RESEARCH ARTICLE

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Immunomodulatory Effect of a Polyherbal Formulation (Imusil) on Cyclophosphamide Induced Experimental Animal Model

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Abstract

Objective: In the present study, we investigated the immunomodulatory effect of a polyherbal formulation referred to as Imusil (IM) on cyclophosphamide (CP) induced immunosuppression model. **Methods:** CP induced experimental animal model was used for evaluating the immunomodulatory effect of IM. For the study, animals were divided into four groups. Group I is served as the normal control, group II is treated only with CP, group III is treated with the standard drug, levamisole and group IV is treated with IM. The experimental duration was 30 days. At the end of the study, we had evaluated various parameters such as immune organ index, liver marker enzymes, antioxidants, haematological analysis, Th1/Th2 cytokine balance and humoral immune responses were examined using ELISA kits, T-lymphocyte subsets by flow cytometry, and histopathological analysis of the liver, spleen and thymus by H&E staining. **Results:** The results obtained from the study revealed that the treatment of immunosuppressed animals with IM significantly ($p<0.05$) reversed the immune response in a positive manner. Treatment with IM properly shields the immune organs and triggers the cell-mediated and humoral immune responses accordingly. Thus, no significant changes were observed in the haematological parameters. Moreover, IM supplementation helps to boost up the antioxidant activity, thereby preventing oxidative stress-mediated damage, and also protects the liver from the toxicity induced by CP. **Conclusion:** The results suggest that IM has the ability to counteract the immunosuppressive effect of chemotherapeutic drugs by stimulating the immune system, along with its potent antioxidant and hepatoprotective properties.

Keywords: Immune responses- immunomodulators- cytokines- antioxidants- herbal medicine

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Introduction

Cancer is a global burden that continues to be the second leading cause of mortality. Our immune system has the ability to recognise cancer through various mechanisms, but the cancerous cells employ strategies to avert this immune surveillance and thereby suppress the immune response (Munn et al., 2016). Thus, when the immune system is functioning properly, it plays an important role in protecting the body from outside threats. However, when it is compromised, it can result in the emergence of a number of chronic disorders (Al-Haddad et al., 2014). Chemotherapeutic agents are widely used in the treatment of cancer to inhibit the uncontrolled proliferation of cancer cells.

Cyclophosphamide (CP) is one such anticancer drug that acts as a nonspecific cytotoxic agent (Haque et al., 2001). It has been reported that the CP not only attacks cancerous cells but also normal cells and can induce oxidative stress by disrupting redox balance (Pratheeshkumar and Kuttan, 2012). Therefore, due to the presence of a large concentration of polyunsaturated fatty acids in the membranes of immune cells, they are particularly vulnerable to oxidative damage and, as a result, hasten immunosenescence (Chahar et al., 2012). Apart from this, CP can also disturb the Th1/Th2 balance and lead to the reduction of major immune cells like T and B cells (Rabinovitch et al., 1994; Yu et al., 2015). Thus, CP is a well-established inducer of immune suppression in animals to evaluate the immunomodulatory effect of a sample of interest (Wang et al, 2011). Moreover, immunity has now become the topmost health concern among people. In recent years, more importance has been given to the identification of immunostimulating agents from nature because they are being considered as prospective agents to replace conventional drugs in therapeutic regimens (Jantan et al., 2019). Among the different modes of therapeutics, synergy based polyherbal

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formulations gained more attention from researchers. This is because different diseases have complex underlying pathophysiology and are initiated by a variety of factors. As a result, these formulations are thought to be able to increase the bioavailability of active ingredients, amplify their therapeutic effects, and reduce their harmful consequences (Zhou et al., 2016).

For decades, Picrorhiza kurroa, Tinospora cordifolia, and Emblica officinalis, have been used as traditionally valued herbal medicines. Picrorhiza kurroa (Kutki) is a medicinal herb used traditionally for the treatment of liver diseases, pyrexia, inflammatory diseases, allergic diseases, and chronic diarrhea. The major component of Picrorhiza kurroa is apocyanin, which possesses miraculous pharmacological benefits like inhibition of reactive oxygen species synthesis and also inhibits the synthesis of pro inflammatory cytokines like IL-2, IFN-γ, and TNF-α (Sharma et al., 2012). Tinospora cordifolia (Guduchi) is another traditional herbal medicine used in Ayurveda that has anti-microbial, anti-inflammatory, antioxidant, anti-allergic, and anti-cancer properties. The immunostimulatory effect of this plant was studied by several researchers, and it was found that the immunostimulatory effect is acquired by augmenting macrophage chemotaxis and phagocytosis as well as by promoting interaction with other immune regulatory lymphoid cells. The various chemical constituents, such as glycosides, phenolics, alkaloids, sesquiterpenoids, and aliphatic compounds present in Tinospora cordifolia are responsible for imparting their pharmacological activities (Bishayi et al., 2002). Another important and commonly used herbal medicine is Emblica officinalis (Indian gooseberry), which is a rich source of Vitamin C, which helps to boost immune defence by augmenting epithelial barrier function against pathogens and inducing phagocytosis for microbial destruction, etc. due to the presence of tannins like embilicanin A, embilicanin B, punigluconin, and pedunculagin, etc. (Carr and Margini, 2017). Therefore, in the present study, we aimed to evaluate the immunomodulatory effect of a polyherbal formulation consisting of Picrorhiza kurroa, Tinospora cordifolia, and Emblica officinalis (hereinafter referred to as Imusil) against cyclophosphamide-induced immunosuppressed animal model.

