



# Protective effect of *Echinacea purpurea* (Immulant) against cisplatin-induced immunotoxicity in rats

Abdelazeem Ali Khalaf<sup>1</sup> · Shaymaa Hussein<sup>2</sup> · Adel Fathy Tohamy<sup>1</sup> · Sherif Marouf<sup>3</sup> · Hanan Dawood Yassa<sup>4</sup> · Amr Reda Zaki<sup>5</sup> · Anupam Bishayee<sup>6</sup>

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## Abstract

**Purpose** Cisplatin, one of the most effective anticancer drugs, is known to cause undesirable adverse effects, including immunotoxicity. *Echinacea purpurea* is an important medicinal plant with immunostimulatory and anti-inflammatory activities. We have investigated the protective effect of an herbal formulation (Immulant) containing *E. purpurea* extract against cisplatin-induced immunotoxicity in rats.

**Methods** Forty mature albino rats were randomized into four groups (10 rats/group). Control (group 1) animals were subjected to intraperitoneal (i.p.) injection of saline solution (0.2 ml) once every 3 days. Group 2 animals received cisplatin (3.5 mg/kg, i.p.) once every 3 days for successive 2 weeks. Group 3 rats received oral Immulant (150 mg/kg) once daily for 2 weeks. Group 4 animals received oral Immulant treatment as in group 3 in addition to cisplatin as in group 2. Serum level of total protein and albumin, total and differential leukocytic count, phagocytic activity of monocytes, humoral activity and splenic histopathology and immunohistochemistry were used as diagnostic markers of immunotoxicity.

**Results** Cisplatin induced marked inhibition of cellular immunity as exhibited by significant decrease of leukocytic count, lymphocyte percentage and phagocytic activity with marked increase in neutrophil percentage. Humoral immunity represented by marked inhibition in total protein and  $\gamma$ -globulin concentration and significant inhibition in antibody titer against *Mycoplasma gallisepticum* were recorded. Histopathological and immunohistochemical observation of the spleen of cisplatin-treated rats revealed obvious pathological findings of marked depletion and degeneration of lymphoid tissue. Co-oral administration of Immulant resulted in substantial improvement of various immunotoxicological indices compared to cisplatin control.

**Conclusion** The herbal medicine Immulant is an immunostimulant which could be used to treat the immunotoxic effects of cisplatin.

**Keywords** *Echinacea purpurea* · Immulant · Cisplatin · Immunotoxicity · Rats

## Introduction

Cisplatin (also known as cisplatinum or *cis*-diamminedichloroplatinum) is a platinum-containing compound which inhibits synthesis of RNA, DNA and protein in cells. Cisplatin is one of the most effective anticancer drugs

used for the treatment of various oncologic diseases, including testicular, cervical, ovarian, mammary, head and neck, esophageal, lung, and brain cancers [1]. Despite its effective anti-cancer efficacy, cisplatin exerts many undesirable adverse effects, including myelosuppression, hepatotoxicity, nephrotoxicity, spermiotoxicity, and immunotoxicity [2]. The toxic

✉ Amr Reda Zaki  
amrzaki2030@yahoo.com

✉ Anupam Bishayee  
abishayee@lecom.edu; abishayee@gmail.com

<sup>1</sup> Department of Toxicology and Forensic Medicine, Faculty of Veterinary Medicine, Cairo University, Cairo, Egypt

<sup>2</sup> Department of Cytology and Histology, Faculty of Veterinary Medicine, Cairo University, Cairo, Egypt

<sup>3</sup> Department of Microbiology, Faculty of Veterinary Medicine, Cairo University, Cairo, Egypt

<sup>4</sup> Department of Anatomy and Embryology, Faculty of Medicine, Beni-Suef University, Beni-Suef, Egypt

<sup>5</sup> Department of Forensic Medicine and Clinical Toxicology, Faculty of Medicine, Beni-Suef University, Beni-Suef, Egypt

<sup>6</sup> Lake Erie College of Osteopathic Medicine, 5000 Lakewood Ranch Boulevard, Bradenton, FL 34211, USA

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 [3]. 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. Most anticancer drugs are found to suppress hematopoiesis in bone marrow and cause myelosuppression and lymphocytopenia, resulting in reduction or inhibition of lymphocytic responses [4]. Cisplatin has a cytotoxic effect on immune cells when they are rapidly dividing. Treatment of ovarian cancer patients with cisplatin caused reduction in the number of T-helper cells with an increase in the number of T-suppressor/cytotoxic cells [5].

