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Research Paper:

Septilin, A Polyherbal Formulation Against the Immunosuppression Induced by Cyclophosphamide and Cisplatin in Swiss Albino Mice

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ABSTRACT

Background: Septilin (Spt) is an ayurvedic drug formulation from Himalaya Drug Company and is well-known for its antibacterial, anti-inflammatory and immunomodulatory activities. Interest in the use of medicinal plants and herbal medicine as immunomodulators has currently been the subject of scientific investigation worldwide. Cyclophosphamide (CP) and Cisplatin (Csp) are widely used chemotherapeutic drugs and are known for their immunosuppressive effects.

Methods: The present study evaluated the immune-stimulating activity of Spt (125, 250 and 500 mg/kg; PO/7 days) against CP (50 mg/kg) and Csp (10 mg/kg) induced immunosuppression in mice sensitized with sheep Red Blood Cells (RBC) by measuring Hemagglutination Antibody (HA) titre values and by determining the haematological parameters, such as Haemoglobin (Hb) content, White Blood Cells (WBC), RBC and platelet counts. Thymus index and differential counts of leukocytes were also determined.

Results: Upon HA assay, the titre was significantly decreased in CP (59.40%) and Csp (62.16%) in the treatment groups ($P<0.001$). An increase in the HA titre value in Spt-treated mice showed the stimulation of humoral immune response ($P<0.001$). The results of haematological study in Spt-treated mice indicated stimulation of total leukocytes, RBC and platelet counts. Moreover, Spt treatment prior to the administration of CP and Csp prevented the loss of body weight and minimized their adverse effect on the mice thymus.

Conclusion: Our experimental evidence suggests the immunostimulatory potency of Spt against the immunosuppression induced by chemotherapeutic drugs in mice. The study results are comparable with the immune-potentiating effects of standard immunomodulatory drug Levamisole (Lev). Hence, Spt may be used as an adjuvant to obviate the immune suppression induced by chemotherapeutic medications.

Keywords: Cisplatin, Cyclophosphamide, Antibody titre, Immunomodulation, Septilin

Introduction

Immune system is the major defence mechanism evolved in our body to fight against harmful invading agents [1]. Most of the chemotherapeutic agents affect the hemopoietic system, resulting in the suppression of bone marrow [2], which is the most affected organ after chemotherapy. Loss of stem cells and inability of bone marrow to regenerate new blood cells result in thrombocytopenia and leukopenia [3]. Thus, Cyclophosphamide (CP) is considered as the most effective immunosuppressive agent,

which exerts its suppressive effect by cross linking the DNA of actively dividing cells, leading to various types of infections. The immunosuppression induced by Cyclophosphamide (CP) occurs primarily through the inhibition of cellular and humoral immune response [4]. Cisplatin (Csp) is a routinely used chemotherapeutic drug, which causes both structural and functional damage to the lymphoid organs. The mechanism involved in the immunotoxic effect of Csp is generally by the formation of DNA adducts and through cleavage of disulphides bonds in proteins [5, 6].

Humoral immunity refers to the production of antigen-specific antibodies, which plays a vital role in the immune defence [7]. Hemagglutination Antibody (HA) titre assay is one of the simple methods used to measure the specific antibody response towards a given antigen and is commonly used in blood grouping and viral quantification. Antigen-antibody reactions are visualized by the formation of agglutination [8]. Changes in the cellular components of the blood are the factors that influence the immunosuppression [9]. Evaluation of haematological parameters, lymphoid organ weight and the histopathology are considered as immunological endpoints in subchronic and chronic rodent studies [10]. Septilin (Spt) is an ayurvedic herbomineral formulation, the traditional Hindu system of medicine, produced by Himalaya Drug Company, India. It consists of the extracts from medicinal plants such as, *Maharasanadi qoath* (130mg), *Tinospora cordifolia*, (98mg), *Rubia cordifolia* (64mg), *Emblica officinalis* (32mg), *Moringa pterigosperma* (32mg), *Glycyrrhiza glabra* (12mg) and the powders of *Balsamodendron mukul* (0.324mg) and *Shankha bhasma* (64mg) [11]. In traditional medicine, Spt is used as an antibacterial, anti-inflammatory and im munomodulatory agent [12-15].

Our previous research provided new insight into the anticlastogenic effects of Spt against CP-induced cytogenetic damage to bone marrow cells in mice [16]. In addition, our previous study revealed the modulatory effect of Spt on cisplatin-induced cytotoxicity in a human cell line from breast adenocarcinoma [17]. Information available on the therapeutic applications of herbo-mineral formulation Spt encouraged us to investigate its immunomodulatory activities against the immunosuppression induced by two potent anticancer drugs, i.e., CP and Csp, in Swiss albino mice.