Materials and Methods

Chemicals

All the chemicals and reagents used in the experiment was analytical grade purchased from Merck Ltd. Cyclophosphamide was procured from HiMedia Laboratories, Mumbai, India. The standard drug lavamisole tablet was purchased from local medical store. Cytokines and Immunoglobulins ELISA kits were provided by Elabscience Biotechnology, China and Abcam Company, USA. Antibodies for flow cytometry was purchased from BD bioscience, USA.

Preparation of polyherbal formulation (Imusil)

Tinospora cordifolia and Emblica officinalis were collected from Uttar Pradesh, India, while Picrorhiza kurroa was collected from the Himalayan region, India. All the plant materials were authenticated by a taxonomist. Finely ground plant material powder was weighed and mixed with deionized water, then incubated for 24 hours with continuous agitation. Colorless water, indicating extraction completion, was obtained after overnight maceration. Insoluble particles were filtered using a 0.22-micron syringe filter. The remaining filtrate was lyophilized and stored at 2–4 degrees Celsius until needed.

Imusil (IM) contain 200mg of rhizome of Picrorhiza kurroa (Kutki) extract, 60mg of stem of Tinospora cordifolia (Guduchi) extract and 60mg of fruit of Emblica officinalis (Gooseberry) extract. The preparation and characterization of Imusil was previously reported (Ratheesh et al., 2022).

Animals

Male wistar rats were used for the study weighing 150-200g. The animals were maintained in an environment with 24-26°C temperature, 55-60% humidity and 12:12 h light - dark cycle. Sterile condition was maintained throughout the experiment and they were supplemented with commercially available rodent pellet diet and tap water ad libitum. The experiments were conducted as per the guidelines of the animal ethics committee CPCSEA (Registration CAF/Ethics/839/2021) according to Government of India accepted principles for laboratory animals' use and care.

CP Induced immunosuppression in rats

Immunosuppression model using CP was established based on the previous work described by Duggina et al., 2015. Before conducting the experiment, all the animals were acclimatized to the laboratory condition and fasted overnight prior to the experiment.

For the experiment, rats were randomly divided (complete randomized method) into four group with 6 animals per group:

Group I: Normal control received only normal saline (NC)

Group II: Cyclophosphamide alone treated animals orally (10mg/Kg b wt) (CP)

Group III: Cyclophosphamide+ Levamisole treated animals (50mg/Kg b wt) (CP+Lev)

Group IV: Cyclophosphamide+ Imusil treated animals (600mg/Kg b wt) (CP+IM)

CP, Lev and IM were administrated orally using intra gastric tube at their respective doses for 30 consecutive days. After the experimental duration, rats in each group was sacrificed via cervical dislocation, blood samples were taken for haematological and serological analysis. The lymphoid organs such as spleen and thymus and liver tissues were dissected out for further investigations.

Determination of immune organ Index

Before sacrifice, each rat was weighed and then the thymus and spleen were dissected out and the weight of thymus and spleen was noted after removing the fat and connective tissue. For calculating the organ index, organ

weight in mg was divided by body weight in g.

Hematological analysis

Blood was withdrawn from each animals after the 24hrs of last dose. Using a fully automated haematology analyser (Abomed biosystems Pvt Ltd, India), White blood cells (WBC), Red blood cells (RBC), platelets (PLT), neutrophils (NEU) and lymphocytes (LYM) were analysed.

Estimation of cytokines and immunoglobulins by ELISA

Enzyme-linked immunosorbent assays (ELISA) were used to measure the cytokine concentration in the serum. By following the instructions of manufacturer, level of cytokines such as IL-2, IFN-γ, IL-10 and IL-4 (Elabscience Biotechnology, China) and the immunoglobulins such as IgG and IgA were tested (Abcam Company, USA).

Estimation of SGOT, SGPT and ALP

The serum was used to test the level of liver enzymes such as glutamic pyruvic transaminase (SGPT), glutamic oxaloacetic transaminase (SGOT), alkaline phosphatase (ALP). The analyses were performed with the diagnostic kits from the Agappa Diagnostic Company, India.

Antioxidant assays

Antioxidant analysis includes the evaluation of catalase (CAT) activity by following the method of Maehly and Chance (1954), superoxide dismutase (SOD) activity was evaluated by the procedure of Kakkar et al., (1984), and glutathione content (GSH) was estimated using the procedure of Benke (1974).

