyclophosphamide

Cyclophosphamide (Endoxan) was obtained from Baxter Oncology GmbH, kantstrasse 2, D-33790 Halle, Germany.

2.5. Materials used for clinicopathological examinations:

Dipotassium salt of ethylene diamine tetra-acetic acid (EDTA): The anticoagulant was obtained from Sigma Chemical Company, (USA) and used according to Feldman et al., (2000). Automatic cell counter (H.A vet Clindiag, Belgium) was used for complete blood count. Commercial diagnostic kits used for estimation of concentration of different biochemical parameters were obtained from Qumica Clinica Alpicada (QCA) (Spain). The diagnostic kits of interleukin (IL)-1β, interleukin (IL)-10, IgG and IgM were obtained from MBS Company (USA). The diagnostic kits of IL-1β, IL-10, IgG and IgM were measured by using Spectra 2000 (USA).

2.6. Experimental design:

Group (1): control negative. It contained 15 rats treated with sterile saline (1 ml / 200 gm body weight). Group (2): (cyclophosphamide control group) treated with 50 mg / k.g B.w intramuscular (Wojcik, 2014). Group (3): *E.coli*-infected rats. 15 rats were injected with *E.coli* concentration ($(1x10^9)$ intraperitoneally at 18^{th} day. Group (4): *E.coli* + cyclophosphamide group 15 rats injected with cyclophosphamide 50 mg / k.g B.w for 3 days (16th , 17th and 18th days) + then experimentally infected with *E.coli* (1×10^9 CFU) by intraperitoneal injection (one dose) at 18^{th} day (Wojcik, 2014). Group (5): *E.p* + cyclophosphamide + *E.coli* group. 15 rats gavaged with E.p 130 mg/k.g B.w for 15 days then injected with cyclophosphamide 50 mg / k.g B.w for 3 days (16th , 17th and 18th days) then experimentally infected with *E.coli* (1×10^9 CFU) by intraperitoneal injection (one dose) at 18^{th} day. *E.p* continued with the same dose till the end of the experiment (Zhai et al., 2007 and Wojcik, 2014).

Group (6) control *Echinacea purprea*: gavaged with *E.p* 130 mg/k.g B.w all over the experimental period.

2.7. Blood Samples:

Blood samples were collected by the retroorbital bleeding method from 5 rats from each group at 24 hrs., 96 hrs. and 14 days of experiment and blood samples were divided into 2 parts, first part (0.5-1 ml) collected on dipotassium salt of EDTA-tubes for complete blood count and second part (3 ml) collected on plain tubes for serum separation.

2.8. Statistical Analysis:

Statistical analysis was performed using the statistical software package SPSS for Windows (Version 16.0; SPSS Inc., Chicago, Ill.). Student's *t*-test was used to determine significant differences between two experimental groups. The significance of differences between more than two groups was evaluated by one-way analysis of variance (ANOVA). If one-way ANOVA indicated a significant difference, then differences between individual groups were estimated using Fishe's least significant difference (LSD) test. Results are expressed as the mean ± standard error of mean (SEM). A *P*-value of less than 0.05 was considered significant (Kinner and Gray, 2008).

3. RESULTS

3.1. Changes in leukogram among the experimental rat groups:

WBCs, Granulocytes, Lymphocytes and monocytes counts in the blood of control, *E. coli*-infected, cyclophosphamide-treated rats in the absence and presence of *Echinacea* treatment were examined after 24 hours, 4 and 14 days of injection of *E.coli* and third dose cyclophosphamide (table 1).

3.2. Changes in serum cytokines (IL-1 and IL-10) and immunoglobulins (IgG and IgM) among the experimental rat groups:

Serum IL-1β, IL-10, IgG and IgM levels of control, *E.coli*-infected, cyclophosphamide-treated rats in the absence and presence of *Echinacea* and treatment were examined after 4 and 14 days of injection of *E.coli* and third dose cyclophosphamide (table 2).

Table (1): Changes in the mean values ± SE of leukogram in different experimental groups:

Parameters	Groups Collection	Control	*E.coli*	Cyc.	*E.coli* + cyc	Treated Ech.	C. Ech.
Leucocytes	First	7.16 ±0.55^{b}	10.62 ±1.69^{a}	1.44 ±0.22^{c}	1.52 ±0.45^{c}	0.9 ±0.14^{c}	–
	Second	6.7 ±0.35^{b}	10.08 ±0.43^{a}	0.85 ±0.11^{c}	0.94 ±0.24^{c}	1.32 ±0.35^{c}	–
	Third	9.84 ±0.69^{b,c}	13.88 ±1.91^{a,b}	7.45 ±0.85^{c}	15.8 ±2.45^{b,c}	18.45 ±1.87^{a}	17.18 ±1.09^{a}
Lymphocyte	First	5.70± 0.70^{a}	6.59± 1.00^{a}	1.01± 0.17^{b}	1.26± 0.41^{b}	0.68± 0.11^{b}	–
	Second	4.47± 0.54^{a}	5.25± 0.72^{a}	0.50± 0.11^{b}	0.41± 0.06^{b}	0.74± 0.18^{b}	–
	Third	6.38± 0.44^{a}	6.79± 1.22^{a}	12.18± 0.22^{a}	8.28± 1.25^{a}	9.03± 2.22^{a}	8.32± 0.34^{a}
Monocyte	First	0.94± 0.16^{a}	0.90± 0.12^{a}	0.11± 0.02^{b}	0.11± 0.03^{b}	0.06± 0.01^{b}	–
	Second	0.84± 0.27^{a}	0.72± 0.10^{a}	0.05± 0.01^{b}	0.06± 0.01^{b}	0.10± 0.02^{b}	–
	Third	0.90± 0.08^{a}	1.25± 0.17^{a}	2.06± 0.51^{a}	1.41± 0.24^{a}	1.6± 0.40^{a}	1.37± 0.10^{a}
Granulocyte	First	2.96± 0.65^{a}	3.16± 0.59^{a}	0.36± 0.04^{b}	0.21± 0.03^{b}	0.21± 0.01^{b}	–
	Second	3.04± 0.56^{a}	3.14± 0.44^{a}	0.25± 0.07^{b}	0.52± 0.20^{b}	0.53± 0.15^{b}	–
	Third	4.01± 0.37^{a,b}	5.88± 0.57^{a,b}	12.26± 0.44^{a,b}	6.14± 0.98^{b}	8.67± 1.33^{a}	8.56± 1.55^{a}

Table (2): Changes in the mean values ± SE of IL-1β, IL-10, IgG, and IgM in different experimental groups:

Parameters	Groups Collection	Control	*E.coli*	Cyc.	*E.coli* + cyc	Treated Ech.	C. Ech.
IL-1β	First	–	–	–	–	–	–
	Second	40.93±0.84^{c}	72.16±3.03^{b}	39.1 ±1.03^{c}	59.20±4.11^{b}	108.46±3.46^{a}	–
	Third	45.05 ±2.65^{f}	64.35 ±1.67^{e}	68.8 ±2.08^{d}	66.45 ±2.65^{e,d}	86.65 ±2.08^{c}	92.05±2.08^{a,b}
IL-10	First	–	–	–	–	–	–
	Second	18.13 ±0.46^{b}	27 ±2.95^{a}	28.9 ±2.63^{a}	17.63 ±0.65^{b}	27.43 ±1.12^{a}	–
	Third	34.75 ±2.00^{d}	32.05 ±2.32^{d,e}	29.5 ±1.55^{e}	41.8 ±0.97^{b}	46.5 ±1.46^{b}	55.95 ±2.24^{a}
IgG	First	–	–	–	–	–	–
	Second	571.50 ±71.43^{a}	529.53 ±19.59^{a}	663.63 ±18.35^{a}	625.03 ±78.44^{a}	617.33 ±22.46^{a}	–
	Third	738.25 ±20.37^{d}	822 ±5.96^{b,c}	759.65 ±9.92^{c,d}	985.4 ±5.79^{a}	768.85 ±43.80^{d}	868 ±10.85^{b}
IgM	First	–	–	–	–	–	–
	Second	19.7 ±1.12^{c}	37.33 ±2.25^{b}	21.9 ±1.4^{b}	26.53 ±1.43^{b}	60.1 ±4.52^{a}	–
	Third	25.45 ±1.51^{d}	30.25 ±0.77^{c}	34.4 ±0.98^{b}	34.7 ±0.65^{b}	36.8 ±0.48^{b}	44.95 ±1.18^{b}