Materials and Methods

Experimental animals: In this study, 8-10-week-old Swiss albino mice, belonging to *Mus musculus* species, were used for the experiments. They weighed 25±2g and were bred and kept in the institutional animal house at Mangalore University, Konaje, India. The experimental animals (n=95) were divided into 19 groups of five mice each (3 females & 2 males). Both genders were used to explore the effects of Spt on either sex in mice. The animals were housed in polypropylene shoe box type cages, bedded with rice husk and kept in air-conditioned room, at 23±2°C, relative humidity 50±5%. They had free access to a pelleted diet (Amruth Feeds, India) and water under 12hr light-dark cycle. The animal care and experimental procedures were conducted per the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), India [18]. All animal groups were kept under an optimal hygienic condition per the established procedures and by observing the necessary ethical standards.

Experimental materials: The drug Spt was purchased from Himalayan Drug Company, India. The CP was procured from Endoxan-N Baxter Oncology, Germany, and Csp was procured from Sigma Life Science (Saint Louis, MO, USA). Levamisole (Lev) was purchased from Khandelwal laboratories, India and other chemicals were obtained from Merck, SRL and Hi-media in India.

Dosage and treatment schedule: The mice were given with a single injection (0.1 mL) of immunosuppressing drugs CP (50 mg/kg) and Csp (10 mg/kg), dissolved in distilled water and normal saline, respectively. The Spt was used as test agent and its LD_{50} value in the mice was found to be 1250 mg/kg [13]. Accordingly, the three doses of Spt selected for use in this study were 125, 250 and 500 mg/kg. The experimental animals were divided into 19 groups, consisting of five mice in each group. They were sensitized with $1x10^8$ cells/mL of sheep Red Blood Cells (RBCs). The day of sensitization was referred as day 0. From days 1-7, animals were orally administered with 0.2 mL of Spt and standard immunomodulatory drug, Lev (50 mg/kg) dissolved in 0.5% Carboxymethyl Cellulose (CMC). The drugs CP and Csp were administered in 0.1 mL aliquot Intraperitoneally (IP) on the 6^{th} day, 1hr after the treatment with the test agents. On day 7^{th}, 2hr after the last dose, blood samples were collected through heart puncture and used for HA titre and haematological studies. The animal groups administered with 0.5% CMC, distilled water and normal saline were maintained separately, and were considered as the negative controls.

Thymus index: Once sacrificed, the mice's thymus glands were weighed and the relative weight was determined, using the following Equation [19]:

Thymus Index (TI)=Thymus weight (mg)/Body weight (g)×100

Haemagglutination titre assay: We employed the Mediratta et al. [20] method for the HA assay to measure the relative concentration of antibody in the sera. The HA titre assay was estimated using micro-titre plates. Two-fold dilutions of antibodies in the sera was done in micro-titre plates, using phosphate buffered saline. A 50µL of 1% sheep RBC suspension was added to each well. The plates were shaken gently, then incubated at room temperature for 2hr, and examined visually for agglutination. The antibody titre values were expressed in the graded manner, with the minimum dilution (1/2) being ranked as one. The highest number of dilutions as evident by visual agglutination was expressed based on the HA titre [21].

Haematological studies: The haematological parameters, such as total RBC, WBC, platelet and Hb counts were found to be the vital constituents of the immune system and were analysed using a haematology analyser (Unitron Bio-Medicals, India). To determine the differential WBC counts, a drop of blood was thinly smeared over a glass slide, air dried and fixed with methanol for 2 min. The fixed slides were stained with Giemsa diluted with phosphate buffer (1:9). A total of 100 cells were counted, classified as neutrophil, eosinophil, basophil, lymphocyte and monocyte, and the percentages were recorded.

Statistical analyses: The statistical significances among the data were determined, based on one-way ANOVA and Dunnett's post hoc tests, using GraphPad Prism 5 software (GraphPad Software, Inc., CA, USA). Statistical differences with a $P \leq 0.05$ were considered as significant. We designated the significances as $P<0.05$, $P<0.01$ or $P<0.001$, which were considered to be either significant, very significant or highly significant, respectively.