Flow cytometry

Peripheral blood was collected in heparinised tube and centrifuged at room temperature to spin down the cells. Then, the RBC cells were removed using 1X RBC lysis buffer (according to manufacturer's instruction). The total number of lymphocytes was counted by Trypan blue exclusion assay using hemocytometer. The specific cell surface staining was done using fluorochrome conjugated monoclonal antibodies: PE mouse anti-rat CD3, FITC mouse anti-rat CD8 alpha and APC mouse anti-rat CD4. The samples were incubated in ice for 45min with intermittent tapping. After centrifugation, cells were suspended in FACS buffer (1% FBS in PBS) solution. The analysis of stained cells were done using BD FACSVerse configured with 3 lasers and 8 fluorescence parameters and using the BD FACSDiva software.

Histopathological analysis of spleen, thymus and liver tissue

Using a 10% formalin solution, the spleen, thymus and liver tissues were preserved for 24 hours at room temperature. The pathological samples were analysed by haematoxylin and eosin staining. Alterations were observed using microscope and images were taken. The slides were analysed by pathologist in blinded fashion.

Statistical Analysis

Statistical analysis was performed using the program SPSS/PC+, version 20.0 (SPSS Inc., Chicago, IL, USA). All the data were evaluated by utilizing one-way analysis of variance (ANOVA) for comparing significant differences among groups. Pair fed comparisons between the groups was made by Duncan's multiple range tests. $P < 0.05$ was considered as statistically significant.

Results

Effect of IM on immune organ index

The organs of the immune system, such as the spleen and thymus, are always obsessed with the initiation of an immune response in order to evade foreign invaders. Immune organ indices are a representation of immune system functioning and a prognosis of the state of the immune system. Thus, to evaluate the immunomodulatory effect of IM on cyclophosphamide induced immunosuppressed rats, we evaluated the spleen and thymus index. The result obtained from the analysis showed that the spleen and thymus index were significantly reduced in the CP induced group as compared with the normal control ($p<0.05$). However, these changes were significantly reverted in the rats treated with IM, which was more than that of the LEV treated group (Figure 1).

Effect of IM on haematological

We had evaluated the effect of IM on blood composition because blood and blood components are highly involved in the modulation of the innate immune response. Immunosuppressive agents like cyclophosphamide usually reduce the number of immunity mediating cells. The results obtained from the blood analysis also showed that the count of RBC, WBC, and platelets and the percentage of lymphocytes, neutrophils, monocytes, basophils, and eosinophils were reduced considerably as compared with the normal control ($p<0.05$). In the treatment group, the effectiveness of IM was very substantial, as it helped to improve the blood parameters significantly as compared to the CP group ($p<0.05$) (Table 1).

Table 1. Effect of IM on Haematological Parameters. Values expressed as average of 6 samples ± SEM in each group. 'a'-Statistical difference with normal group at $P \leq 0.05$. 'b'-Statistical difference with CP treated rats at $P \leq 0.05$. WBC- white blood cell count; RBC- red blood cell count; PLT- platelet count; LYM- lymphocytes; NEUT- neutrophils

Group	RBC (10^6/µL)	WBC (10^3/µL)	PLT (10^3/µL)	LYM (%)	NEUT (%)
NC	7.19±0.08	9.48±0.11	772.16±1.83	79.08±0.14	12.5±0.12
CP	3.36±0.07[a]	6.31±0.10[a]	588±3.18[a]	60.7±0.17[a]	7.41±0.10[a]
CP+LEV	6.7±0.08[b]	8.53±0.14[b]	725.16±2.7[b]	74.25±0.13[b]	11.53±0.09[b]
CP+IM	6.98±0.07[b]	8.75±0.08[b]	735±2.30[b]	77.6±0.16[b]	11.91±0.07[b]

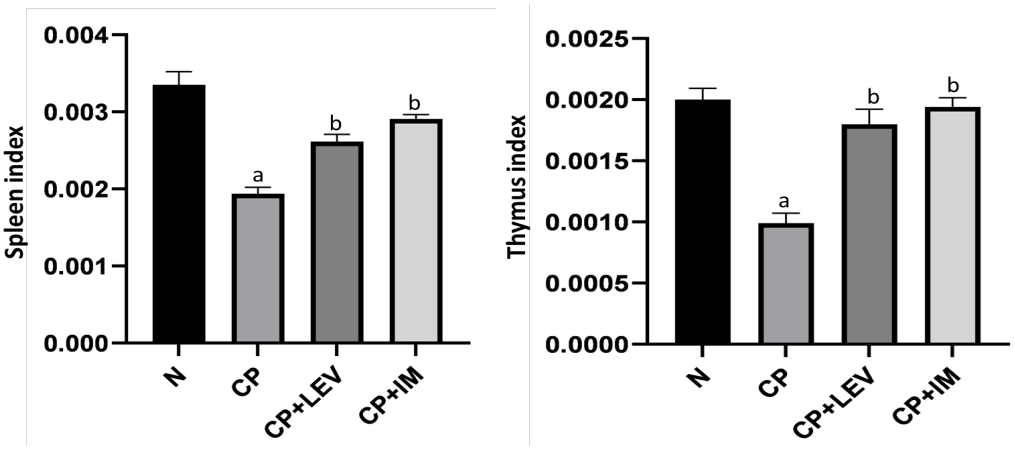

Figure 1. The Effect of IM on the Immune Organ Index. Values expressed as average of 6 samples ± SEM in each group. 'a' -Statistical difference with normal group at P ≤ 0.05. 'b' -Statistical difference with CP treated rats at P ≤ 0.05.

