



Research Journal of Pharmaceutical, Biological and Chemical Sciences

Drugs Responsible For Bone Marrow Suppression and Its Herbal Approaches: An Overview

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ABSTRACT

Some of the medicinal plants valued in Ayurvedic Rasayana for their therapeutic potential have been scientifically investigated with promising results. A number of plant-based principles have been isolated with potential immunomodulatory activity that can explain and justify their use in traditional medicine in the past and can form the basis for further research in the future as well. The aim of this review is to highlight results of research done on immunomodulators of plant origin. The selection of papers was made using the most relevant databases for the biomedical sciences on the basis of their ethnopharmacological use. Many plants and some phytoconstituents responsible for immunomodulation have been explained. The review also discusses biological screening methods for various plant drugs that focus on revealing the mechanism involved in immunomodulation.

Keywords: bone marrow, herbal

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INTRODUCTION

Blood helps to distribute the nutrients, oxygen and hormones that a body needs. It also carries toxins and waste material to the liver and kidney to be removed from your body. Blood cells are made in bone marrow. The bone marrow is a spongy tissue located inside some bones [1]. The bone marrow stroma consists of a heterogeneous population of cells that provide the structural and physiological support for hematopoietic cells. Additionally, the bone marrow stroma contains cells with a stem-cell-like character that allows them to differentiate into bone, cartilage, adipocytes, and hematopoietic supporting tissues. "Stroma", in ancient Greek, means the physical substrate, or something upon which one rests or lies. Hematopoietic cells proliferate, differentiate, and mature upon a meshwork of cell processes and surfaces that together comprise the bone marrow stromal scaffold. In mammals, hematopoiesis is an extravascular process, and therefore is supported by an extravascular marrow stroma as well. The stroma includes all cell types that:

(1) are located between the outer surfaces of marrow blood vessels and the bone surfaces which encase the hematopoietic space and tissue, and

(2) are not of the hematopoietic lineage, and these include marrow adipocytes, Western-Bainton cells, bone-lining cells (inactive osteoblasts), and osteoblastic cells[2].

The bone marrow works like a 'factory' that produces all of the cells that are found in the bone marrow and in the peripheral blood stream. This factory is dependent on the function of the pluripotent stem cells. Pluripotent refers to the ability of a cell to become many different types of cells. The bone marrow has two types of stem cells, mesenchymal and hematopoietic. This process of development of different blood cells from these pluripotent stem cells is known as hematopoiesis. Pluripotent hematopoietic cells can become any type of cell in the blood system. Under the influence of tissue and hormonal factors these cells develop into specific blood cell lines. When these cells differentiate or mature they become the cells that we can recognize in the blood stream[3].

The composition of the HSC bone-marrow microenvironment, known as the HSC niche. During homeostasis, HSCs, and therefore putative bone-marrow HSC niches, are located near bone surfaces or are associated with the sinusoidal endothelium. The molecular crosstalk between HSCs and the cellular constituents of these niches is thought to control the balance between HSC self-renewal and differentiation[4].

Stem cell transplantation (SCT) is an important treatment for several malignant disorders including leukemia and solid tumors, as well as for non-malignant conditions such as metabolic and genetic diseases. The number of stem cell-transplanted patients is constantly increasing due to the broader applicability and ameliorated clinical outcome. SCT requires an intensive preparative conditioning regimen consisting of total body irradiation (TBI), chemotherapy, or a combination of both. Despite a continuous improvement of SCT, several

complications such as sinusoidal obstructive syndrome (SOS), graft versus host disease (GVHD), cardiac toxicity and treatment-related mortality are still major limiting factors[5].