Results

Thymus index: The body weight and that of the thymuses in mice treated with CP were found to be decreased compared to those in the normal control groups, treated with distilled water. The animals administered with Spt and standard allopathic immunomodulatory drug Lev, prior to the injection of CP, showed improvement on these parameters. Of the three doses of Spt tested, the 250 and 500 mg/kg caused significant increases ($P<0.05$) for the thymus weights. Also, the Csp treatment resulted in immunosuppressive effect in the mice by reducing the normal body weight and the primary lymphoid organ weight. Co-treatment of Spt at a 250 mg/kg dose showed significant increases in the thymus weight of Csp-treated mice. The effect obtained by administering Spt was comparable with the results of the standard immunomodulatory drug, Lev (50 mg/kg; Table 1).

Haemagglutination antibody titre: The HA antibody titre was determined to quantify the amount of haemagglutinin in the sera against sheep RBCs. Figure 1 represents the effect of Spt on the HA titre. The oral administration of Spt at 125, 250 or 500 mg/kg, showed an increase in HA titre values, compared to the control group that received 0.5% CMC solvent. This was comparable to the values obtained for the standard immunomodulatory drug, Lev, at 50 mg/kg. The CP treatment showed its immunosuppressive effect on mice by a decrease in the production of antibodies (59.40%). This was evident by a significant decline in the HA titre value ($P<0.001$) compared to that of distilled water treatment in the control group. The pre-treatment with Spt showed significant rises in the HA titre values (32.43%; $P<0.001$) in contrast to those observed in the CP-treated group. The Spt administration at 250 mg/kg exhibited the maximum enhancement of HA titre values (72.97%), which was almost comparable to the titre values achieved in the standard immunomodulatory Lev-treated group (Lev 50+CP 50 mg/kg) (75.68%) as illustrated in Figure 2.

The impact of Csp on the antibody production against sheep RBC is presented in Figure 3. The Csp injection showed a 62.16% decrease in the HA titre values (2.8), compared to that of the control group that received normal saline ($P<0.001$). The supplementation with Spt increased the antibody titre values (4.0-5.4) and the effect was found to be highly significant, as compared to those found in the Csp-treated group ($P<0.001$). The maximum titre value (5.4) was observed at the Spt dose of 250 mg/kg, which was equivalent to that of the standard drug, Lev (HA titre value: 5.6).

Haematological studies: The Spt and standard drug, Lev, caused improvement in the WBC counts while there was not much variations in the concentration of Hb and the RBC and platelet counts, compared to the effect of 0.5% CMC vehicle agent administered to the control group (Figure 4). The CP injection to mice caused significant reductions in the percentages of Hb, WBC, RBC and platelet counts, compared to those noted for the use of distilled water in the control group ($P<0.001$). The injection of Spt and Lev showed significant increases in all the above parameters in contrast to those observed in the CP-treated group. Figure 5 represents the immunostimulatory effect of Spt on CP-induced bone marrow suppression in the mice.

The Csp treatment showed significant decreases in the Hb concentration (26.83%) and WBC (27.12%), RBC (29.19%) and platelet counts (13.30%), respectively, compared to the control group that received normal saline

Table 1. Effect of septilin, cyclophosphamide and cisplatin on thymus and body weight of mice (n=5)

Treatment (mg/kg)		Mean±SE		
		Body Weight on Day 0 (g)	Body Weight on Day 7 (g)	Thymus Index
I	D. Water	25.8±0.20	26.7±0.24	1.50±0.02
II	0.9% Saline	24.7±0.14	26.1±0.22	1.54±0.01
III	0.5% CMC	26.0±0.24	27.0±0.26	1.53±0.02
IV	Lev	25.9±0.12	27.1±0.12	1.86±0.02
V	Spt125	26.2±0.19	27.2±0.37	1.73±0.03
VI	Spt250	25.5±0.20	26.8±0.27	1.80±0.02
VII	Spt500	26.3±0.13	27.5±0.08	1.60±0.02
VIII	CP50	26.1±0.19	24.6±0.19	1.30±0.01
IX	CMC+CP50	26.3±0.34	24.8±0.33	1.30±0.01
X	Lev+CP50	26.5±0.31	25.3±0.31	1.72±0.09[b]
XI	Spt125+CP50	25.8±0.18	23.7±0.20	1.60±0.00
XII	Spt250+CP50	26.0±0.12	24.2±0.14	1.68±0.10[b]
XIII	Spt500+CP50	26.4±0.16	24.3±0.14	1.67±0.01[b]
XIV	Csp10	26.3±0.15	24.0±0.09	1.24±0.02
XV	CMC+Csp10	26.5±0.20	24.7±0.22	1.27±0.01
XVI	Lev+Csp10	25.7±0.16	24.1±0.20	1.74±0.04[d]
XVII	Spt125+Csp10	26.1±0.24	24.6±0.50	1.59±0.04
XVIII	Spt250+Csp10	25.8±0.20	24.0±0.14	1.67±0.02[d]
XIX	Spt500+Csp10	26.0±0.29	24.0±0.20	1.57±0.01

One-way ANOVA followed by Dunnett's post hoc test. [a]P<0.05 when compared to distilled water treated mice; [b]P<0.05 when compared to CP treated mice, [c] P<0.05 when compared to 0.9% saline treated mice; [d]P<0.05 when compared to Csp treated mice.