Effect of IM on humoral immune response

The humoral immune response is mediated by the release of antibodies by plasma cells. Among those antibodies, immunoglobulins perform several roles, and the levels of immunoglobulins like IgG and IgA are considered important biomarkers for evaluating the status of the humoral immune response. In the present study, we examined the serum levels of IgG and IgA in each group by ELISA. As shown in Figure 2, treatment with CP sharply reduced the levels of IgA and IgG. However, the IM treated group showed increased levels of IgA and IgG as compared with the CP and Lev treated groups, suggesting the efficacy of IM on the humoral immune response.

Effect of IM of cytokine level

Cytokines have a major role in the initiation of the immune response. Th1 and Th2 cytokines perform their specific functions during the time of immune reaction. Among the various cytokines, IFN-γ and IL-2 are commonly known as Th1 cytokines, whereas IL-4 and IL-10 are called Th2 cytokines. As shown in figure 3, CP administration significantly affects the level of these cytokines by causing their reduction in comparison with normal control ($p<0.05$). The analysis of the effect of IM on the release of Th1/Th2 cytokines showed that the treatment of IM enhanced the release of IFN-γ, IL-2, IL-4, and IL-10 in immunosuppressed rats. Therefore, the results imply that IM stimulation has the ability to trigger the secretion of Th1 and Th2 cytokines and prompt a protective immune response.

Effect of IM on the level of liver marker enzymes

In addition to other immune organs, the liver is also considered a frontline immune organ that plays a unique role in innate immune responses. In order to evaluate the proper functioning of the liver, we estimated the levels of the liver marker enzymes SGOT, SGPT, and ALP. As presented in Figure 4, CP intoxication elicited a rise in the serum levels of SGOT, SGPT, and ALP in comparison with the normal group. This significant change was minimised and maintained at a normalised level in the immunosuppressed animal treated with IM, which pointed out the potent hepatoprotective ability of IM.

Effect of IM on the activity of endogenous antioxidants

Reactive oxygen species have a major role in the immune response through the activation of T cells, but their excess production results in a negative impact. Therefore,

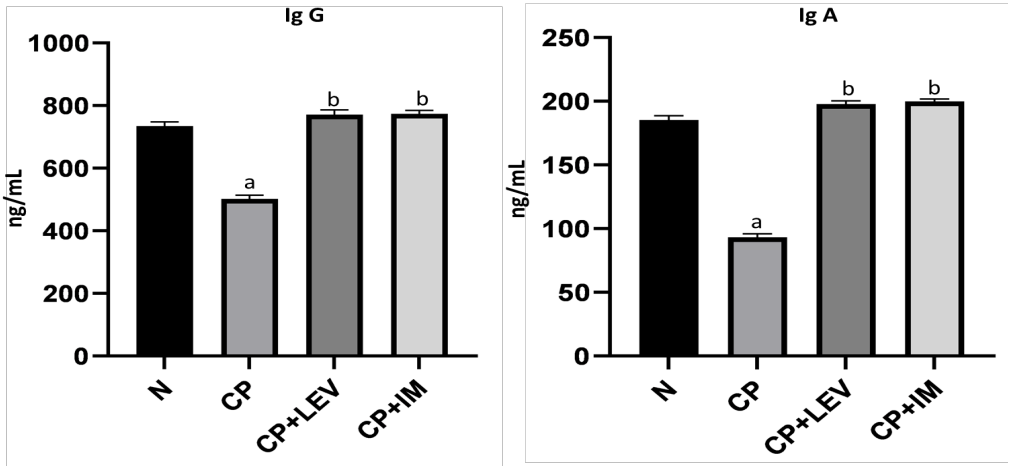

Figure 2. Effect of IM on Humoral Immune Response. Values expressed as average of 6 samples ± SEM in each group. 'a' -Statistical difference with normal group at P ≤ 0.05. 'b' -Statistical difference with CP treated rats at P ≤ 0.05.

