

Preliminary Investigation for Immunomodulation of Methanolic Extracts of Leaves and Flowers of *Pongamia Glabra* Vent. in Mice Model

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Abstract Evaluation of the immunomodulatory activity methanolic extracts of leaves and flowers of *Pongamia glabra* Vent. in cyclophosphamide induced myelosuppressed mice. Methanolic extracts of leaves and flowers of *Pongamia glabra* Vent. at the doses of 250mg/kg and 500mg/kg p.o. were administered for 13 days to normal adult albino mice of either sex and cyclophosphamide (30mg/kg i.p.) was injected on 11th, 12th and 13th days 1 hour after the administration of the treatment with extract. On 14th day blood was collected by retro orbital puncture and the activity was evaluated by determining the RBC, Hb%, Platelet, total WBC and differential counts. Methanolic extracts of leaves and flowers of *Pongamia glabra* Vent. showed dose dependant highly significant ($p < 0.001$) counteracting effect to cyclophosphamide induced reduction in total WBC, DLC and platelet counts and significant ($p < 0.01$) effect to that of reduction in RBC counts and Hb%. The significant immunostimulant activity of the methanolic extracts of *Pongamia glabra* Vent. leaves and flowers at the doses of 250mg/kg and 500mg/kg p.o. in cyclophosphamide induced myelosuppression may be attributed towards the presence of bioactive components, viz.- saponins, sterols, tannins and flavonoids in the extracts.

Keywords *Pongamia Glabra* Vent., Cyclophosphamide, Immunomodulatory, Myelosuppression

1. Introduction

Modulation of immune responses to alleviate the diseases has been of interest for many years in Ayurveda under the concept of 'Rasayana' (Chemical rejuvenators)[1]. Immunomodulation is a procedure which regulates the immune system of an organism by suppression and stimulation of the cells and organs of the immune system[2].

Immunostimulation in a drug induced immunosuppression model can be said to be true immunomodulation[3]. Certain agents called immunomodulatory agents have the ability to normalize or modulate pathophysiological processes[4]. A number of medicinal plants such as *Ocimum indicum*, *Withania somnifera*, *Tinospora cordifolia*, *Mangifera indica*, etc.[5] have been systematically screened for their immunomodulatory activity. Cyclophosphamide is an alkylating agent widely used in anti-neoplastic therapy of a variety of cancers such as lymphoma, myeloma and chronic lymphocytic leukemia[6]. Cyclophosphamide brings up general depletion of immune competent cells by acting on both cyclic and intermitotic cells[7]. Cyclophosphamide

induced immunosuppression (myelosuppression) is reported to cause various types of infections[8].

Pongamia glabra Vent. (Syn. *Pongamia pinnata*) Fam. Leguminosae (Figure 1), commonly called in regional language as Karanja, a tree found all over India bearing imparipinnate leaves and pinkish white colored flowers[9]. Leaves of the plant are reported to contain a bitter alkaloid. The ancient folklore uses of the plant claimed are as follows—The juice of the leaves is used to treat diarrhea, cough, dyspepsia, flatulence, leprosy and gonorrhoea[10]. Leaves are used as antiparasitic and insect repellent[11]. In review of the wide range of medicinal applications, the present study was aimed to screen methanolic extracts of leaves and flowers of *Pongamia glabra* Vent. for immunomodulatory activity in cyclophosphamide induced immune suppressed albino mice.

2. Materials and Methods

2.1. Plant Material

Leaves and flowers of *Pongamia glabra* Vent. were collected from local areas of Gulbarga, North Karnataka and a voucher specimen has been deposited at the departmental herbarium. The plant was identified and authenticated by Dr. Srinathrao, Prof. and HOD, Dept. of Botany, Gulbarga

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University, Gulbarga and Ref. No. GUG/BOT/Herbarium/2008-09/09. The mentioned parts of the plant were dried and powdered and the powdered drug was first defatted with petroleum ether (40-60°C) and extracted with methanol using Soxhlet apparatus for 48h to obtain methanolic extracts of leaves and flowers of the plant respectively. The filtrates of the extracts were concentrated to dryness under reduced pressure using a rota flash evaporator. The yields of the methanolic extracts of leaves and flowers were 10.28gm (14.68%w/w) and 43.68gm (55.96%w/w) respectively.



Figure 1. Image of *Pongamia glabra* Vent

2.2. Preliminary Phytochemical and Thin Layer Chromatography studies

The methanolic extracts of leaves and flowers were subjected for preliminary qualitative chemical tests and the presences of major phytoconstituents were confirmed by TLC studies [12, 13].

- TLC profile for flavonoids

Solvent system: ethyl acetate: formic acid: glacial acetic acid: water (10:1.1:1.1:2.6).