HUMAN HEMATOPOIESIS AND LYMPHOPOIESIS

With the exception of lymphocytes, blood-cell formation in normal human adults occurs exclusively in the bone marrow. All mature blood cells have a finite life, with the majority of cells being terminally differentiated and unable to replicate (Bagby, 1994). To maintain steady-state levels, formation of cells in the marrow must equal the rate of cellular senescence and elimination. This formation of blood cells is supported by a small population of pluripotent stem cells that exhibit the capacity to self renew and are capable of extensive proliferation. These cells also can reconstitute all hematopoietic lineages and are capable of long-term reconstitution of the hematopoietic system of recipient animals. The primitive pluripotent stem cells are estimated to comprise 1 in 100,000 bone-marrow cells and give rise to multi potent and committed progenitor cells that represent about 2 to 5 per 1000 marrow cells. Under the control of various growth factors, the primitive hematopoietic stem cell either can self replicate or replicate to form pluripotent stem cells[6]. These cells, in turn, can divide to form multi potent progenitor cells that are committed to either the myeloid or lymphoid lineages (Bagby, 1994). The multi potent myeloid progenitor cell (CFU-GEMM) can give rise to colony-forming cells of each myeloid lineage (erythrocyte, BFU-E; megakaryocyte, BFU-meg; monocyte/neutrophil/eosinophil, CFU-GM; basophil, CFU-baso). The CFU-GM cell can undergo further differentiation to form colony-forming cells that are restricted to the monocyte (CFU-M), neutrophil (CFU-G), and eosinophil (CFU-Eos) lineages[7]. Their in vitro clonogenic counterpart is represented by Colony Forming Units-fibroblasts (CFU-f), which in turn give rise to Bone Marrow Stromal Cells (BMSC) and Mesenchymal Stem Cells (MSC), possibly corresponding to a single cell population. BMSC in particular provide the absolutely essential support for hemopoiesis through both direct contact with cell surfaces and stromal cell derived soluble mediators[8].

Migration of HSCs occurs at specific times during development (i.e., seeding of fetal liver, spleen and eventually, bone marrow) and under certain conditions (e.g., cytokine-induced mobilization) later in life. The latter has proven clinically useful as a strategy to enhance normal HSC proliferation and migration, and the optimal mobilization regimen for HSCs currently used in the clinic is to treat the stem cell donor with a drug such as cytoxan, which kills most of his or her dividing cells. Normally, only about 8% of LT-HSCs enter the cell cycle per day,95,96 so HSCs are not significantly affected by a short treatment with cytoxan[9].

DRUGS RESPONSIBLE FOR BONE MARROW SUPPRESSION:

Peripheral cytopenia from bone marrow suppression is a frequent dose limiting side effect of chemotherapy and can manifest as acute and chronic marrow damage.4 Chemotherapy may result in the destruction of activity of proliferating haematopoietic precursor cells, leading to deprivation of formed elements, and incidence of life threatening haemorrhage and infection. Carboplatin, Chlorambucil, Oxaliplatin, Cyclophosphamide,

Cytarabine, Vinorelbine, Ifosamide, Dactinomycin, Mitomycin, Procarbazine, Fludarabine, Mustine, Topotecan, FU, Mitoxantrone, Vinblastine, Melphelan, Oxaliplatin, Idarubicin, Irinotecan, Rituximab, Transtuzumab, Doxorubicin, Gemcitabine, Methotrexate, Daunorubicin, Paclitaxel, Hydroxyurea[10].