Csp: Cisplatin; CMC: Carboxymethyl Cellulose; CP: Cyclophosphamide; HA: Hemagglutination Antibody; Hb: Haemoglobin; IP: Intraperitoneal; Lev: Levamisole; PO: Per Os (by mouth); RBC: Red Blood Cells; Spt: Septilin; TI: Thymus Index; WBC: White Blood Cells.

(P<0.001). However, the co-administration of Spt minimized the Csp-induced the mice suppression of bone marrow, showing significant improvement in all the above-mentioned haematological parameters. These effects of Spt were comparable with the results of the group that received standard immunomodulatory drug, Lev at 50mg/kg combined with Csp at 10 mg/kg. There was not much variations in haematological parameters between the three doses of Spt tested but improvement in the blood parameters was observed with Spt at 250 mg/kg (Figure 6).

Leukocytes' differential counts: the differential WBC counts indicated the relative percentage of each type of WBCs, which reflected the induced inflammatory responses by CP and Csp. The oral administration of Spt increased the percentage of lymphocytes, the effect of which was similar to that of the standard drug Lev-administered group. In the Spt-treated mice, the increased percentage of lymphocytes was comparable to those of the CP- and Csp-treated groups. The CP injection decreased the percentage of lymphocytes significantly, representing an 11.6% increase in the neutrophil counts compared to that of the distilled water in the control group. The Spt pre-treatment stabilized the differential leukocytes' counts to the normal level in contrast to that seen in CP-treated group. In the Csp-treated animals, a 10% decrease in the lymphocyte counts was observed plus negligible variations in the monocytes and eosin-

Table 2. Effects of septilin, cyclophosphamide and cisplatin on the differential counts of white blood cells in mice (n=5)

Treatment (mg/kg)	Mean±SE (%)				
	Neutrophil	Lymphocyte	Monocyte	Eosinophil	Basophil
Dist. water	31.4±0.24	66.0±0.32	0.8±0.20	1.6±0.24	0.4±0.24
0.9% Saline	31.0±0.45	66.4±0.24	1.0±0.44	1.2±0.21	0.4±0.25
0.5% CMC	30.2±0.37	66.8±0.58	1.0±0.31	1.8±0.20	0.4±0.24
Lev	23.2±0.49	72.8±0.80	1.6±0.40	2.2±0.59	0.2±0.20
Spt125	24.8±0.58	72.8±0.66	0.8±0.37	1.2±0.58	0.2±0.20
Spt250	23.4±0.24	73.4±0.32	1.2±0.20	2.0±0.00	0.2±0.20
Spt500	24.6±0.51	73.0±0.84	1.0±0.32	1.2±0.38	0.2±0.20
CP50	45.6±0.51[a]	53.4±0.40[a]	0.2±0.20[a]	0.6±0.41[a]	0.0±0.00
CMC+CP50	43.0±0.44	56.4±0.51	0.2±0.21	0.8±0.25	0.0±0.00
Lev+CP50	31.0±0.55[b]	65.4±0.40	1.4±0.24	2.0±0.31	0.2±0.20
Spt125+ CP50	28.2±0.66[b]	68.8±0.66	1.2±0.21	1.6±0.24	0.2±0.20
Spt250+ CP50	30.8±0.37[b]	65.6±0.51	1.6±0.21[b]	1.8±0.37	0.2±0.20
Spt500+ CP50	28.2±0.80[b]	68.9±0.51	1.2±0.56	2.0±0.20	0.2±0.20
Csp10	43.2±1.39[c]	56.4±1.21[c]	0.0±0.00	0.4±0.40	0.0±0.00
CMC+ Csp10	40.8±0.37	58.6±0.24	0.0±0.00	0.6±0.40	0.0±0.00
Lev+Csp10	32.0±0.31	65.2±0.58[e]	1.0±0.00f	1.6±0.58	0.2±0.20
Spt125+Csp10	32.0±0.84[e]	65.0±0.71[e]	1.4±0.20[d]	1.6±0.25	0.0±0.00
Spt250+Csp10	31.2±0.73[e]	65.4±0.51[e]	1.2±0.38[d]	1.8±0.24[f]	0.2±0.20
Spt500+Csp10	34.0±0.71[e]	63.2±0.37[e]	1.0±0.32[d]	1.6±0.24	0.2±0.21

100 cells scored from each animal. One-way ANOVA followed by Dunnett's post hoc test; [a]P<0.001 when compared to distilled water treated mice, [b]P<0.001 when compared to CP treated mice, [c]P<0.001 when compared to 0.9% saline treated mice, [d]P<0.05 when compared to Csp treated mice, [e]P<0.01 when compared to Csp treated mice, [f]P<0.001 when compared to Csp treated mice.