Medicinal plants are known to possess various biological and pharmacological activities that have immense value in pharmaceutical industry for the discovery and development of drugs with appreciable efficacy and acceptable side effects. *Echinacea purpurea* (family: Asteraceae) is an important medicinal plant with various pharmacological properties [6]. *E. purpurea* was found to exhibit significant antioxidant, anti-inflammatory, and immunoregulatory activities [6]. Several active constituents, such as flavonoids, caffeic acid derivatives (alkamides), essential oils, and poly acetylenes, were isolated from this plant. Among various phytochemicals, alkamides were found to possess immunoregulatory effects [7]. Nevertheless, limited information is available on the potential of *E. purpurea* in combating immunotoxicity of chemotherapeutic drugs, such as cisplatin. Therefore, the purpose of the present study was to investigate the protective effect of a formulation containing *E. purpurea* extract (Immulant) against cisplatin-induced immunotoxicity in rats.

## Materials and methods

### Chemicals

Cisplatin was prepared in the form of Unistin vial (EIMC United Pharmaceutical, Cairo, Egypt). *E. purpurea* extract (in the form of Immulant syrup, 16.7 mg/ml) was obtained from Arab Company for Pharmaceuticals and Medicinal Plants (Cairo, Egypt). All other chemicals and reagents used in this study were of analytical grade and obtained from local firms.

### Animals

Forty mature male albino rats (about 3 months old, weighing 180–200 g) were obtained from the animal house, Faculty of Veterinary Medicine, Cairo University,

Egypt. The rats were kept under appropriate environment of temperature ( $25 \pm 2$  C), humidity (60–70%), and light (12-h dark/light cycles), with free access to a commercial balanced diet and tap water *ad libitum*.

### Experimental design

All animal procedures were conducted according to the protocol approved by the Institutional Animal Care and Use Committee at Cairo University, Cairo, Egypt (IACUC, CU-II-F-10-19). After a 2-week acclimatization period, the rats were randomly divided into four groups (10 rats/group) in separate plastic cages and subjected to various treatments as follows. Group 1: Control group was injected intraperitoneally (i.p.) once every 3 days with 0.2 ml saline solution. Group 2: Cisplatin group was injected i.p. with cisplatin (3.5 mg/kg) once every 3 days for successive 2 weeks. The dose of cisplatin was selected based on a previous study [8]. Group 3: Immulant control rats in this group were orally administered Immulant (150 mg/kg) once daily for consecutive 2 weeks following the procedure of Zhai et al. [9]. Group 4: Immulant and cisplatin group was given orally once daily Immulant (150 mg/kg) as in group 3. Additionally, cisplatin (3.5 mg/kg) was administered as in group 2 in animals 30 min following Immulant administration. The experimental dose for Immulant was selected based on conversion of therapeutic human dose to animal dose according to the method described previously [10, 11]. After 2 weeks, rats of all experimental groups were inoculated subcutaneously with 0.5 ml  $\log^{10}$  50% embryo infectious doses (EID<sub>50</sub>) of *M. gallisepticum* (National Health Institute, Doki, Giza, Egypt).

### Sample collection

Ten days after inoculation of *M. gallisepticum*, the rats were anesthetized by ether inhalation and a midline thoraco-abdominal incision was performed to expose their viscera. Blood samples were collected in heparinized sterile tubes via direct cardiac puncture. The samples were used for total and differential leukocytic count and measurement of phagocytic activity, while the sera were collected and stored in a freezer at  $-20$  C for subsequent assessment by immunoassay. Splenic tissues of rats were excised, cleaned from their surroundings, washed with normal saline, and subjected to histopathological and immunohistochemical examination.

### Hematological and biochemical analysis

Total and differential leukocytic count was performed following the methods of Schalm et al. [12]. Total protein and albumin concentration were measured by the adapted methods of Gornal et al. [13] and Doumaset et al. [14], respectively. Electrophoretic patterns of serum proteins ( $\alpha$ -,  $\beta$ , and  $\gamma$ -

globulin fractions) were carried out according to the method of Kaplan and Saveloy [15].

