IMMUNOMODULATORY ACTIVITY OF PONGAMIA GLABRA

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Summary

Many herbal remedies individually or in combination have been recommended in various medical treatises for the cure of different diseases. However, the use of plant remedies, known to possess natural antioxidant, immunomodulatory and other activities, has increased in the last decade in human and animal medicine, as it is perceived as a natural approach to treat disease. Traditionally, leaves of Pongamia glabra are claimed to posses immunomodulatory activity and hence the reason behind evaluating the immunomodulator activity of aqueous extract of Pongamia glabra (AEPG). The immunomodulator activity of AEPG was evaluated by using various methods as Carbon clearance test, Effect on serum immunoglobulins and Cyclophosphamide induced neutropenia. In all these paradigms 400mg/kg dose of AEPG was shows more significant result than its 200 mg/kg of dose. These results suggest that Pongamia glabra can be used as immunomodulator.

Keywords: Immunomodulator, Pongamia glabra, Carbon clearance test, serum immunoglobulins, neutropenia

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Introduction

*Pongamia glabra* commonly known as ‘Karanj’ is a medium sized evergreen tree with a spreading crown and a short bole [1]. Traditionally the fruits and sprouts are used in folk remedies for abdominal tumors in India, the seeds for keloid tumours in Sri Lanka and a powder derived from the plant for tumors in Vietnam. In Sanskrit India, seeds were used for skin ailments. Today, the oil is used as a liniment for rheumatism. Leaves are active against *Micrococcus*; their juice is used for cold, coughs, diarrhea, dyspepsia, flatulence, gonorrhea and leprosy. Roots are used for cleaning gums, teeth, and ulcers. Bark is used internally for bleeding piles. Juices from the plant as well as the oil are antiseptic. It is said to be an excellent remedy for itch, herpes and pityriasis versicolor. Powdered seeds are valued as a febrifuge, tonic, in bronchitis and whooping cough. Flowers are used in treatment of diabetes. Bark has been used for beriberi. Juice of the root is used for cleansing foul ulcers and closing fistulous sores. Young shoots have been recommended for rheumatism [2]. It has been reported that *P. glabra* plant is used as anti-inflammatory [3], antiplasmodial [4], anti-hyperglycaemic, anti-lipidperoxidative [5], anti-diarrhoeal [6], anti-ulcer [7], antioxidant and anti-hyperammonemnic [8] properties. On the basis of all above reference various medicinal uses aim of the present work is to estimate immunomodulatory potential of *P. glabra* aqueous extract by using various methods.

Materials and Methods

Plant material

The leaves of *Pongamia glabra* was collected from Bhor Dist-Pune and authenticated at Botanical Survey of India, Pune (Voucher specimen number MTS -11).

Preparation of extract

The leaves were dried in shade at room temperature and coarsely powdered. The dried powdered material of leaves was boiled with distilled water. The boiled leaf extract was then filtered through muslin cloth. The aqueous extract obtained was evaporated to get dry powder mass. The aqueous extract yielded semisolid, viscous, dark brown coloured mass to yield 33.182 % w/w.

Drugs and Chemicals

The following drugs and chemicals were used. Cyclophosphamide, Zinc sulphate, Barium Sulphate (PCL India.)

Preliminary phytochemical study

The preliminary phytochemical study of aqueous extracts of leaves of *Pongamia glabra* was performed as per Khandelwal [9].

Experimental animals

Albino Wistar rats weighing between 180– 220 g and Swiss albino mice weighing between 25–35 g was used. Institutional Animal Ethics Committee approved the experimental protocol. Animals were maintained under standard conditions in an animal house approved by the Committee for Purpose of Control and Supervision on Experiments on Animals (CPCSEA). The animals were given pellet food and water *ad libitum*. 
Treatment
The animals were distributed into three groups consisting of six animals each. The first group served as control, the second and third group received low dose and high dose of aqueous extract of *Pongamia glabra* (AEPG) at 200 mg/kg, p.o. and 400 mg/kg, p.o. respectively.

Determination of Acute oral toxicity
Three female mice were randomly selected and marked for individual identification. Animals were fasted 24 hrs prior to dosing of aqueous extract of *Pongamia glabra* were administered in a single dose orally. Toxicity study was carried out using a starting dose of 5000 mg/kg body weight. Animals were observed individually after dosing at least once during the first 30 min. periodically during the first 24 hrs, with special attention given during first 4 hrs, and subsequently daily for 14 days. The observation comprised the behavior and according to the Guidelines of the Organization for Economic Cooperation and Development (OECD 1998).