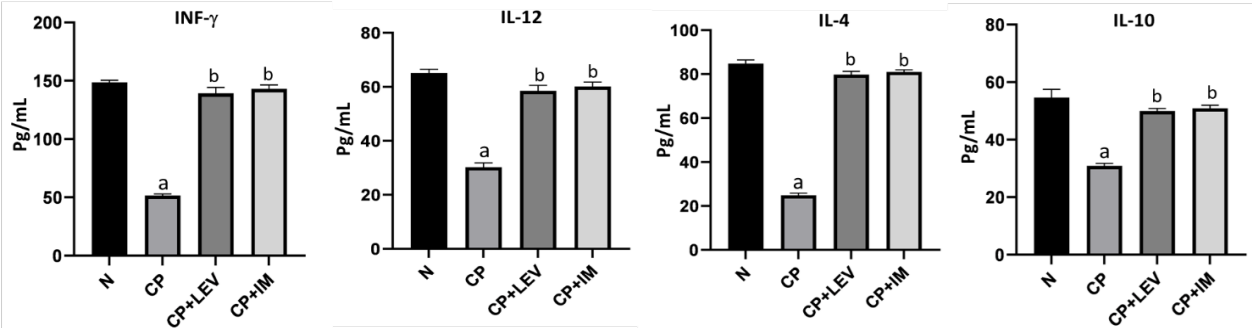

Figure 3. Effect of IM on Th1/Th2 Cytokines. Values expressed as average of 6 samples ± SEM .in each group. 'a'-Statistical difference with normal group at P ≤ 0.05. 'b'-Statistical difference with CP treated rats at P ≤ 0.05.

evaluating the status of endogenous antioxidants can provide a clear reflection of oxidative stress in the body. The result of the stress marker study revealed that in CP-administered animals, the antioxidant activity declined significantly as compared to normal, which implies that CP can induce oxidative stress and lead to a subsequent reduction of antioxidants. In contrast to this, treatment with IM helps protect animals from oxidative stress by enhancing the antioxidant activity of SOD and CAT and also improving the GSH content as compared with the CP group ($p<0.05$) (Figure 5).

Flow cytometry analysis

To evaluate the effect of IM on the differentiation of T cells, we analysed the percentages of total T cells and T cell subsets in rats by performing immunophenotyping of the different cells present in the blood using flow cytometry analysis. The results were reported in Figure 6. Our data indicated that the expression of T helper cell CD4+ and cytotoxic T cell CD8+ was significantly enhanced in the

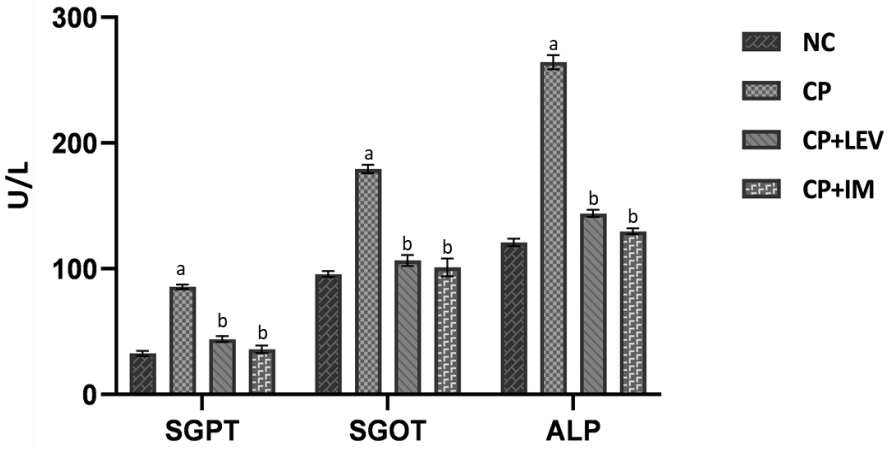

Figure 4. Effect of IM on Liver Enzyme Markers. The values are expressed as mean± SEM of six rats in each group. a - Statistical difference with control group at P< 0.05. b – Statistical difference with CP treated rats at P< 0.05. U: SGOT- µmol of oxaloacetate liberated /min/mg protein. U: SGPT- µmol of pyruvate formed /min/mg protein. U: ALP- amount of enzyme to decompose 1 µmole of P-NPP/minute at 25° C.

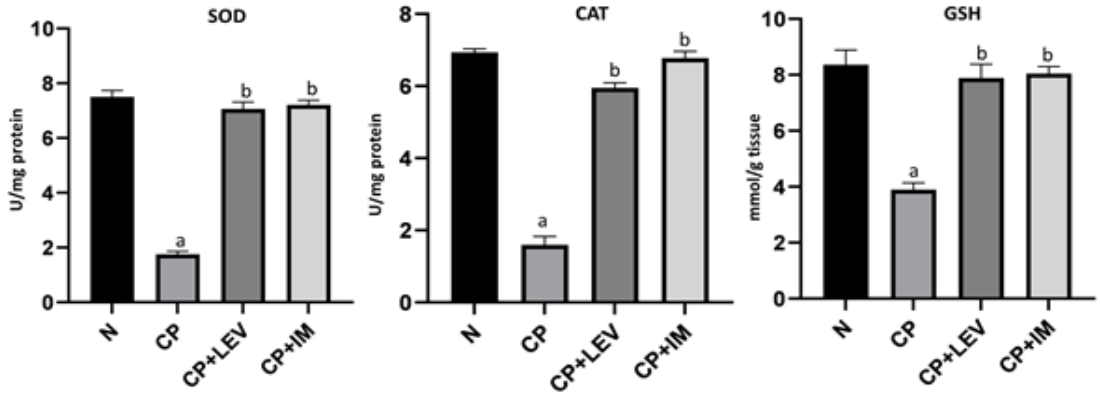

Figure 5. Effect of IM on the Antioxidant Activities of SOD and CAT and the Level of GSH. The Values expressed as average of 6 samples ± SEM in each group. 'a'-Statistical difference with normal group at P ≤ 0.05. 'b'-Statistical difference with CP treated rats at P ≤ 0.05.