### Assessment of phagocytic activity

Phagocytic activity of peripheral blood monocytes employing *Candida albicans* was measured as described previously [16, 17]. Peripheral blood mononuclear cells were separated using Ficoll-Hypaque density gradient was carried out according to the procedure of Boyum [18]. Subsequently, the mononuclear cell layer was collected, washed and re-suspended in RPMI-1640 medium supplemented with 10% fetal calf serum, and viability was recorded according to Hanks and Waalace [19]. The phagocytic percentage and index were calculated using the following formulas:

$$\text{Phagocytic\%} = \frac{\text{Number of macrophages ingesting } C. albicans}{\text{Total number of macrophages}} \times 100$$

$$\text{Phagocytic index} = \frac{\text{Number of macrophages ingesting more than 3 blastopores}}{\text{Total number of macrophages with ingested blastopores}}$$

### Measurement of antibodies for humoral activity

Serum samples of all groups were examined for humoral activity using *M. gallisepticum* Antibody Test Kit (BioChek, Westbrook, Maine, USA).

### Histopathological studies

Spleen from all treated groups were carefully dissected out and quickly fixed in aqueous Bouin's solution, dehydrated in ascending grades of ethyl alcohol, cleaned using xylene, impregnated in paraffin wax, and sections of 5–7  $\mu\text{m}$  thickness were prepared. The deparaffinized sections were stained with Harri's hematoxylin and eosin (H&E) as well as Crossmon's trichrome stain following Gomori's reticulin method according to Bancroft and Gamble [20]. Histological sections were examined under a light microscope and images were captured by a high-definition microscopic camera (Leica Microsystems, Wetzlar, Germany).

### Immunohistochemical examination

Immunohistochemistry for detection of caspase-3 was performed using paraffin sections mounted on coated glass slides. Antigen retrieval was performed in citrate buffer (pH 6.0) using microwave digestion (2 cycles of 12 min each). Subsequently, endogenous peroxidase was blocked using 0.05%  $\text{H}_2\text{O}_2$  for 30 min. Following incubation with normal horse serum (1:20 dilution), the slides were incubated with primary antibodies (AB3623, rabbit anti-active caspase-3, from Merck Millipore, Billerica, MA, USA) overnight at

4 °C. After that, the sections were treated with secondary antibodies associated with streptavidin-biotin-peroxidase complex. Diamino-benzidine was used as a chromogen. All tissue sections were counter-stained with hematoxylin. The sections were then washed with phosphate buffered saline following each step. Negative controls were used using non-immune serum instead of the primary or secondary antibodies as per the technique of Ramos-Vara [21].

### Statistical analysis

Results were expressed as mean  $\pm$  standard error of mean (SEM). Comparison of means was performed by one-way analysis of variance (ANOVA) followed by Student's *t* test. Values of *P* less than 0.05 were considered statistically significant. Statistical analysis was performed using SPSS version 16.0 software (SPSS Inc., Chicago, IL, USA).

## Results

### Hematological results

Table 1 presents results on total and differential leukocyte count in various groups. There was a significant decrease in total leukocyte (leukopenia) and lymphocyte counts (lymphocytopenia) as well as increase in neutrophil count (neutrophilia) in rats treated with cisplatin in comparison with control animals. In the group that received *E. purpurea* extract in the form of Immulant in addition to cisplatin, all aforementioned parameters were reversed compared to cisplatin alone. In rats that received Immulant only, the results are similar to those in control group.

### Serum proteins

Data recorded in Table 1 reveals significant decrease in mean value of serum total protein in rats that received cisplatin in comparison with control group. Also, significant decrease was observed in  $\gamma$ -globulin concentration in cisplatin-treated rats, while no changes were recorded in,  $\alpha$ - and  $\beta$ -globulin concentration in this group in comparison with the control. Significant improvement was observed in  $\gamma$ -globulin in cisplatin plus Immulant group compared to cisplatin alone group and the values were nearly similar to those of control group. In Immulant-treated group, electrophoretic pattern of all serum proteins was similar to those of control rats.