Evaluation of Immunomodulatory activity:

1. Carbon clearance test $^{[10,11]}$: The four groups of Swiss albino mice were administered drug or vehicle for 5 days orally. After 48 hrs of the last dose of the drug, mice were injected with 0.1 ml of Indian ink via the tail vein. Blood samples were withdrawn at 0 min and 15 min. A 50 µl blood sample was mixed with 4 ml of 0.1% sodium carbonate solution and the absorbance of this solution was determined at 660 nm. The phagocytic index K was calculated using the following equation:

$$K = \frac{(\log_{e} OD_1 - \log_{e} OD_2)}{15}$$

Where - OD$_1$ and OD$_2$ are the optical densities at 0 and 15 min respectively.

2. Effect on serum immunoglobulin $^{[12, 13]}$: Albino rats were treated with the drug or vehicle orally for 21 days. Six hours after the last dose, blood samples were collected and the serum was separated by centrifugation, the collected serum was used for estimation of immunoglobulin levels. Briefly, for each serum sample to be analyzed, a control tube containing 6 ml of distilled water and a test tube containing 6 ml of zinc sulphate solution were prepared. To each, 0.1 ml of serum was added from a pipette. They were inverted to enable complete mixing of the reagents and left to stand for 1 hrs at room temperature in plugged tubes. The pH of the solution was monitored through out the experimental period using pH meter. The first tube served as blank and the second tube was taken as sample. The turbidity developed was measured. The turbidity obtained (sample-blank) was compared with that obtained with standard barium sulphate (BaSO$_4$) solution. The turbidity obtained with this solution was expressed as 20 zinc sulphate turbidity (ZST) units.

3. Cyclophosphamide induced neutropenia $^{[14, 15]}$: Swiss albino mice received the drug or vehicle orally for 10 days. On 10th day, a neutropenic dose of Cyclophosphamide (200 mg/kg, sc) was administered and this day was labeled as day zero. Blood samples were collected through retro-orbital vein. The total leucocyte count (TLC) and DLC were performed prior to and on day 3 after injection of Cyclophosphamide. The TLC and DLC in treated groups were compared with the values of the control group.
Results

Phytochemical screening
The aqueous extract of *Pongamia glabra* showed the presence of alkaloids, glycosides, saponin, flavonoids, steroids, and phenolic compounds.

Acute oral Toxicity
It was observed that extract of the *Pongamia glabra* was not lethal effect even at the dose of 2000mg/kg body weight. Hence 200mg/kg and 400 mg/kg were fixed as dosage.

1. Carbon clearance assay
Administration of AEPG at doses 200mg/kg and 400 mg/kg produced increase in clearance of carbon particles from blood as indicated by a significant increase in phagocytic index (P<0.01) as shown in table 1.

2. Serum immunoglobulin levels
The administration of AEPG at doses 200 mg kg (P<0.05) and 400 mg/kg (P<0.01) significantly increased the serum immunoglobulin levels when compared to control as shown in table 1.

Table1. Effect of AEPG on Phagocytic index and Immunoglobulin level.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Phagocytic index</th>
<th>Serum immunoglobulin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.006 ± 0.000254</td>
<td>16.94 ± 0.2410</td>
</tr>
<tr>
<td>AEPG 200</td>
<td>0.071 ± 0.001064**</td>
<td>24.02 ± 0.3758*</td>
</tr>
<tr>
<td>AEPG 400</td>
<td>0.423 ± 0.00118**</td>
<td>27.09 ± 0.5014**</td>
</tr>
</tbody>
</table>