Csp: Cisplatin; CMC: Carboxymethyl Cellulose; CP: Cyclophosphamide; HA: Hemagglutination Antibody; Hb: Haemoglobin; IP: Intraperitoneal; Lev: Levamisole; PO: Per Os (by mouth); RBC: Red Blood Cells; Spt: Septilin; TI: Thymus Index; WBC: White Blood Cells

ophils, compared to the effects of normal saline in the control group. The oral administration of Spt for seven consecutive days minimized the effect of Csp on the leukocytes' differentiation. The administration of Spt significantly increased the percentage of lymphocytes at all doses tested (P<0.001). The maximum improvement in the percentage of lymphocyte (65.4%) was observed at 250 mg/kg of Spt. This effect was equivalent to the values noted for the standard immunomodulatory drug Lev and Csp-treated group (65.2%). See details in Table 2.

Discussion

The results obtained by the current study demonstrated the immunosuppressive effects of CP and Csp on mice, as evident by decreases in both the thymus and body weights (Table 1). The reduction in the thymus weight represents the low production of T-lymphocytes involved in cell-mediated immunity. Figures 5 and 6 show the haemato-suppressive effects of CP and Csp on sheep RBC sensitized mice. Specifically, there were decreased percentages of Hb, RBCs, WBCs and platelets counts compared to those in normal mice. The suppression of

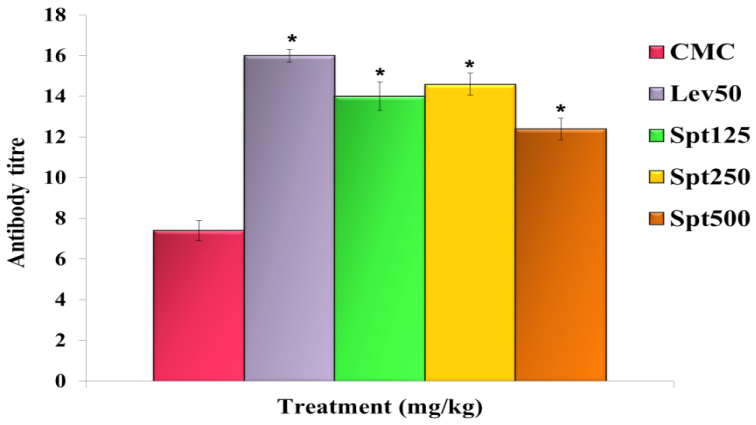

Figure 1. Effect of Spt on HA titre in SRBC sensitized mice

Values are Mean±SE, (n=5), One-way ANOVA followed by Dunnett's Post Hoc Test, *P<0.001 when compared to 0.5% CMC treated mice.

the bone marrow that was induced by CP and Csp was largely due to the action of free radicals on the immune cells. Binding of Csp to proteins and enzymes might have altered certain biochemical mechanisms, enhancing the generation of Reactive Oxygen Species (ROS) [22].

In animals treated with CP and Csp, the number of neutrophils increased with the concurrent decline in the percentage of lymphocytes. The increased number of neutrophils in these animals might be due to a decrease in their adherence to the vascular endothelia, reduced emigration into inflamed tissues without having cytotoxic and phagocytic activities [23].

To protect our immune system from CP and Csp-induced ROS, natural antioxidants serve as immunomodulators [24]. Allopathic drugs may be used to boost the immune response along with immunosuppressive drugs. However, herbal medicines are gaining popularity as the modulator of the immune system due to their less undesirable side effects [25, 26]. Hence, the impetus behind the present study to evaluate the immunomodulatory effects of Spt against the immunosuppression induced by CP and Csp in Swiss albino mice. The CP and Csp injections significantly decreased the weights of the thymus and body of the treated mice.