### Immunological results

Table 2 shows the immune response (Ab titers and phagocytic activity) against *M. gallisepticum* in various rat groups. In cisplatin-treated group, the values of Ab titers,

**Table 1** Effects of Immulant on various hematological parameters in control and cisplatin-treated rats

Groups Parameters	Control	Cisplatin	Immulant	Cisplatin+Immulant
Leukocytes ( $\times 10^3$ )	10.2 $\pm$ 0.15	6.3 $\pm$ 0.42 <sup>a</sup>	9.9 $\pm$ 0.61	8.9 $\pm$ 0.46 <sup>c</sup>
Neutrophil (%)	23.6 $\pm$ 1.2	29 $\pm$ 1.3 <sup>a</sup>	24.6 $\pm$ 2.02	25.6 $\pm$ 1.45 <sup>c</sup>
Lymphocyte (%)	65.0 $\pm$ 2.6	55.8 $\pm$ 1.3 <sup>a</sup>	64.0 $\pm$ 2.09	62.0 $\pm$ 1.15 <sup>c</sup>
Monocytes (%)	11.0 $\pm$ 1.34	12.4 $\pm$ 0.6	11.2 $\pm$ 0.71	12 $\pm$ 0.92
Eosinophil (%)	1.1 $\pm$ 0.05	2.1 $\pm$ 0.25	0.8 $\pm$ 0.02	1.8 $\pm$ 0.31
Basophil (%)	0.8 $\pm$ 0.02	0.4 $\pm$ 0.01 <sup>b</sup>	0.5 $\pm$ 0.02	0.3 $\pm$ 0.02
Total protein (mg/dl)	7.55 $\pm$ 0.17	6.4 $\pm$ 0.01 <sup>a</sup>	8.13 $\pm$ 0.01	7.3 $\pm$ 0.1
Albumin (mg/dl)	4.61 $\pm$ 0.04	4.07 $\pm$ 0.06	5.15 $\pm$ 0.03	4.49 $\pm$ 0.01
$\alpha$ -Globulin (mg/dl)	0.62 $\pm$ 0.03	0.59 $\pm$ 0.02	0.81 $\pm$ 0.01	0.57 $\pm$ 0.01
$\beta$ -Globulin (mg/dl)	0.30 $\pm$ 0.01	0.29 $\pm$ 0.02	0.51 $\pm$ 0.03	0.28 $\pm$ 0.01
$\gamma$ -Globulin (mg/dl)	2.14 $\pm$ 0.09	0.59 $\pm$ 0.02 <sup>b</sup>	2.3 $\pm$ 0.04	1.96 $\pm$ 0.03 <sup>c</sup>

Values are expressed as mean  $\pm$  SEM. <sup>a</sup> P<0.05 and <sup>b</sup> P<0.01 compared to control. <sup>c</sup> P<0.05 compared to cisplatin

phagocytic index and percentage showed significant reduction in comparison with the control group. Administration of Immulant before cisplatin treatment caused significant improvement in the immunological parameters in comparison with the cisplatin group. In Immulant-treated group, the Ab titers, phagocytic index and percentage were higher than the control, but the results were not statistically significant.

### Histological observations

Examination of the spleen sections of control rats showed normal architecture (Fig. 1a). It contained white and red pulps surrounded by a capsule of dense connective tissue (Fig. 1b). White pulp was consisted of lymphoid nodules with the central artery located eccentrically. Lymphoid nodules of white pulp separated from red pulp with well visible marginal zone (Fig. 1c). Red pulp was composed of splenic cords and sinusoids. The spleen sections from rats treated with cisplatin only revealed obvious pathological changes, including disorganization of lymphoid follicles, hyperplasia in white pulp (Fig. 1d), depletion of lymphocytes in red pulp with edema, and some necrotic cells in white and red pulps (Fig. 1e). Increased hemosiderosis as well as fibrosis in the red pulp and some lymphoid follicles were also noticed. Additionally, dilated and congested blood vessels were observed (Fig. 1f). Thickening in the reticular fiber of the capsule and the trabeculae was also noticed (Fig. 1g). Immulant plus cisplatin rat group showed hypocellularity in the red pulp with the appearance of germinal center (Fig. 1h) and depletion of periarteriolar lymphatic sheath (Fig. 1i). Additionally, it decreased dilated and congested splenic vein and blood sinusoids, reduced hemosiderin deposition and restored the normal architecture of the tissue (Fig. 1j). Immulant-treated spleen simulated spleen of control group (Fig. 1k).