1. **Etoposide:** Etoposide is a well characterised anti-neoplastic, semi-synthetic podophyllotoxin derivative which has been used for the treatment of a variety of malignancies such as lymphomas, testicular cancer and lung cancer. Etoposide specifically inhibits DNA topoisomerase II and this can subsequently lead to the inhibition of RNA and DNA synthesis. Etoposide induces S or G2 phase arrest thus prolonging cell cycle time and inhibiting cell proliferation. (Dose-dependent increase or decrease of somatic intrachromosomal). The primary limitation of its use is myelosuppression. Etoposide reduced the expression of vascular cell adhesion molecule-1 (VCAM-1), but not of fibronectin, on stromal cells in primary human bone marrow cultures. Both VCAM-1 and fibronectin are ligands for the beta-1 integrin very late antigen-4 (VLA-4), expressed on hemopoietic precursor cells (HPC). These molecules mediate the adherence of HPC to the stroma, an essential step in HPC development. The viability of stromal cells was unaffected but the capacity of the stroma to support myelopoiesis and erythropoiesis was reduced and there was a marked inhibition of the adhesion of HPC to the stroma[11].
2. **1,3-Bis(2-chloroethyl)-1-nitrosourea (BCNU):** BCNU is a lipophilic nitrogen mustard agent used in the treatment of a variety of malignancies, particularly those of the central nervous system. bone marrow is cumulative with multiple doses, indicative of long-term, perhaps permanent changes (Drugs toxic to the bone marrow that target the stromal cells). A large number of reactive metabolites are produced during BCNU decomposition and further work is needed to determine the importance of each in human genotoxicity. The major modifications in DNA produced by BCNU have been well characterized and at least in mammalian cells the O6-alkylguanine lesion and related 1-(3-cytosinyl),2(1-guanyl)ethane crosslink play a dominant role in genotoxicity as evidenced by results from point mutation assays and cytogenetic methods[12]. The DNA repair protein AGT4 (1) plays an important role in the protection of cells from the cytotoxic effects of alkyl nitrosoureas and methylating agents. AGT removes adducts from the O6 position of guanine in DNA through covalent binding of the alkyl group to a cysteine residue on the protein within the active site. During this process, irreversible inactivation of the protein occurs and the synthesis of new protein molecules is required to regenerate AGT activity. There is an inverse relationship between the level of AGT and the sensitivity of tumor cells grown in culture and as xenografts to the cytotoxic effects of alkyl nitrosoureas[13].
3. **Busulfan (BU):** BU, a sulfonic acid ester, is an alkylating agent used for the treatment of chronic myelogenous leukemia and as a conditioning drug in pediatric bone marrow transplantation. Alkylating agents form covalent DNA interstrand cross-links that inhibit DNA synthesis[11]. Stromal damage may indeed contribute to BU-induced marrow

failure. Busulfan is an alkylating agent that produces preferential depletion of early haemopoetic stem cells (HSCs)[14]. These agents are known to suppress natural killer cell activity, and BU produces a specific loss of early stem cells. If the concept that stem cells require biologic space is correct, then the reduction of mouse stem cells might prove helpful in promoting stem cell engraftment[15].

4. **Cyclophosphamide (CYP):** CYP, a phosphoramidate mustard, and the active analogue 4-hydroperoxycyclophosphamide (4-HC), are alkylating agents that are used in pediatric solid tumors and some forms of lymphomas and leukemias. Cyclophosphamide (Cy) induced the appearance of a population of suppressor cells in the bone marrow and spleens of mice[16]. Cyclophosphamide can limit proliferation without impairing inter mitotic functions of sensitized lymphocytes, such as the release of MIF. Results of this study suggest that cyclophosphamide does not impair development of a population of specifically sensitized T-type lymphocytes. However, several components of the expression phase are affected; the resultant anergy is probably due to a summation of effects on lymphocytes and macrophages[17]. Its severe toxicities induced mainly by oxidative stress[18]. However, despite its wide spectrum of clinical uses, it exhibits severe cytotoxicity to normal cells both in humans and experimental animals. Its metabolites can interact with the cellular macromolecules such as proteins, membrane lipids, RNA, as well as DNA and induce apoptosis. One of its metabolites, namely acrolein, induces oxidative stress that leads to DNA damage of normal cells and cause toxicities to various organs. One of the worst affected sites is the haematopoietic compartment of bone marrow. Cell cycle analysis revealed that the decreased bone marrow and spleen cell counts in the cyclophosphamide group was due to an elevated hypoploidy peak. In fact it is well established that metabolism of cyclophosphamide generates active alkylating metabolites such as 4-hydroxycyclophosphamide, aldophosphamide mustard and acrolein, which interfere with cellular DNA synthesis in rapidly dividing cells and ultimately lead to cell death. Apart from cancer cells it also damages the DNA of the healthy tissues with high cellular turnover such as the bone marrow[19].
5. **Doxorubicin (adriamycin):** Doxorubicin, also named adriamycin, is one of the most popular chemotherapeutic drugs used in the treatment of a variety of cancers. It is effective against solid and non-solid malignant tumors and is used in oncology protocols against malignancies. The preferential target of doxorubicin is the DNA of dividing cells by generating free radicals and apoptosis; the drug intercalates within DNA strands causing cell cycle blockage in the G2 phase, single-strand breaks and inhibition of the activity of some nuclear proteins, such as DNA and RNA-polymerase and DNA-topoisomerase II. It has molecule involved in chromosome stability and transcription, the DNA methyl-transferase 1-DNMT1, inducing apoptosis. It causes significant increase in the bone marrow DNA damage[20]. A range of different growth factors and cytokines, including interleukin-3 (IL-3), granulocyte macrophage colony stimulating factor (GM-CSF), and granulocyte colony stimulating factor (G-CSF), have been developed and shown capable of stimulating hematopoiesis and supporting peripheral recovery of