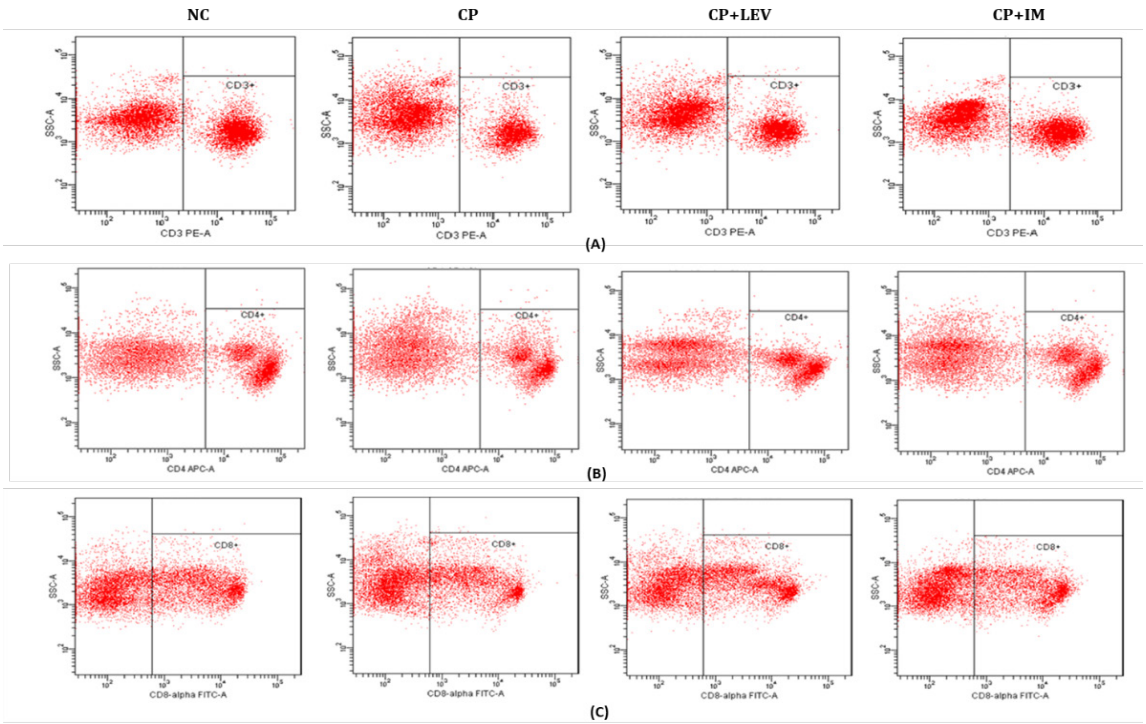

Figure 6. Effect of IM on the Population Change in T Lymphocyte Subset in CP Induced Immunosuppressed Rats. T lymphocytes were detected using PE-, APC- and FITC- conjugated monoclonal antibodies specific for the rat's T cell markers CD3 (A), CD4 (B), and CD8 (C) respectively. Stained cells were analysed by flow cytometry using a BD FACSDiva software.

animals supplemented with IM at a dose of 600 mg/kg as compared with the CP control group and LEV treated group. Similarly, the CD3+ cells were also found to be higher in the IM treated group than in the CP and LEV supplemented groups. These results demonstrated that IM can regulate the cellular immune response and maintain normal physiological functions.

Histopathological observation of spleen tissue

It was widely recognised that immune organ damage frequently occurred in conjunction with the failure of the immune system. Therefore, to further explore the protective effect of IM on the spleen, thymus, and liver ultrastructure, histopathological analysis by H&E staining was carried out. As shown in Figure 7, the spleen histology of the IM treated group was found to be arranged intact with clear nuclei and less intercellular space, which was similar to the normal group and LEV group. Likewise, in the case of the thymus, IM treated rats showed better results, as the area of the cortex and medulla was almost clear and