### Immunohistochemical observations

Negative caspase-3 immunohistochemical reactivity was observed in the spleen of control group (Fig. 2a). In cisplatin-treated group, intense immunoreaction in the white and red pulp revealed apoptotic events within the lymphoid follicles and to lesser extent, the periarteriolar lymphoid sheath (PALS) (Fig. 2b, c). Mild or negative immunoreactivity was detected in cisplatin- and Immulant-treated group (Fig. 2d). Immulant-treated group resembled control spleen (Fig. 2e).

### Discussion

Immunotoxicity is the adverse effect of most cytotoxic chemotherapy on the immune system, prompting a suppression of the immune response. Cisplatin is one of the most effective drugs used for chemotherapy of various cancers. Nevertheless, the clinical utility of this drug is limited due to many adverse effects, including severe impact on the immune system. In this study, administration of cisplatin to mature rats resulted in marked immunotoxic effects represented by leukopenia, lymphocytopenia, neutrophilia as well as decrease in total protein,  $\gamma$ -globulin, antibody titers and phagocytic activity (percentage and index). Bone marrow is susceptible to damage by cytotoxic drugs since they cause neutropenia, lymphopenia, thrombocytopenia, and anemia [22]. Parket al. [23] revealed that cyclophosphamide used in various malignancies produced immunosuppressive effect as hematopoietic dysfunction, which manifests leukopenia, lymphopenia, anemia and thrombocytopenia. Our results were also supported by the previous data of Awadallah et al. [24] who indicated that administration of therapeutic doses of cisplatin to adult male rabbits resulted in leukopenia, lymphocytopenia, and neutrophilia.

**Table 2** Effect of Immulant on various immunological parameters in control and cisplatin-treated rats

Control	Cisplatin		Immulant		Cisplatin +Immulant						
	Ab Titers	Phagocytic %	Ab Titers	Phagocytic %	Ab Titers	Phagocytic %					
800 ± 8.12	56 ± 3.15	0.24 ± 0.04	644 ± 7.11 <sup>a</sup>	20 ± 2.76 <sup>a</sup>	0.19 ± 0.01 <sup>a</sup>	874 ± 11.9	61.5 ± 2.71	0.27 ± 0.02	720 ± 10.45 <sup>b</sup>	37.8 ± 2.83 <sup>c</sup>	0.21 ± 0.02 <sup>c</sup>
		index		index				index			index

Values are expressed as mean ± SEM. <sup>a</sup> P<0.05 compared to control. <sup>b</sup> P<0.05 and <sup>c</sup> P<0.01 compared to cisplatin