granulocytes after chemotherapy. beta glucan enhanced cytotoxicity and synergized with a specific anti-tumor monoclonal antibody in killing tumor cells[21]. It is also found to increase lipid peroxide level responsible for bone marrow suppression[22]. It eliminates bone marrow stem cells (BMSCs) and expands breast cancer stem cells (BCSCs). These suggest that quantification of ABCG2+ BMSCs is a direct approach to assess myelosuppression sensitively[23].

6. **Methotrexate:** MTX is a folic acid antagonist specifically inhibits dihydrofolate reductase. used in the treatment of leukemias, lymphomas and choriocarcinoma[11]. Methotrexate (4-amino-10 methyl folic acid) is an antimetabolite and an analogue of folic acid. The drug enters the cells via an active transport system for reduced folates and, due to a relatively irreversible binding, the drug inhibits the enzyme dihydrofolate reductase which catalyses the reductive process of folic acid into tetrahydrofolic acid. The inhibited formation of tetrahydrofolates results in an interference with DNA synthesis, repair and cell replication. The affinity of dihydrofolate reductase for methotrexate is far greater than its affinity for folic or dihydrofolic acid and, therefore, even very large amounts of folic acid given simultaneously will not reverse the effects of methotrexate. The drug seems also to cause an increase in intracellular deoxyadenosine triphosphate, which is thought to inhibit ribonucleotide reduction and polynucleotide ligase, an enzyme concerned in DNA synthesis and repair[24]. Methotrexate is widely used in the management of rheumatoid arthritis patients and is considered a first line drug[25]. MTX chemotherapy at a high dose is known to cause bone growth defects in growing bones, effects of its chronic use at a low dose on growing skeleton remain less clear[26]. Other problems associated with methotrexate are skin rashes, sensitivity of the skin to sunlight, Nausea and vomiting, Itchy eyes, Temporary effect on liver function, Mouth sores and ulcers, Diarrhoea [27].

COMPLICATIONS WITH BONE MARROW SUPPRESSION:

Most immunosuppressive agents also damage bone marrow cells. Whereas the main consequences of the latter are deficiencies of red cells, polymorphs and platelets, it must be remembered that the bone marrow is to a greater or lesser extent the source of stem cells for the lymphoid system and also for the body's macrophages[28]. Drugs that cause toxicity to the bone marrow are a heterogeneous group of compounds that act by various mechanisms. The etiology of this pathology is poorly understood but the highly proliferative nature of the hematopoietic cells is assumed to make the bone marrow more sensitive to toxicity. Recent evidence suggests that drugs can also affect specific aspects of stromal cells and the extracellular matrix that they establish. The data support the view that characteristics other than a high proliferation rate could confer susceptibility of the bone marrow to the toxic effects of drugs.

Highly simplified two-dimensional cross-section of postulated three-dimensional microenvironment of the bone marrow. Abbreviations: ECM, extracellular matrix. The stroma is multilayered, consists of several cell types, including fibroblasts, endothelial cells, macrophages

and adipocytes and secretes both stimulatory and inhibitory cytokines that regulate development of the hematopoietic precursor cell HPC.. Inhibitory cytokines include TNF-alpha, TGF-beta and IFN-gamma. Stimulatory cytokines include SCF, G-CSF, M-CSF, GM-CSF, IL-3 and IL-6. Several of these growth factors, including SCF, GM-CSF and IL-3, are attached to heparan sulphate proteoglycans of the ECM. Adhesion molecules on the HPC include ICAM-1, CD11a-c, LFA-1, CD44 and VLA-4. Adhesion molecules on stroma include fibrinogen, collagen, VCAM-1 and ICAM-1, 2 and 3. The role of accessory cells has been incompletely defined but they are known to secrete cytokines e.g., IL-1. that stimulate the release of growth factors e.g., GM-CSF[11].