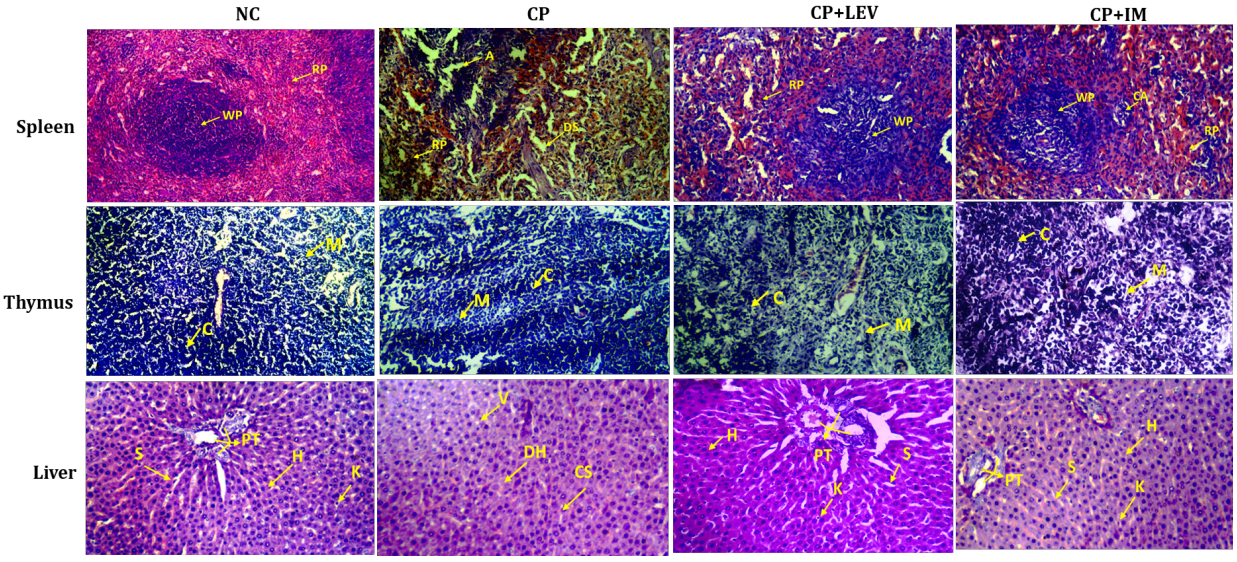

Figure 7. Histopathological Analysis of Rat Spleen, Thymus and Liver. Tissues were stained with H&E and examined by optical microscopy. WP- White pulp; RP- Red pulp; CA- Central artery; DS- Dilated sinusoid; A-Atrophy; M- Medulla; C- Cortex; H- Hepatocytes; K-Kupffer cells; PT- Portal triad; S- Sinusoidal space; V-Vacuole; DH-Degenerated hepatocytes; CS-Congested sinusoidal space.

the number of thymocytes was increased compared to that of the CP control group. To evaluate the hepatoprotective effect of IM, we performed a histopathological analysis of liver tissue. In the cyclophosphamide treated group, the normal architecture of hepatocytes was lost, and moderate to severe hepatocyte degeneration, necrosis, and some fatty infiltration were also observed. The sinusoidal space was found to be congested. These changes were not found in the animals treated with IM and LEV and seem to be more similar to the liver of the normal control group. Thus, the findings suggested that the CP induced spleen, thymus, and liver cell damage could be reversed by IM.

Discussion

It has been demonstrated that using immunomodulators to improve host defence responses can be a successful strategy for boosting disease resistance (Zhao et al., 2014). Thus, the active area of current research is now focused on natural immunomodulators to keep the body in a state of equilibrium. By stimulating or deactivating compromised immunological responses, phytocompounds can strengthen the host's defence system (Wagner et al., 1984).

The spleen and thymus are the primary sites of immune response activation. The thymus can regulate lymphocyte differentiation, maturation, and the production of immunological responses, whereas the spleen helps in the synthesis of bioactive substances and B and T cell colonisation (Meng et al., 2019; Liu et al., 2008). As immune function declines, the spleen and thymus will contract and lose volume (Huang et al., 2016). Thus, the thymus and spleen indices reflect the state of immune function. In our study, the CP induced group showed reduced organ indices, but IM protected the spleen and thymus and diminished CP-induced immunosuppressive activity, suggesting that IM could strengthen an organism's immune system.

The production of cytokines by various immune cells is crucial for the differentiation of lymphocytes, the regulation of inflammation, cell survival, and defence against microbial infection, apoptosis, and other immunological responses (Lacy and Stow, 2011; Van der Meide and Schellekens, 1996). Depending on the function and variations in the secreted cytokines, Th cells that have been activated in response to antigen recognition are divided into Th1 and Th2 cells (Zhou et al., 2008; Osugi et al., 1997). Th1 cells release IFN-γ and IL-2 whereas Th2 secretions include IL-4 and IL-10 (Lacy and Stow, 2011). In general, Th1 cells support inflammatory responses and tumour immunity (Nishimura et al., 2000; Haabeth et al., 2011) and Th2 cells support humoral immunity via B cells against external pathogens (Mosmann and Sad, 1996). Thus, these cytokines can control the immune balance of Th1 and Th2 because of their ability to interchange the cell-mediated immune response into a humoral response and vice versa. Some studies show that pharmaceutical substances can change the Th1-Th2 balance, which might be advantageous for the host. Because the type of response needed depends on the nature of the disease agent (Burns et al., 2009), the result obtained from the present study revealed that IM administration significantly enhanced the production of Th1 and Th2 cytokines, which was reduced in CP induced immunosuppressed animals. According to this result, we propose that the augmentation of these cytokines by IM may be responsible for inducing cell-mediated and humoral immune responses.