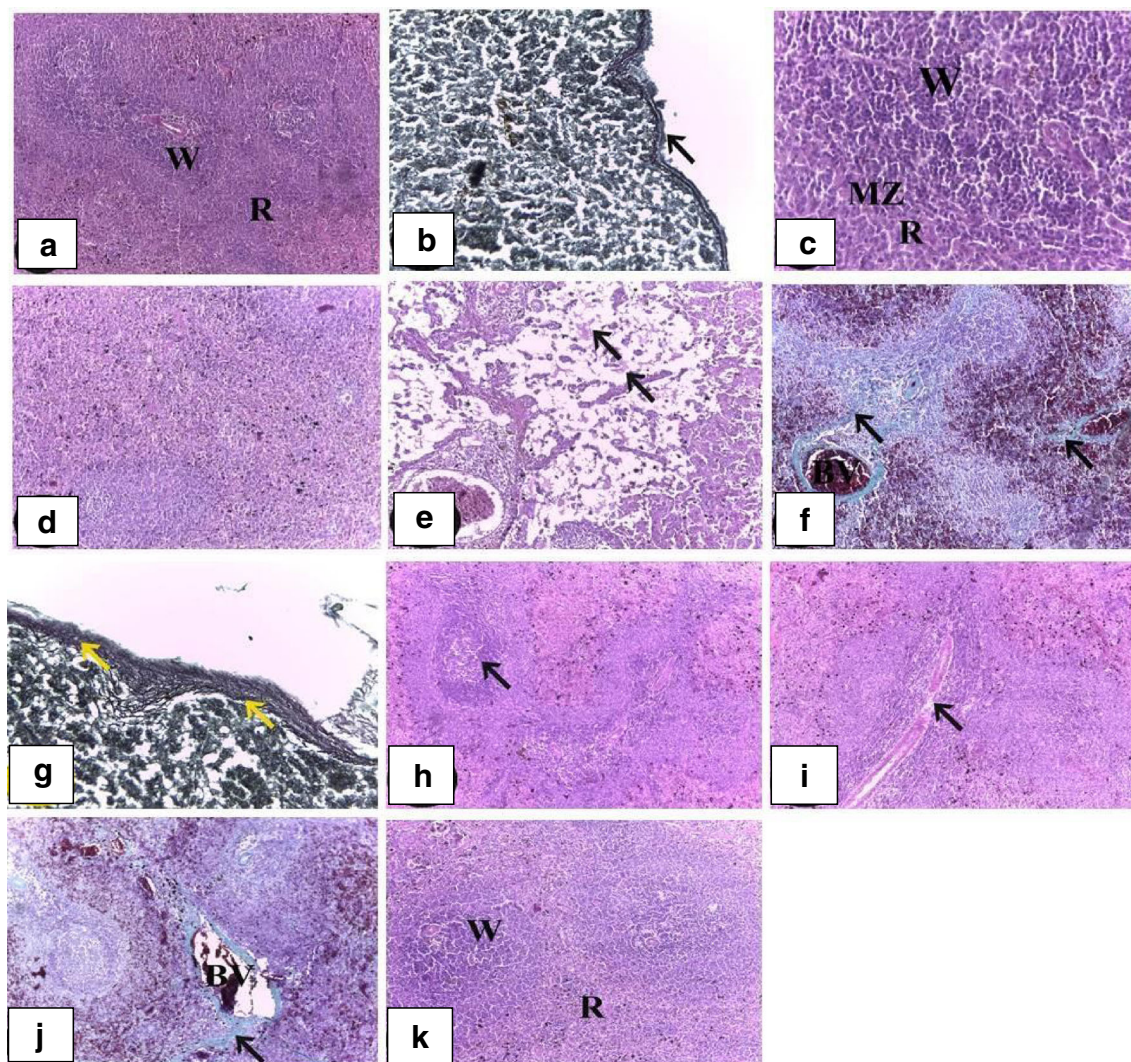
Cisplatin induced immunosuppression and suppressed both humoral and cell-mediated immune response [25]. The hematotoxic effect of cisplatin may be attributed to its myelotoxicity as it binds with cellular DNA, affecting cell replication. Moreover, cisplatin produces reactive oxygen species and triggers apoptosis of the circulating blood and bone marrow cells [26]. This is in contrast with Markovic et al. [27] who reported that cisplatin increased the number of leukocytes due to the consequence of infection and inflammation.

In this study, exposure of male rats to cisplatin resulted in significant decrease in total protein and  $\gamma$ -globulin concentration. Based on a previous study, immunotoxic effect of cisplatin was manifested by significant decrease in antibody titers and phagocytic activity against *M. gallisepticum* [28]. Our results are supported by Jílek et al. [29] who reported reduction of  $\gamma$ -globulin in blood possibly due to the cytotoxic effect of cisplatin on the immunocompetent cells, especially B lymphocytes and plasma cells. Li et al. [30] showed that cisplatin can induce immunosuppressive effects through inhibition of T cell activity. Additionally, Kouchi et al. [31] reported that cisplatin blocked splenic T lymphocyte function more than splenic B lymphocyte function.

The reduction in antibody titers may be attributed to the direct effect of cisplatin on the immune system of rats. This speculation is supported by the histopathological examination of spleen of treated rats which revealed marked depletion and degeneration of lymphoid tissue. The immunotoxic effect of cisplatin was consistent with the mechanism of action of cisplatin which is a bifunctional alkylating agent. It is known to produce DNA strand cross-linking and subsequent inability of DNA to separate during cell division, resulting in cell death [32]. The current study noticed hyperplasia in the white pulp and depletion of lymphocytes in the red pulp in agreement with other investigators who observed reduced the extramedullary hematopoiesis in the red pulp with prominent apoptotic bodies in the lymphoid follicle [33–35]. Cytotoxic drugs suppress the immune response which can lead to diminished host resistance to infectious agents or tumor cells [36]. Immunotoxicity may parallel alterations in the weight of lymphoid organs and/or changes in the normal microscopic architecture [37]. However, splenic immunosuppression may attribute to the decreased lymphatic cells numbers in the spleen and other immune organs [37, 38]. Meneze sand colleagues [39] indicated that all extensive injuries were repaired with collagen fibers which may lead to the fibrosis as observed here. Crăciun and Pașca [40] reported that cisplatin had a significant toxic effect in both central (thymus) and peripheral (spleen) lymphoid organs.