PLANTS USEFUL IN BONE MARROW SUPPRESSION

There are many plants which are reported by various researchers, of having immunomodulator activity as well as hematopoietic activity which is helpful in treatment of chemotherapy causing bone marrow suppression.

1. ***Trigonella foenum-graecum (Methi)***: Protective effect of aqueous extract of *Trigonella foenum-graecum* (Fenugreek) was reported not only on (lipid peroxidation) LPO but also on the enzymatic anti-oxidants. CP-treated animals exhibited a significant decrease in the activities of glutathione S-transferase (GST), glutathione reductase (GR), glutathione peroxidase (GP) and catalase (CAT) when compared to the controls. Level of reduced glutathione (GSH) was also reduced with an increase in LPO in CP-treated animals. L-buthionine-SR-sulfoximine (BSO) treatment depicted an additive toxic effect in CP-treated animals. Pre-treatment of herbal extract restored activities of all the enzymes and thus showed an overall protective effect on additive effect of CP and BSO. Restoration of GSH by extract treatment may play an important role in reversing CP-induced apoptosis and free radical mediated LPO in urinary bladder. Fenugreek, known for its hypoglycemic, anti-inflammatory and immunomodulatory activity, may be a promising protective medicinal herb for consideration in complementary therapy in cancer patients under chemotherapeutic interventions[29].
2. ***Polygoni Multiflori Radix Praeparata***: *Polygoni Multiflori Radix Praeparata* (called Zhishouwu in Chinese) is processed from the root of *Polygonum multiflorum* Thunb. (Polygonaceae). Intraperitoneal administration of *Polygoni Multiflori Radix Praeparata* (PMPP) could increase Th1 type cytokine productions (IL-2) and hematological parameters (RBC, WBC and PLT counts), enhance antioxidant profiles and promote hematopoiesis of splenocytes through up-regulating levels of EPOR and GATA-1 proteins in Cy-induced anemic mice. Efficacy of PMPP provided evidence that this functional plant extract could be developed as a potential immunomodulatory agent for treatment of anemia and immunosuppression[30].
3. ***Argyreia speciosa***: *Argyreia speciosa* Sweet (Convolvulaceae), commonly known as Vryddhadaru in sanskrit, is a woody climber found throughout India and has been used

as a 'rasayana' drug in the traditional Ayurvedic system of medicine. Oral administration of the ethanolic extract of *A. speciosa* root (ASEE), at the doses of 50, 100 and 200 mg/kg in mice, dose-dependently potentiated the delayed-type hypersensitivity reaction induced both by sheep red blood cells (SRBC) and oxazolone. It significantly enhanced the production of circulating antibody titre in mice in response to SRBC. ASEE failed to show any effect on macrophage phagocytosis. Chronic administration of ASEE significantly ameliorated the total white blood cell count and also restored the myelosuppressive effects induced by cyclophosphamide. The present investigation reveals that ASEE possesses immunomodulatory activity.[31]