Detection: UV 365 (blue fluorescent spots).

- TLC profile for alkaloids

Solvent system: toluene: ethyl acetate: diethylamine (7:2:1)

Detection: wagner's reagent (brown colored spots).

- TLC profile for steroids

Solvent system: petroleum ether: acetone (7:3).

Detection: anisaldehyde: sulphuric acid reagent (pink to red colored spots)

- TLC profile for saponins

Solvent system: chloroform: glacial acetic acid: methanol: water (6.4:3.2:1.2:0.8).

Detection: anisaldehyde: sulphuric acid reagent (pink colored spots)

2.3. Animals

Swiss albino mice of either sex, weighing 25-30g housed in standard atmospheric conditions were used. They were maintained by feeding with standard rodent diet and water *ad libitum*. The study was approved by Institutional Animal Ethical Committee, Ref. No. HKECOP/IAEC/45/2011-12.

2.4. Acute toxicity studies

Acute toxicity studies were carried out as per OECD guidelines by 425 method (#26) [14]. The animals did not show mortality at the dose of 5000mg/kg and hence its 1/10th dose i.e., 500mg/kg and 1/20th dose i.e., 250mg/kg p.o. were used as the therapeutic doses for the methanolic extracts of the study undertaken.

2.5. Test Samples

Weighed quantities of the test extracts were suspended in 1% sodium carboxy methyl cellulose to prepare a suitable dosage form [15]. The control animals were administered an equivalent volume of sodium CMC vehicle.

2.6. Drugs

Cyclophosphamide was used as a standard immunosuppressant, Cycloxan® (Biochem-pharmaceutical industries Ltd. Mumbai) containing 200mg – cyclophosphamide, was purchased from the market and dilutions were made using sterile water for injection as labeled on the marketed product.

2.7. Cyclophosphamide Induced Myelosuppression [16]

Animals were divided into six groups of six animals each. Group I served as control group and received the vehicle (1% sodium CMC) for 13 days. Group II (Cyclophosphamide group) received the vehicle (1% sodium CMC) for a period of 13 days and on 11th, 12th and 13th days was injected with cyclophosphamide (30 mg/kg i.p.). Groups III and IV were administered methanolic extracts of leaves of the plant at the doses of 250mg/kg and 500mg/kg p.o. daily for 13 days respectively. Similarly Groups V and VI were administered methanolic extracts of flowers of the plant 250mg/kg and 500mg/kg p.o. daily for 13 days respectively. All the groups III, IV, V and VI were injected with cyclophosphamide (30mg/kg i.p) on the 11th, 12th and 13th days, 1 hour after the administration of the respective treatment. Blood samples were drawn on 14th day of experiment by retro orbital puncture and hematological parameters were studied for RBC, Hb %, platelets, total WBC counts and differential leucocytes counts (DLC).

2.8. Statistical Analysis

Data were expressed as mean ± SEM and differences between the groups were statistically determined by analysis of variance followed by Dunnet's test.

3. Results and Discussion

The preliminary phytochemical screening and TLC studies of the methanolic extracts of leaves and flowers indicated the presence of carbohydrates, glycosides, steroids, saponins, flavonoids, alkaloids and tannins and in addition the presence of proteins was observed in the extract of flowers. Results are represented in Table 1.

Cyclophosphamide at the dose of 30mg/kg. i.p. caused a significant reduction in total WBC count, differential leucocyte counts and platelets along with marginal reduction in RBC and Hb % compared to control group (Group I). Methanolic extracts of leaf and flowers at the doses of 250mg/kg and 500mg/kg p.o. showed dose dependant statistically highly significant ($p < 0.001$) increase in total WBC, DLC and platelets and significant increase in RBC and Hb% ($p < 0.01$) compared with cyclophosphamide group (Group II). However the methanolic extract of flowers showed more significant effect than the methanolic extract of leaves. Results are represented in Table 2 and 3.

Table 1. Thin Layer Chromatography results of *Pongamia glabra* Vent. leaves and flowers methanolic extracts

Extract	Flavonoids	Alkaloids	Steroids	Saponins
	Rf value	Rf value	Rf Value	Rf value
Leaf Ext.	0.57, 0.65 (2spots)	0.94 (1spot)	0.61,0.63, 0.68,0.76 (4spots)	0.55, 0.62 (2spots)
Flower Ext.	0.62, 0.72 (2spots)	0.57 (1spot)	0.63,0.66, 0.72,0.78, 0.81 (5spots)	0.71, 0.78 (2spots)

Table 2. Effect of methanolic extracts of *Pongamia glabra* Vent. leaves and flowers on cyclophosphamide induced myelosuppression in mice