3. Cyclophosphamide induced neutropenia
Administration of Cyclophosphamide (200 mg/kg, sc) produced a decrease in neutrophil count in all the groups. However, the reduction in neutrophil count was less in AEPG 400 treated groups compared to control. The AEPG 400 administration produced a 46.06 % and 13.19% reduction in TLC and Neutrophil count respectively. The AEPG 200 administration produced a 52.25% and 30.96% reduction in TLC and Neutrophil count respectively. Both % reduction observations are compared with control shows 56.91% and 46.03% reduction in TLC and Neutrophil count, as shown in table 2.
Table 2. Effect of AEPG on Cyclophosphamide induced neutropenia.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>TLC Reduction in cell no</th>
<th>% Reduction</th>
<th>% Neutrophil reduction</th>
<th>Neutrophil reduction</th>
<th>% Reduction</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before</td>
<td>After</td>
<td>Before</td>
<td>After</td>
<td>Before</td>
</tr>
<tr>
<td>Control</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5642±34.19</td>
<td>2428±110.90</td>
<td>3208±11.00</td>
<td>56.91</td>
<td>14.06±0.31</td>
<td>7.59±0.11</td>
</tr>
<tr>
<td>AEPG 200</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5487±56.04</td>
<td>2620±70.03</td>
<td>2867±84.23</td>
<td>52.25</td>
<td>12.66±0.13</td>
<td>8.74±0.071</td>
</tr>
<tr>
<td>AEPG 400</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5369±37.22</td>
<td>2896±29.26</td>
<td>2473±21.70</td>
<td>46.06</td>
<td>10.89±0.32</td>
<td>9.37±0.065</td>
</tr>
</tbody>
</table>

Discussion

Immunomodulation is a procedure which can alter the immune system of an organism by interfering with its functions, if it results in an enhancement of immune reactions it is named as an immunostimulative drug which primarily implies stimulation of specific and non specific system, i.e. granulocytes, macrophages, certain T-lymphocytes and different effector substances. Immune-suppression implies mainly to reduce resistance against infections, stress and may occur on account of environmental or chemotherapeutic factor [16].

The carbon clearance test was carried out to evaluate the effect of drugs on the reticuloendothelial system (RES). This is a diffuse system comprising of phagocytic cells, comprising of fixed tissue macrophages and mobile macrophages. The phagocytic cells in this system comprise the mononuclear phagocyte system (MPS), and the macrophage is the major differentiated cell in the mononuclear phagocyte system. Cells of the RES and MPS are known to be important in the clearance of particles from the bloodstream. When colloidal ink containing carbon particles are injected directly into the systemic circulation, the rate of clearance of carbon from the blood by macrophage is governed by an exponential equation [10, 11]. *Pongamia glabra* at both doses showed significant increase in the phagocytic index. Hence, these agents may stimulate the reticuloendothelial system.

The estimation of serum immunoglobulin level is a direct measure to detect the humoral immunity. Serum immunoglobulin refers to a group of serum molecules produced by B-lymphocytes, they are soluble and secreted form of B-cell receptors and are produced to a maximum level to counter the invasion by an antigen and hence they are also called as antibodies. Blood contains three types of globulins as alpha, beta and gamma, based on their electrophoretic migration rate. In the present study, estimation of serum immunoglobulins was carried out using zinc sulphate turbidity test (ZST). This test determines the amount of immunoglobulins present in the serum. A small amount of serum was added to a zinc sulphate solution and allowed to incubate at room temperature for 1 hr. Zinc sulphate causes precipitation of the immunoglobulins, which makes the solution cloudy instead of clear. This test is fairly specific for immunoglobulins, but does not do a very good job of quantitating them and it is difficult to distinguish a borderline problem. However, this test is relatively quick and inexpensive test [12]. Its drawbacks include the dependence of results on a number of factors, such as time, temperature, and particularly pH of the reaction mixture.
A serious drawback is the dependence of the final turbidity on pH of the zinc sulphate solution. Prolonged storage or even a short exposure to atmospheric carbon dioxide considerably changes pH and affects the result of the reaction. The possible ways of overcoming this problem are buffering or the use of pH indicators [13]. The turbidity was expressed as ZST units, which in turn indicate the amount of immunoglobulin present in the sample. *Pongamia glabra* at a low dose showed a significant increase in the serum immunoglobulin level.

Cyclophosphamide induces myelo suppression in the experimental animals. It belongs to nitrogen mustard subclass of alkylating agents and acts as an immunosuppressive agent by causing alkylation of DNA, in turn by interfering in DNA synthesis and function. It is also used extensively as immunosuppressant [14]. *Pongamia glabra* at a 400mg/kg of dose caused a 46.06% reduction in the Cyclophosphamide induced neutropenia suggesting that it may have an effect on the haemopoetic system. The prevention of neutropenia induced by cyclophosphamide may be through activation of macrophages, which secrete a large number of substances including colony stimulating factor and interleukin 1 [14, 15].

From the given data we conclude that aqueous extract of *Pongamia glabra* have immunomodulator activity which may be due to presence of various phytoconstituents like alkaloids, glycosides, saponins, flavonoids, steroids, and phenolic compounds in its aqueous extract.

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**References**