



## IN-VIVO IMMUNOMODULATORY ACTIVITY OF ETHANOLIC EXTRACTS OF AERIAL PARTS OF PHYLLANTHUS AMARUS PLANT IN CYCLOPHOSPHAMIDE INDUCED MICE

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**Objective:** To evaluate the immunomodulatory activity of ethanolic extract of aerial parts of phyllanthus amarus plant in cyclophosphamide induced mice.

**Materials and Method:** To evaluate the immunomodulatory activity of ethanolic extracts of Aerial parts of phyllanthus amarus plant. on cyclophosphamide induced immunosuppression in mice. ethanolic extracts of aerial parts of phyllanthus amarus plant, (500mg/kg p.o.) were administered 13 days to albino mice and cyclophosphamide (30mg/kg i.p.) was administered on 11th, 12th and 13th days 1 hour after the administration of the respective treatment. On the 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. Ethanolic extracts of aerial parts phyllanthus amarus plant. 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 ethanolic extracts of aerial parts of phyllanthus amarus plant on cyclophosphamide induced myelosuppression may be attributed towards the collective presence of saponins, sterols and tannins in the extracts, which suggest the immunomodulatory activities of the ethanolic extracts of phyllanthus amarus plant, leaves, stem and fruits.

**Results:** The results of the study revealed the dose dependent effect of the extracts in counteracting the myelosuppression induced by cyclophosphamide as indicated by increase in RBC total WBC platelet counts, Hb% and DLC in the extract treated group (III & IV), when compared to cyclophosphamide treated group (Group II).

**Conclusion:** Ethanolic extract of phyllanthus amarus would contribute for significant immunostimulating activity of In-Vivo

**Keywords:** Phyllanthus amarus, Cyclophosphamide, Immunomodulatory, Myelosuppression

### Introduction

The phyllanthus amarus family is (Euphorbiaceae), and authenticated by Chairman department of P.G studies in Botany Sharnbasva university Kalburgi Karnataka. Plant is collected in Bidar city of Karnataka. Phyllanthus amarus is broad spectrum medicinal plant that has received worldwide recognition. It is cultivated in waste land in the month of Jun to July India.

The survey of literature reveals that the medicinal plant has been used for the treatment in reducing pain expel investigation to stimulate and promote

digestion, as antihelminthes to expel investigation worms and act as mild laxative.<sup>1</sup> It is also used as chemoprotective, antimutagenic, nephroprotective, cardioprotective, and hepato-protective.<sup>2</sup> Phyllanthus amarus is a small erect herbal plant that grows upto 10-50 cm and it has small leaves, its stem have green capsules with flowers and very small fruits that burst open when dry. This plant is commonly called stonebreaker.<sup>3</sup> Phyllanthus amarus is used in traditional medicine for gastrointestinal, kidney, liver, menorrhagia and other conditions.<sup>4</sup>

Indian traditional systems of medicine like Siddha and Ayurveda have suggested to increase the body's natural resistance to disease under the concept of

'Rasayana'. A number of medicinal plants as Rasayanas such as *Tinospora cordifolia*, *Mangifera indica*, etc. have been claimed to possess immunomodulatory activity[5]. Immunomodulation is any procedure which can alter the immune system of an organism by suppression and stimulation of the cells and organs of the immune system[6].

Immunostimulation is a drug induced immune suppression model and immune suppression in an experimental hyper-reactivity model by the same preparation can be said to be true immunomodulation[7]. Certain agents have been shown to possess activity to normalize or modulate pathophysiological processes and hence are called immunomodulatory agents[8]. A number of medicinal plants have been screened systematically for their immunomodulatory activity such as *Tinospora cordifolia*, *Mangifera indica*[9]. Cyclophosphamide is an alkylating agent widely used in antineoplastic therapy. It is effective against a variety of cancers such as lymphoma, myeloma and chronic lymphocytic leukemia[10]. Cyclophosphamide acts on both cyclic and intermitotic cells, resulting in general depletion of immune competent cells[11]. Cyclophosphamide induced immunosuppression is reported to prompt various types of infections[12]. Survey of literature shows the presence of glycosides, tannins and wax. The present study was aimed at screening of ethanolic extracts of aerial parts of *Phyllanthus amarus*. For immunomodulatory activity on cyclophosphamide induced immune suppressed albino mice.