Animal gr. n=6	RBC ($10^6/mm^3$)	Hb (g%)	Platelets ($10^5/mm^3$)	WBC ($10^3/mm^3$)
I (Control)	6.232±0.070	9.683±0.101	6.300±0.057	4.800±0.096
II (Cyclophosphamide)	5.023±0.056	8.250±0.136	4.767±0.088	2.267±0.244
III(Leaf Ext. 250mg/kg)	5.345±0.187	9.050±0.067	5.100±0.068	3.367±0.141
IV(Leaf Ext. 500mg/kg)	5.560±0.197	9.177±0.079	5.300±0.186	3.633±0.267
V (Flower Ext. 250mg/kg)	5.452±0.200	9.067±0.056	5.117±0.083	3.300±0.086
VI(Flower Ext. 500mg/kg)	5.583±0.130	9.133±0.091	5.417±0.087	3.350±0.182

Bone marrow is a source of cells involved in immune activity and is the sensitive organ most affected during any immunosuppression therapy with this class of cytotoxic drugs. Stem cells degeneration and inability of bone marrow to regenerate new blood cells will give rise to thrombocytopenia and leucopenia[17].

Immunomodulatory activity of methanolic extract of leaves and flowers of *Pongamia glabra* Vent. was explored on cyclophosphamide induced myelosuppression in mice at the doses of 250mg/kg and 500mg/kg p.o. Results of the

experimental study proved the dose dependant counteracting effect of the extracts to the cyclophosphamide induced myelosuppression, as indicated by increase in RBC, total WBC platelet counts, Hb % and DLC in the extract treated groups (Group III, IV, V and VI), compared to cyclophosphamide treated group (Group II). However the significance of counteracting activity to reduction in blood cell counts of the flowers extract was more than that of the leaves extract at the mentioned doses. The potentiated activity of flowers extract may be due to presence of excess number of flavonoids and steroids in the extract. The results clearly indicated modulation of bone marrow activity suppression i.e. myelosuppression when used cyclophosphamide alone and stimulation to counteract the cyclophosphamide induced myelosuppression in pretreated methanolic extract groups of leaves and flowers of *Pongamia glabra* Vent..

Table 3. Effect of methanolic extracts of *Pongamia glabra* Vent. leaves and flowers on cyclophosphamide induced myelosuppression in mice

Animal gr. n=6	Neutro-phils (%)	Lympho-cytes (%)	Eosino-phils (%)	Baso-phils (%)	Mono-cytes (%)
I (Control)	22.00±0.577	71.83±0.654	2.667±0.210	1.500±0.232	1.667±0.210
II (Cyclophosphamide)	12.17±0.703	62.17±0.477	0.666±0.210	0.166±0.166	0.167±0.167
III(Leaf Ex. 250mg/kg)	15.33±0.882	62.00±0.966	1.167±0.307	0.333±0.211	0.333±0.211
IV(Leaf Ex. 500mg/kg)	18.00±0.816	68.67±0.843	1.500±0.223	0.500±0.223	0.666±0.210
V(Flower Ex. 250mg/kg)	15.83±0.703	64.67±1.116	1.337±0.333	0.500±0.224	0.500±0.224
VI(Flower Ex. 500mg/kg)	16.33±0.494	67.67±0.918	1.667±0.210	0.500±0.223	0.500±0.223

*= $p < 0.01$, **= $p < 0.001$
Test drug treated groups were compared with control group (Group I)

4. Conclusions

The phytochemical investigation of the methanolic extracts of leaves and flowers of *Pongamia glabra* Vent. revealed the presence of saponins, steroids, proteins, tannins and flavonoids. Saponins are steroidal glycosides proven as important phytoconstituents to have immunomodulating property. The isolated diosgenyl saponins of *Paris polyphylla* were reported for immunostimulating activity[18]. Tannins are also reported to possess immunostimulating activities. The ingredients of well known Ayurvedic formulation, Chyawanprash was used traditionally as a rejuvenator includes *Terminalia chebula*, *Terminalia*

belerica, *Emblica officinalis* and many other tannin containing drugs, which have been reported for their immunostimulating activity [19]. Hence the collective presence of steroids, saponins, tannins and flavonoids in the methanolic extracts would be attributed for immunostimulating activity. However the flower methanolic extract of *Pongamia glabra* Vent. exhibited more immune-potentiating activity than that of methanolic extract of leaves. However this is a preliminary research work and the precise mechanism of immunomodulatory action exerted by the bio-active constituents of methanolic extracts of leaves and flowers of *Pongamia glabra* Vent. against cyclophosphamide induced myelosuppression needs to be investigated.

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