The mice pre-treated with Spt (250 mg/kg) showed an increase in the thymus index (1.68), compared to those treated with CP alone (1.30; P<0.05). The immunostimulatory effect of Spt on the mice's haematological parameters suppressed by CP and Csp, i.e., RBC, WBC, platelet counts and percentage of Hb, are presented in Figures 4 and 5. Further, the oral administration of Spt in mice prior to CP and Csp injection restored the differentiation of leukocytes to that of the normal levels. The increased monocyte activity in Spt-treated animals, as reflected in Tables 1 and 2, signifies the enhanced sta-

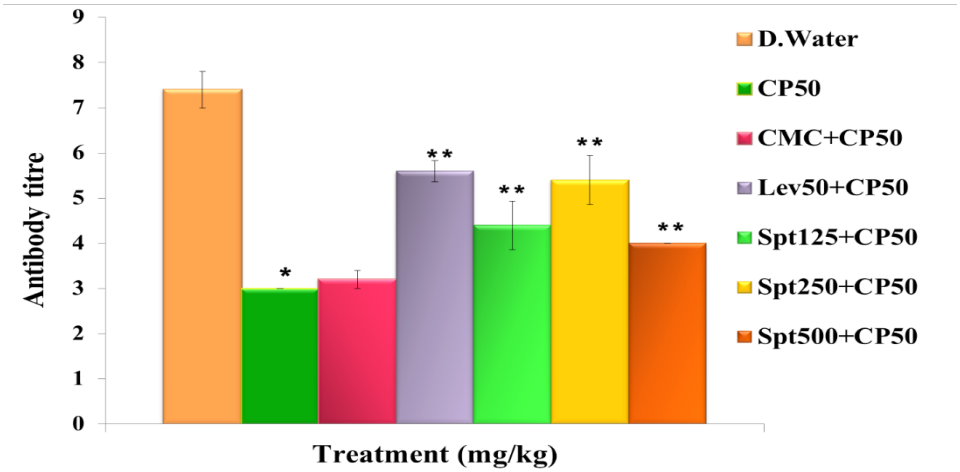

Figure 2. Effects of CP and Spt on HA titre in SRBC sensitized mice

Values are Mean±SE, (n=5), One-way ANOVA followed by Dunnett's Post Hoc Test, *P<0.001 compared to distilled water, **P<0.001 compared to CP treated mice.

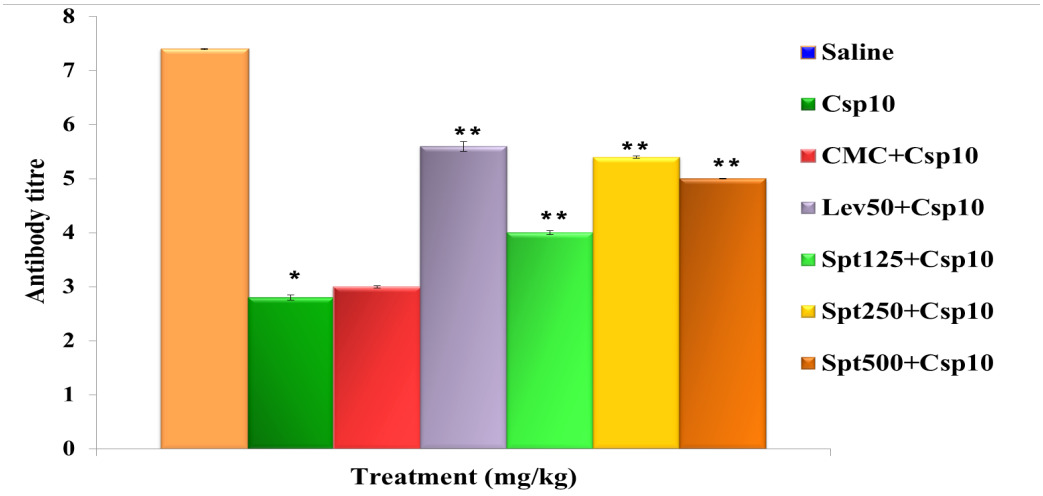

Figure 3. Effect of Csp and Spt on HA titre in SRBC sensitized mice

Values are Mean±SE, (n=5), One-way ANOVA followed by Dunnett's Post Hoc Test; *P<0.001 compared to 0.9% saline treated mice, ** P<0.001 compared to Csp treated mice.

tus of macrophages and stronger immune response [27]. The results of immunostimulatory effect of Spt in mice were comparable with those of the standard allopathic immunomodulatory drug, Lev (Figures 1-6).