**Figure 1:** Image of the plant *Phyllanthus amarus*

## 1. Materials & Methods

### Plant Material

The aerial parts of plant *Phyllanthus amarus* family is (Euphorbiaceae) and authenticated by chairman department of P.G studies in Botany Sharanbasava university Kalburgi Karnataka were collected from local areas of Bidar city Karnataka. The fresh plants

*Phyllanthus amarus* were dried and made powder. By using hammer mill and hand grinder (Avischkar suddal 1995) about 2kg of the dry powder was charged in Soxhlet extractor apparatus and was extracted with polar solvent Alcohol. The extract was collected to dryness rotary evaporators (Rohlf Mumbai) under reduced pressure and controlled temperature (50/60°C) after dryness above extract weight and percentage yield calculated dryness.

### Preliminary Phytochemical Studies

The alcoholic extract of aerial parts of *Phyllanthus amarus* was subjected for qualitative chemical tests the components found various phytochemicals of therapeutic interest<sup>13</sup>. Found presence of Alkaloids, Flavonoids, Carbohydrates, Tannins, Lignans, saponin, Steroids, Glycosides.

### Animals

Swiss albino mice of either sex, weighing 25-30g housed in standard conditions of temperature, humidity and light were used. They were fed with standard rodent diet and water ad libitum. The study was approved by Institutional Animal Ethics Committee. Ref. No. KRES/KCP/77/2019-20. (IAEC) NO.726/PO/Re/S/02/CPCSEA dated 22.03.2017.

### Acute Toxicity Studies

Acute toxicity studies were conducted as per OECD guideline by 425 method to assess LD<sub>50</sub>[14]. The animals did not show mortality at the dose of 5000mg/kg and hence its 1/10th dose i.e., 500mg/kg was used as the therapeutic dose for the methanolic extracts of the study.

### Test Samples

Weighed quantities of test extracts were suspended in 1% sodium carboxy methyl cellulose to prepare a suitable dosage form.<sup>14</sup> The control animals were given an equivalent volume of sodium CMC vehicle.

### Drugs

Cyclophosphamide was used as a standard immunosuppressant, Cyphos (Getwell – pharmaceutical industries Ltd.) containing 200mg – cyclophosphamide, was procured from the market and dilutions were made using sterile water for injection as mentioned on the label of the marketed product.

### Cyclophosphamide Induced Myelosuppression<sup>15-16</sup>

Animals were divided into four groups of six animals each. Group I served as Control group, received the vehicle (1% sodium CMC) for a period of 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 (30mg/kg i.p). Group III, IV were administered ethanolic extracts of aerial part of phyllanthus amarus plant (250/500mg/kg p.o.) daily for 13 days respectively. The animals of groups III, IV were injected with Cyclophosphamide (30mg/kg i.p) on the 11th, 12th & 13th days, 1 hour after the administration of the respective treatment. Blood samples were collected on the 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).

Statistical analysis: Data were expressed as mean  $\pm$  SEM and differences between the groups were statistically determined by analysis of variance followed by Dunnet's test.

### 2. Results and Discussions

Cyclophosphamide at the dose of 30mg/kg. i.p. (group II) caused a significant reduction in total WBC count, differential leukocyte counts and platelets along with marginal reduction in RBC & Hb % as compared to control group (Group I). Ethanolic extracts of phyllanthus amarus plant showed very significant increase in Hb% ( $P < 0.001$ ), increase in total WBC ( $P < 0.002$ ) DLC and Platelets significant ( $P < 0.001$ ). Increase in RBC count compared to cyclophosphamide group when compared. Both tables showed similar levels of significance (Tables 1 and 2)

**Table 1:** Effect of ethanolic extracts of aerial parts of phyllanthus amarus plant on cyclophosphamide induced myelosuppression in mice