4. ***Panax ginseng***: Ginseng, the root of *Panax ginseng* C.A. Meyer, is traditionally used as a restorative, anti-diabetic, anti-vomiting, and anti-cancer agent worldwide. The major active ingredient responsible for the actions of panax ginseng is ginsenoside, a four-ring, steroid-like structure with sugar moieties[32]. Traditional clinical practice recommends prescriptions containing ginseng in conjunction with chemotherapy to reduce the side-effects of anti-cancer drugs[33]. In this study, we have demonstrated that Korean *Panax ginseng* (KG) significantly enhances myelopoiesis in vitro and reconstitutes bone marrow after 5-fluorouracil-induced (5FU) myelosuppression in mice. KG promoted total white blood cell, lymphocyte, neutrophil and platelet counts and improved body weight, spleen weight, and thymus weight. The number of CFU-GM in bone marrow cells of mice and serum levels of IL-3 and GM-CSF were significantly improved after KG treatment. KG induced significant c-Kit, SCF and IL-1 mRNA expression in spleen. Moreover, treatment with KG led to marked improvements in 5FU-induced histopathological changes in bone marrow and spleen, and partial suppression of thymus damage[34].
5. ***Cissampelos pareira***: *Cissampelos pareira* Linn. (Menispermaceae) is a climbing shrub distributed throughout warm parts of Asia, East Africa, and America. The alkaloidal fraction (AFCP) of roots of *Cissampelos pareira* Linn. AFCP was found to have significant immunosuppressive activity at lower doses (25 and 50 mg/kg) while no activity was observed at higher doses (75 and 100 mg/kg).[35]
6. ***Combretum racemosus***: *Combretum racemosus* is confined to Tropical Africa and represented in Nigeria by a climbing shrub. It was observed that gavaging the animals with *Combretum racemosus* enhanced the Hb level, platelet count, TWBC, PCV and RBC count which was depleted by cyclophosphamide treatment in the cyclophosphamide control group. The myeloprotective effect of this plant extract may be due to free radical scavenging activity or direct interference with the formation of the active metabolite of cyclophosphamide through inhibition of cytochrome P-450 enzyme system[36].
7. ***Buchanania lanzan***: *Buchanania lanzan* Spreng. (*B. lanzan*) belongs to the family Anacardiaceae, is commonly known as 'Chaar' in India and 'Almondette tree' in English. Pre-treatment with *B. lanzan* 250, 500 and 1000 mg/ kg, p.o., daily for 7 days

significantly reduced the chromosomal damage and lipid peroxidation with concomitant changes in antioxidants and detoxification systems. These results point out the presence of chemopreventive phytoconstituents in the crude extract offering protection against cyclophosphamide induced genotoxicity and oxidative stress in mice[37].

8. ***Ganoderma lucidum***: *Ganoderma lucidum* (known in China as Lingzhi and in Japan as Reishi) is a fungus of the family Polyporaceae that is regarded by Chinese people as the “miraculous king of herbs” and has attained a reputation in the East as a beneficial herbal substance. *Ganoderma lucidum* polysaccharide fraction (GLB) combined with 5-FU decreased the toxicity of 5-FU on BMCs[38]. Rather than directly stimulating hematopoietic progenitor proliferation and inhibition of apoptosis in BMC, GI-PS promoted myelopoiesis by selective binding to BMSC which stimulated HGF secretion to enhance the clonogenic activity of hematopoietic progenitor cells and improving the hematopoietic micro environment by enhancing CFU-F formation. Further research is necessary to determine whether GI-PS promote myelopoiesis by preventing HSC cycling, thereby protecting them and enhancing the long-term reconstitution of hematopoiesis after Cy-induced myelosuppression[39].
9. ***Ficus Glomerata***: Methanolic extracts of leaves, fruits & bark of *Ficus glomerata* Roxb. showed very significant ($p < 0.001$) counteracting effect to cyclophosphamide induced reduction in total WBC, DLC and platelet counts & significant ($P < 0.01$) effect to that of reduction in RBC counts and Hb %. The significant immunostimulant effect of the methanolic extracts of *Ficus glomerata* Roxb. leaf, fruit & barks on cyclophosphamide induced myelosuppression may be attributed towards the collective presence of saponins, sterols and tannins.[40]
10. ***Pongamia glabra***: Methanolic extracts of seeds and barks was assessed by determining the RBC, Hb%, platelet, total WBC and differential counts. Methanolic extracts of seeds and barks of *Pongamia glabra* Vent. showed dose dependent highly significant counteracting effect ($p < 0.001$) to cyclophosphamide induced reduction in total WBC and platelet counts and significant ($p < 0.01$) effect to that of reduction in RBC counts, Hb% and DLC. The significant immunostimulant effect of the methanolic extracts of *Pongamia glabra* Vent. Seeds and bark on cyclophosphamide induced myelosuppression may be attributed towards the collective presence of saponins, sterols, tannins and flavonoids in the extracts.

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