4. DISCUSSION

Regarding leukogram, TLC and granulocytes percent in *E. p* treated and *E. p* control groups showed significant increase when compared with *E. coli* + cyclophosphamide - treated group and control group; respectively. These results agreed with *(Mishima et al., 2004).* The recovery in the leukocyte count appeared to be due to the ability of polysaccharides and echinacocide to increase the number of leukocytes, and the ability of cichoric acid and echinacin to activate macrophages and to stimulate bone marrow and the reformation of hematopoietic stem cells. These results reflected cell-mediated immune responses *(Mishima et al., 2004)* at the same time changes in hematological parameters in this study could be attributed to the fact that the active ingredients in *Echinacea purpurea* play stimulatory roles on the level of nucleus of many cells in the body *(*Alban *et al., 2002).* On the other hand, Mayahi *et al., (2011)* found non-significant differences in blood picture due to *Echinacea purpurea* as feed additive, the same results reported in different species by *Wagner et al., (1985).* Our results revealed non-significant changes in monocytes and lymphocytes counts in *Echinacea* treated and *E. p.* control groups when compared with *E. coli* + cyclophosphamide treated and control groups respectively these results disagreed with *Zhai et al., (2007)* these results may be attributed to different species selection(mice) also our results disagreed with *Cundell et al., (2003)* who reported significant increase in the percentage of circulating mononuclear cells (lymphocytes and monocytes) associated with a significant decrease in the percentage of circulating neutrophils, because they fed aging rats with Echinacea for 8 weeks, in a period longer than which used in our experiment. Regarding the results of cytokines, the levels of IL-1β in the serum of *E. p* treated group were significantly increased when compared with *E.coli* + cyclophosphamide-treated group at the 4th and 14th days after injection of *E.coli* and third dose cyclophosphamide, similarly *E. p* control group showed significant increase in levels of IL-1β when compared with control group, these results consistent with reports obtained from both human by *Barak et al., (2002)*; *in vitro* studies *(Burger et al., 1997)* investigated that human macrophages incubated in vitro with a lyophilized and reconstituted

Fresh-pressed juice or a reconstituted dried juice of the above-ground parts of *E. purpurea,* harvested at the peak of flowering, produced the cytokines TNF-α, IL-1β, and 1L-10 at concentrations comparable with, or higher than, those seen with LPS stimulation; however, our

findings disagreed with three teams found no effect of *E.p* extracts on these cytokines *(Schwarz et al., 2002).* These differences might be due to species selection, plant organ selection, extraction method, mode of administration or other experimental choices *(Senchina et al., 2005).*

Regarding the results of IL-10 in *Echinacea* treated group, the levels of IL-10 were significantly increased when compared with *E. coli*+ cyclophosphamide treated group at the 4th day after injection of *E. coli* and third dose cyclophosphamide. Importantly, the levels of IL-10 in *Echinacea* control group were significantly increased when compared with negative control group. Our results came in agreement with *McCann et aL, (2007),* they found that fresh-pressed juice, reconstituted dried juice of *E. purpurea,* harvested at the peak of flowering, freshly prepared extracts of *E. purpurea* or even two years stored *Echinacea purpurea* augmented the production of the cytokines IL-10 at concentrations comparable with, or higher than, those seen with lipopolysaccharides (LPS) stimulation, the accumulated data implied that most Echinacea extracts harbored the ability to augment IL-10 production, and this concurred with the studies indicating anti-inflammatory properties of *E. p. (Fusco et al., 2010).*

In assessing the effect of *E. purpurea* on immunoglobulins, the levels of IgM in *Echinacea* treated group, increased significantly when compared with *E. coli* + cyclophosphamide treated group at the 4th and 14th days after injection of *E. coli* and third dose cyclophosphamide. Importantly, the levels of IgM in *Echinacea* control group were significantly increased when compared with negative control group. Our results came in harmony with *Percival, (2000); Barrett, (2003)* who found that there was an increase in immunoglobulin IgM response against sheep red blood cells (sRBC) in the mice treated with a glycerine extract of *E. p,* meanwhile our findings not consistent with *Mishima et al., (2004)* they explained that *E. purpurea* activated macrophages to_stimulate Interferon-γ (IF gamma) production in association with the secondary activation of T lymphocytes,- resulting in a decrease in IgG and IgM production.

The data of IgG levels obtained in our study revealed significant decrease in the levels of IgG in *E. p* treated group and *M o* treated group when compared with *E. coli* + cyclophoaphamide group at 14th day after injection of *E.coli* and third dose cyclophosphamide this result agreed with that obtained by *Mishima et al., (2004),* meanwhile our finding disagreed with *Reitman et al., (1999)* who found that *E. p* administration for six weeks increased IgG production in the early to middle term in rats. *E. p* has interferon (IFN)-like activity to induce and activate macrophages and T lymphocytes, Cytokines released from macrophages in rats peripheral blood after *E. purpurea* administration activated helper T cells to proliferate. In addition, activated macrophages in association with the secondary T lymphocyte activation increased IFN-y production and stimulated proliferation of cytotoxic T cells and suppressor T cells. These results reflected cell-mediated immune responses, which may explain the difference between the two results *(Mishima et al., 2004).*

It is concluded that *E. purpurea* have immunostimulation effect in E. coli+ cyclophosphamide injected rats.

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