Animal group n=4	RBC ( $10^6/\text{mm}^3$ )	Hb(g%)	Platelets ( $10^5/\text{mm}^3$ )	WBC ( $10^2/\text{mm}^3$ )
(Control) I	8633 $\pm$ 0.149	11.47 $\pm$ 0.353	1.34 $\pm$ 0.026	6.113 $\pm$ 0.2214
(Cyclophosphamide) II	9.523 $\pm$ 0.022	10.36 $\pm$ 0.306	1.087 $\pm$ 0.050	4.85 $\pm$ 0.2157
Extract 250mg III	10.042 $\pm$ 0.245	12.67 $\pm$ 0.232	1.222 $\pm$ 0.077	5.213. $\pm$ 0.1645
E.E.P.A Extract 500mg IV	10.668 $\pm$ 0.216	13.32 $\pm$ .228	1.095 $\pm$ 0.095	5.428 $\pm$ 0.1821

Values are expressed as Mean  $\pm$ SEM  $P < 0.01$   $P < 0.001$

**Table 2:** Effect of ethanolic extracts of aerial parts of phyllanthus amarus on differential leucocyte counts in cyclophosphamide induced myelosuppression model

Animal Gr. n=4	Neutrophils (%)	Lymphocytes (%)	Eosinophils (%)	Basophils (%)	Monocytes (%)
(Control) I	32.58 $\pm$ 0.3135	32.670 $\pm$ 0.507	0.25. $\pm$ 0.0428	21.32 $\pm$ 0.348	13.33 $\pm$ 0.338
(Cyclophosphamide)II	31.53 $\pm$ 0.4379	25.23 $\pm$ 1.716	0 $\pm$ 0	18.33 $\pm$ 0.62	24.57 $\pm$ 0.697
Extract 250mg III	44.65 $\pm$ 0.7673	39.42 $\pm$ 0.537	0.9967 $\pm$ 0.4394	14.47 $\pm$ 0.295	0.2433 $\pm$ 0.0226
E.E.P.A Extract 500mg IV	53.23 $\pm$ 0.6168	26.35 $\pm$ 0.690	0.3733 $\pm$ 0.0729	18.45 $\pm$ 0.525	1.092 $\pm$ 0.0245

Values are expressed as Mean  $\pm$  SEM. \* $P < 0.01$ , \*\* $P < 0.001$  as compared to group II

## Discussion

Immunomodulatory activity of ethanolic extract of aerial parts of *Phyllanthus amarus* was explored by evaluating their effects on cyclophosphamide induced myelosuppression in mice at dose levels 250mg/kg and 500mg/kg p.o. In comparison to the study done by Sanjeev Heroor, Arun Kumar Beknal, Nitin Mahurkar on immunomodulatory activity of methanolic extracts of fruits and bark of *Ficus glomerata* Roxb. In mice on human neutrophils which showed significant counter effecting to cyclophosphamide induced myelosuppression, our study revealed the dose dependent effect of the extracts of *Phyllanthus amarus* in counteracting the myelosuppression induced by cyclophosphamide as indicated by increase in RBC, total WBC platelet counts, Hb % and DLC in the extract treated groups (Group III & IV), when compared to cyclo-phosphamide treated group (Group II).

## 3. Conclusions

From the phytochemical investigation, it was found that the major chemical constituents of the ethanolic extracts of aerial *Phyllanthus amarus* contains steroids, saponins, tannins, proteins and carbohydrates. Saponins are either triterpenoid or steroidal glycosides proven as important phytoconstituent with different pharmacological activities such as anti-allergic, cytotoxic, antitumour, antiviral, immunomodulating, anti-hepatotoxic, and antifungal activities. Recently three diosgenyl saponins isolated from *Paris polyphylla* reported for immunostimulating activity. Tannins are also known to possess immunostimulating activities. The well known ayurvedic formulation, *Triphala Churna* contains *Terminalia chebula*, *Terminalia belenica* and *Embolica officinalis*, which are rich in tannins have been reported for immunostimulating activity. Hence the collective presence of steroids, saponins and tannins in the ethanolic extracts of *Phyllanthus amarus* contribute significant immunostimulating activity of *In-Vivo*.

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