Natural products, including bioactive phytochemicals, can augment the effectiveness of chemotherapeutic drugs and reduce their toxic effects. Natural herbal products can





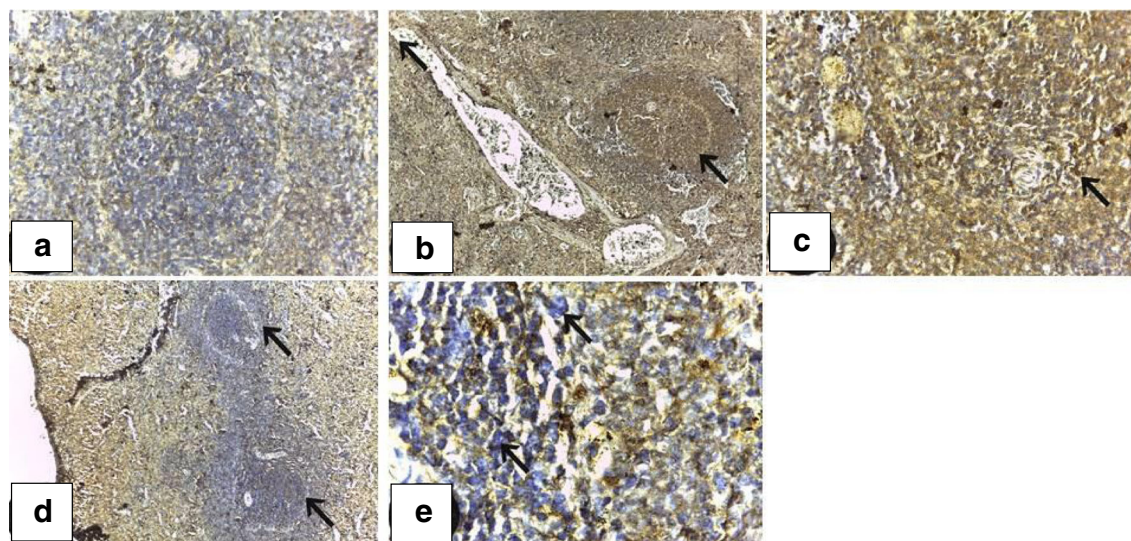
**Fig. 1** Histopathological findings on spleens from various experimental groups. **a** A section of spleen of the control group showing normal histological architecture into white (W) and red pulps (R) (H&E;  $\times 100$ ). **b** A section of spleen of the control group showing thin reticular fiber in the capsule (arrow) (Gomori's reticulin;  $\times 400$ ). **c** A section of spleen of the control group showing white pulp (W) separated from red pulp (R) with well visible marginal zone (MZ) (H&E;  $\times 400$ ). **d** A section of spleen of the cisplatin-treated group showing disorganization of lymphoid follicles (H&E;  $\times 400$ ). **e** A section of spleen of the cisplatin-treated group showing depletion of lymphocytes in red pulp with edema (arrow), and some necrotic cells in white and red pulps (H&E;  $\times 100$ ). **f** A section of spleen of the cisplatin-treated group showing increasing of hemosiderosis, fibrosis (arrow) in the red pulp and some lymphoid

follicles, dilated and congested blood vessels (BV) (Crossmon's trichrome;  $\times 400$ ). **g** A section of spleen of the cisplatin-treated group showing thickening in the reticular fiber of the capsule and the trabeculae (Gomori's reticulin;  $\times 400$ ). **h** A section of spleen of the cisplatin- and Immulant-treated group showing hypocoellularity of the red pulp and germinal center (arrow). (H&E;  $\times 100$ ). **i** A section of spleen of the cisplatin- and Immulant-treated group showing depletion of periarterial lymphatic sheath (arrow). (H&E;  $\times 100$ ). **j** A section of spleen of the cisplatin- and Immulant-treated group showing decrease dilated and congested blood vessels (BV) and collagen fibers (arrow). (Crossmon's trichrome;  $\times 100$ ). **k** A section of spleen of the Immulant-treated group showing white (W) and red pulps (R) (H&E;  $\times 100$ )