To further clarify the action of IM as an immunomodulatory drug, T-lymphocyte populations were examined by flow cytometric assay. Based on the phenotype and function of T-lymphocytes, they are divided into different subsets, and each subset expresses a particular set of surface markers. All T lymphocytes display CD3, whereas inducer-helper T cells (Ti/Th) and suppressor cytotoxic T cells (Ts/Tc) express CD4 and CD8 respectively, on their surfaces. Thus, CD3+ T cells represent the total population of T lymphocytes, whereas CD4+ and CD8+ are the important functional subsets of T cells. Helper T cells, or CD4+ cells, support lymphocyte differentiation and antibody production, and cytotoxic T cells, or CD8+ cells, are responsible for killing the infected cells (Oda et al., 2014). In this study, we found that IM was successful in increasing the proportion of CD3+, CD4+, and CD8+ T cell subsets. Thus, the outcome showed that IM can significantly improve cellular immune function in an immunosuppressed condition.

In addition to cell-mediated immunity, humoral immunity is also important in the host immune response. Major immunoglobulins, such as IgG and IgA, induce a humoral immune response by eliminating the invaded pathogens through opsonization, complement activation, and the neutralisation of poisons, germs, and organisms (Schroeder and Cavacini, 2010). The toxic nature of CP can be observed at different levels, as there was a remarkable decrease in IgA and IgG levels after the administration of CP, showing its negative impact on the immune system, but on the other side, the condition got reversed when treated with IM, representing its ability to enhance the humoral immune response.

The hematopoietic system serves as a crucial indicator of physiological and pathological status in both human and animal investigations (Mukinda and Syce, 2007). A complete blood analysis can help to identify the disease condition as well as the severity of that disease. In a diseased condition, there will be a tremendous change in blood count (Zhang et al., 2015). In immunosuppressed animals, mature blood cells such as RBCs, WBCs, and PLTs, and additionally, NEU and LYM, were also decreased due to the effect of immune suppressors on hematopoietic stem cells (Agrawal et al., 1999). On the administration of IM, the alteration in the level of haematological parameters was maintained at a normal level.

Furthermore, CP administration not only impairs the immune system but also induces an oxidative stress condition by producing highly reactive metabolites (Manda and Bhatia, 2003). The interaction of acrolein (a reactive metabolite of CP) can activate intracellular ROS and the production of NO, which leads to the production of peroxynitrite (Bhatia et al., 2008). The combination of acrolein and peroxynitrite decreases the antioxidants. In order to get rid of the damaging effect of reactive oxygen

species (ROS), trigger the cellular defence system and thereby elevate the anti-oxidant enzymes in the body. SOD and catalase are the main free radical scavengers, as SOD converts O_2 to H_2O_2, which is in turn converted to H2O by catalase, thus preventing the production of hydroxyl radicals (Wei et al., 2011). To change the hydroxyl radical into a nontoxic product, the reduced glutathione (GSH) gets oxidised into glutathione disulfide and then reduced to GSH by glutathione reductase (Liguori et al., 2018). The results of the present study are in agreement with prior studies reporting that CP reduces the antioxidant status, but IM exhibits a potent anti-oxidant potential by increasing antioxidants like SOD, CAT, and GSH.

Apart from the toxic effect of CP on the major immune organs, hepatotoxicity is another well-known side effect of CP (DeLeve, 1996). The liver is the major site where CP activation takes place and is then broken down into its various cytotoxic metabolites (acrolein and phosphoramide mustard) with the help of cytochrome P450 enzymes. Thus, the pharmacological reports show that these metabolites, along with the ROS modulate all the liver marker enzymes, such as SGOT, SGPT, and ALP significantly (Sheetla et al., 2013). The liver enzymes are very significant in assessing the condition of the liver. From this study, we found that IM supplementation prevents liver damage and thereby controls the level of liver enzymes. The histopathological analysis of liver tissue also lends support to this result.

In conclusion, the results assimilated from the present study strongly portrayed that the IM is a potent immunostimulator as it is involved in the modulation of both humoral and cell mediated immune responses, enhances the antioxidant activities, and also possesses good hepatoprotective effect. Thus, the severe side effects and immunosuppressive effect of chemotherapeutic drugs can be controlled by the consumption of IM, as it is a promising alternative for immunity related health problems.

Author Contribution Statement

Conceptualization, Methodology: RM; Investigation: SS, AA; Writing - original draft, review & editing: RM, SS, SPJ; Validation: SS, JT, TJ; Data curation: JKJ, TJ: Formal analysis: JT, MA. All authors reviewed and approved the final draft of the manuscript.

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Ethical committee

This study was approved by the Institutional animal ethics Committee of Indian Institute of Science, Bangalore, India.

Availability of data

The data that support the findings of this study are available from the corresponding author on request.

Conflict of Interest

All the authors are disclosing the conflict of interest. Imusil is a registered and patent pending product of Glowderma Lab Pvt. Ltd. Mumbai, India.

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