Antibodies stimulate effector functions through binding to antigens, which either inhibit them or destroy them by forming cross-linked clusters that are ultimately ingested by phagocytes [21]. Our HA titre assay confirmed the immunosuppressive effects of CP on mice, shown by the low production of antibodies against sheep RBC with low HA titre values (Figure 1). In animals treated with Csp, a 62% reduction in the HA tire value was achieved, compared to the control group treated with normal saline (Figure 2). The CP and Csp treatments of the mice showed a decrease in the antibody production against sheep RBC, as evidenced by the reduced HA titre value, indicating their suppressive effects on the mice humoral immunity. However, pretreatment of the animals with Spt stimulated the production of antibody with a one-fold increase in HA titre value in contrast to those observed in the groups treated with CP or Csp (Figures 2 and 3). These results indicate the immunostimulatory effects of Spt against the suppression induced by CP and Csp in mice.

The excessive lymphoid organ damage caused by CP and Csp is likely due to the over production of NO, which promotes inflammation through eliciting lipid peroxidation in the immune cells [28]. In this study, Spt reduced the inflammation caused by the effect of immunosuppressive drugs on the animals' thymus. This effect may be due to the scavenging ability of NO and the presence of diverse phytochemicals, including tannin, sugars, alkaloids,

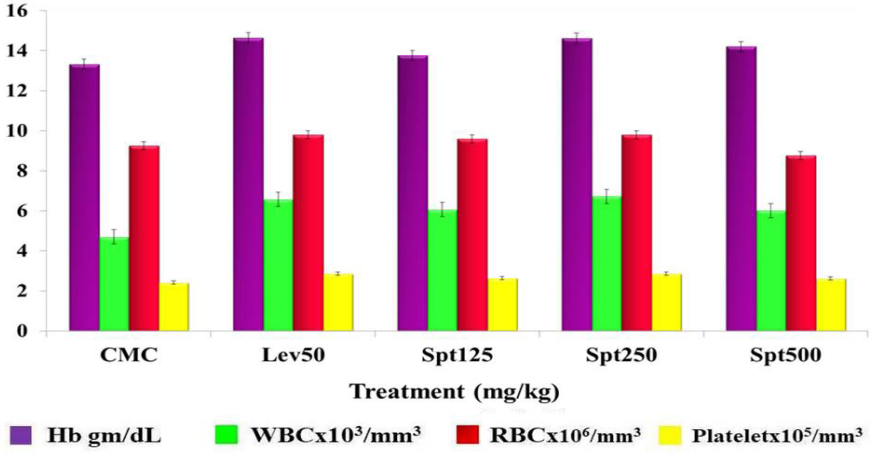

Figure 4. Effect of Spt on % of haemoglobin, total WBCs, RBCs and platelets count in mice

Hb: Haemoglobin; WBC: White Blood Cells; RBC: Red Blood Cells.

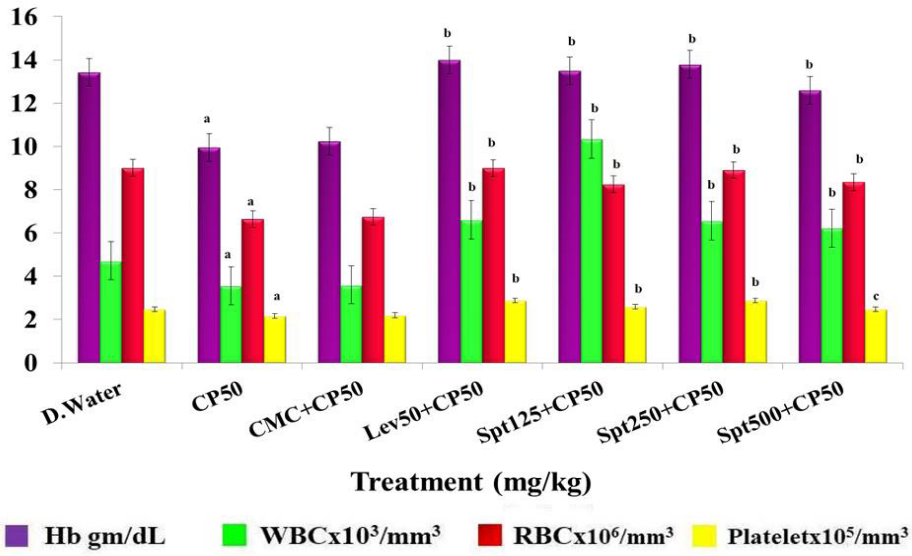

Figure 5. Effect Spt against CP induced changes in haematological parameters in mice