be used as immunomodulators to enhance the host defence mechanism against the immunotoxicity of chemotherapy. Such substances might provide immunoprotection against cisplatin toxicity [41]. Omega-3 fatty acids, such as eicosapentaenoic acid and docosahexaenoic acid, are polyunsaturated fatty acids. Omega-3 fatty acids have been found to exert immunomodulatory effects due to their ability to reduce interleukin-6 levels [42]. Glucans are present in the bran of grasses, such as barley, oats and wheat, and

exhibit strong protective activities against the immunosuppressive effects of mutagenic agents, such as cisplatin. Glucans stimulate immune response through their ability to trap free radicals which are released during the biotransformation of mutagens [43]. Gallic acid, a triphenolic compound present in various fruits and plants, has been considered as an effective antioxidant agent. Gallic acid has been found to enhance the immune function against the immunotoxicity of cisplatin [44].





**Fig. 2** Immunohistochemical profile of caspase-3 expression in spleen in various experimental groups. **a** A section of spleen of the control group showing negative caspase-3 immunoreactivity (caspase-3;  $\times 100$ ). **b** A section of spleen of the cisplatin-treated group showing intense caspase-3 reaction expressed in white and red pulp (caspase-3;  $\times 100$ ). **c** A section of spleen of the cisplatin-treated group showing intense caspase-3

reaction expressed in white and red pulp (caspase-3;  $\times 400$ ). **d** A section of spleen of the cisplatin- and Immulant-treated group showing mild to negative caspase-3 immunohistochemical expression (caspase-3;  $\times 100$ ). **e** A section of spleen of the Immulant-treated group showing negative caspase-3 immunohistochemical expression (caspase-3;  $\times 1000$ )

This study investigates the protective effect of Immulant (an herbal formulation containing an extract of medicinal plant *E. purpurea*) against the immunotoxicity of cisplatin in mature male rats. Animals that received cisplatin plus Immulant showed significant protective effect against hemotoxic and immunotoxic effect of cisplatin. These protective effects were manifested by increased percentage of total leukocytes, lymphocytes and decreased percentage of neutrophil. Immulant is frequently used as a dietary supplement to improve the function of immune system. It is used as herbal medicine to treat malignant tumors, respiratory infections, and various other inflammatory conditions [45]. Immulant is known to activate non-specific cellular and humoral immunity as well as the complement system [46]. Immulant has also been found to stimulate the immune system by increasing the production and activation of leukocytes, lymphocytes, monocytes, and cytokines [47].

The immunostimulation of Immulant may be linked to its polysaccharide fraction which has been found to stimulate the activity of macrophage and several other functions linked to cytokine production [48]. Various phenolic compounds and alkamides were found to exhibit antiviral and antifungal properties [49]. Immulant contains alkamides, caffeic acid esters, polysaccharide, and polyacetylenes [50]. Purified polysaccharide isolated from *E. purpurea* extract elicited an immunostimulatory effect when immune cells were exposed to this natural agent [51]. Several animal and human studies reported stimulation of neutrophil and macrophage phagocytic functions by *E. purpurea* [46].

Co-administration of Immulant with cisplatin induced marked improvement in the histopathological and histochemical changes in the spleen of treated rats. This observed effect may be due to the antioxidant properties of various constituents (e.g., polysaccharides and flavonoids) present in the *E. purpurea* extract.

The precise mechanisms of the observed immunoprotective effects of Immulant are not known at this time and require additional in-depth investigation. Various phytoconstituents present in *E. purpurea*, 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 [52]. 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 [52]. Hence, it is plausible that Immulant may confer immunoprotection through multifactorial immunomodulatory effects which could be achieved through chemical synergy of various bioactive constituents.

## Conclusions

In conclusion, the results of this study showed that cisplatin treatment induced a substantial immune damage in rats and pretreatment with Immulant provided protective effect against cisplatin-induced immunotoxicity. However, further clinical investigation is warranted to understand the full potential of Immulant as an adjunct to cisplatin therapy.

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**Authors' contributions** All authors participated in the design, interpretation of the study, analysis of the data, and review of the manuscript.

## Compliance with ethical standards

**Ethics approval** All animal studies were performed in accordance with the procedure approved by the Institutional Animal Care and Use Committee of Cairo University (IACUC, CU-II-F-10-19).

**Consent for publication** Not applicable.

**Competing interests** The authors declare that they have no competing interest.

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