Values are Mean±SE (n=5), One-way ANOVA followed by Dunnett's post hoc test; [a] P<0.001 compared to distilled water treated mice, [b] P<0.001 compared to CP treated mice, [c] P<0.01 compared to CP treated mice, [d] P<0.05 compared to CP treated mice. Hb: Haemoglobin; WBC: White Blood Cells; RBC: Red Blood Cells.

flavonoids and other proteins [29]. In this context, Kumar et al. [30] have reported the modulatory effect of Spt against CP-induced variations in the blood parameters including leukocyte counts and Hb levels. The pre-treatment with Spt improved the total WBC counts, Hb level and bone marrow cell proliferation in contrast to the effects noted after treatment with CP. The augmentation of cellular and humoral immune defence by Spt in Balb-C mice was also observed by Praveenkumar et al. [31].

The immunostimulatory effect of Spt against the suppressive drug, prednisolone, was also studied by Sharma and Ray [32]. In the latter study, Spt increased the production of antibodies in mice immunized with sheep RBC and counteracted the suppressive effects of prednisolone. Daswani and Yagnanarayan [12] documented the immunomodulatory effects of Spt against E. coli sepsis in Swiss albino mice and rats. These authors reported that Spt exhibited dual effects on the immune system. It caused immune-stimulation at low dosage but served

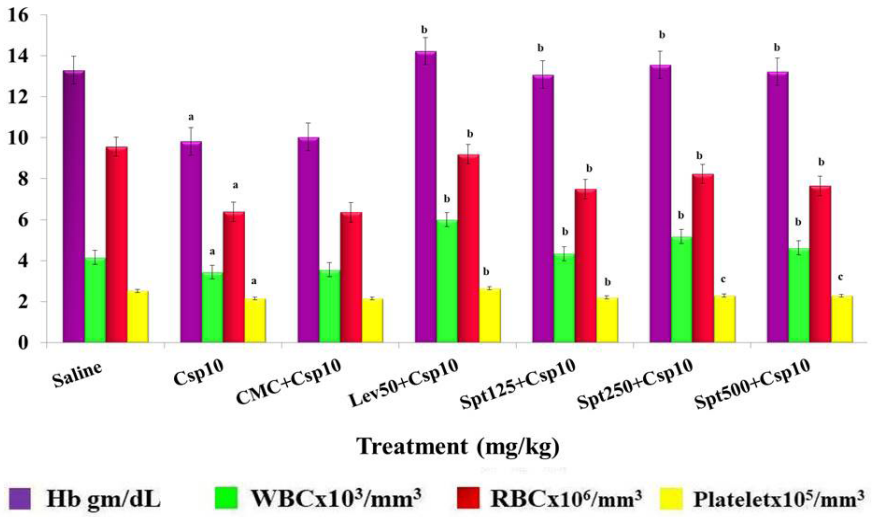

Figure 6. Effect of Spt on Csp induced changes in %Haemoglobin, Total WBCs, RBCs and platelets count in mice

Values are Means±SE (n=5), One-way ANOVA followed by Dunnett's post hoc test; [a] P<0.001 compared to saline, [b] P<0.001 compared to Csp, [c] P<0.01 compared to Csp. Hb: Haemoglobin; WBC: White Blood Cells; RBC: Red Blood Cells.

as an immunosuppressant at high doses. Therefore, Spt may be a potential immunomodulatory agent, whose activities are likely to be attributed to the complex nature of its phytochemical constituents. Hence, further quantitative phytochemical analyses are warranted to elucidate the unknown properties of this compound.

Conclusion

The current study uncovered the potent stimulatory activities of Spt against the immunosuppression caused by drugs used for chemotherapy. This compound imparted a stimulatory effect on mice immune system at the three doses tested in this study. Of the three doses, Spt showed a maximal improvement in the immunological parameters at 250 mg/kg. Our findings support the available data on the therapeutic application of Spt in traditional medicine as an immunomodulatory agent. This contention is evidenced by the increased antibody production and modulation of haematological parameters in mice treated with CP and Csp. The immunomodulatory activity of Spt may be attributed to its various constituents, such as flavonoids, tannins, alkaloids, saponins and phenolic compounds. Finally, we can conclude that Spt has the potential to serve as an effective immunomodulatory agent in averting the suppressive effects of chemotherapy drugs on the immune system.

Ethical Considerations

Compliance with ethical guidelines

The ethical approval to conduct this study was received from the Institutional Animal Ethics Committee of Mangalore University, Karnataka, India (MU/AZ/504/IAEC/2015-2016; dated September 23, 2015).

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Authors' contributions

Both authors equally contributed to preparing this article.

Conflict of interest

The authors declared no conflict of interests.

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