Immunomodulatory Effects of Gallic Acid against Cyclophosphamide- and Cisplatin-induced Immunosuppression in Swiss Albino Mice

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Gallic acid is a triphenolic acid, widely distributed in fruits, vegetables and plants and is reported to produce antioxidant, antiinflammatory, antifungal, antiviral and antitumor effects. In the present study, immunomodulatory effect of gallic acid was tested against cyclophosphamide and cisplatin; two widely used anticancer agents induced immunosuppression in Swiss albino mice. Cyclophosphamide and cisplatin are known immunosuppressive agents, which elicit variety of immune responses. In recent years much attention is given for the identification of plants or their bioactive compounds as immunomodulators. Three different doses of gallic acid i.e., 100, 200 and 400 mg/kg weight were administered orally for 7 consecutive days. Cyclophosphamide (50 mg/kg) and cisplatin (10 mg/kg) were administered intraperitoneally as single dose. Levamisole 50 mg/kg was used as standard immunomodulatory drug. 0.5 % carboxymethyl cellulose was used as solvent control. Evaluation of immunomodulatory property of gallic acid was done by using haemagglutination antibody titre response and haematological parameters such as white blood cells, red blood cells, platelet counts and haemoglobin levels. Relative weight of thymus an important lymphoid organ was also determined. Augmentation of antibody titre values and haematological end points clearly indicated immunomodulatory effect of gallic acid against cyclophosphamide and cisplatin-induced myelosuppression in Swiss albino mice. Results indicate that, gallic acid could be used as an adjuvant with immunosuppressive drugs to reduce their adverse effects on immune system.

Key words: Gallic acid, cyclophosphamide, cisplatin, immunomodulatory, Swiss albino mice

The immune system is a major defence mechanism evolved in an organism to protect against the foreign invaders and to eliminate diseases\[1\]. Immune system involves various types of cells of which some are immunostimulants and others are immunosuppressors in their functions\[2\]. Immunomodulation is the enhancement of immune reactions by immunostimulant agents. This primarily involves the stimulation of non-specific systems, i.e. granulocytes, macrophages, complement, certain T-lymphocytes and different effector substances. Suppression of the individual elements of the immune system; may allow the pathogenic organisms to surge over the host, which lead to secondary infections\[3\]. Immunosuppression is the suppression of body’s immune response owing to some environmental or chemotherapeutic influences\[4\]. Hence it is necessary to evaluate the mechanisms of immunotoxicity induced by a drug, whereas a change in cellular components of the blood is one of the events leading to immunosuppression\[5\].

In recent years, lot of importance is given for the identification of better immunostimulating agents from natural sources to impart better immune responses\[6\]. Immunomodulation using medicinal plants is a novel approach in phytomedicine to enhance the host defence mechanism during some autoimmune disorders\[1\]. In Indian traditional system of medicine many of the plants and their bioactive components are known to possess immunomodulatory properties, hence they are being used as an alternative approach to minimise the...
irreversible effects of modern drugs like adjuvants, synthetic agents and antibody reagents in the immune system[7,8]. Whole plants or their secondary metabolites are being used as drugs for the treatment of various ailments and in efficiency of immune response. In Ayurveda, rasayana drugs consisting of various plants are used to improve the defence mechanism and immunomodulatory activity[9-11]. Literature indicated that the Indian medicinal plants are rich source of immonomodulators. Also, plant derived components such as proteins, lectins, polysaccharides, alkaloids, flavonoids and phenolic substances have shown immunomodulatory properties along with their antioxidant and antiinflammatory properties[12,13]. Some of the plants with established immunomodulatory properties are Eclipta prostrata, Phyllanthus emblica, Glycyrrhiza glabra, Piper longum, Aloe vera, Allium sativum, Withaia somnifera, Emblica officinalis, Tinospora cordifolia and Ocimum sanctum[8,14,15].

Bone marrow is the primary lymphoid organ where all types of immunocytes originate. Thymus is another primary lymphoid organ where immune-competent T-cells develops and matures, hence; it plays a primary role in adaptive immune responses[16]. Mature cells migrate from lymphoid organs to the periphery to perform their functions. If any damage occurs to two primary lymphoid organs, production of immunocytes may be reduced leading to and induce immune dysfunction[17,18]. Evaluation of haematological parameters, lymphoid organ weight and histopathology are considered as immunological endpoints in subchronic and chronic rodent studies (OCSPP guidelines, 2013)[19]. These endpoints have been used by many investigators to study possible effects on immune function[20-23].

Humoral immunity refers to production of antigen specific antibodies and it plays a vital role in the immune system[24]. Haemagglutination titre assay is one of the simple assays used to measure specific antibody response towards the given antigen and it is commonly used in blood grouping and viral quantification. Antigen-antibody reactions can be visualised with the formation of agglutination[25,26]. There are many of using haemagglutination titre assay as one of the test parameters to study the immunomodulatory effects of plants or their bioactive molecules[1,21,27,28]. In our study haemagglutination titre assay and thymus weight and haematological parameters were used to study the immunomodulatory effects of gallic acid.

Cyclophosphamid is a widely used alkylating drug used in the treatment of various types of cancers such as lymphoma, myeloma and chronic lymphocytic leukaemia[29]. However, it is also effective immunosuppressive agents, which cross links the DNA of actively dividing cells thereby inhibiting the both cellular and humoral response immunity[30,31]. Cyclophosphamid-induced immunosuppression is reported to prompt various types of infections[13,32]. As per researchers, cyclophosphamid can be used as immunosuppressive agent to study the immunomodulatory effects of plant extracts[10,16,22,33].

In contrast, cisplatin is the first platinum based potent chemotherapeutic drug, widely used for the treatment of testicular, ovarian, bladder and other carcinomas[34,35]. A few reports are available on the effects of cisplatin on the cellular components of immune systems, but it might have potential in the control of inflammatory and autoimmune diseases[36]. Cisplatin has shown immunosuppressive and immunotoxic effects by affecting the body fluid components[37,38]. Investigator has been reported the immunosuppressive potential of cisplatin and protective role of plant extracts against it[37].

Gallic acid, (3,4,5-trihydroxybenzoic acid) found in fruits and plant materials in the form of free acids, esters, catechin derivatives and hydrolysable tannins also it is one of the major bioactive phenolic compounds present in the plants[39,40]. These plant bioactive compounds are considered as effective antioxidants. Even, the antioxidant property of gallic acid as a specific compound or as bioactive compound was well recognised by researchers[41-44]. Reactive oxygen species (ROS) and nitrogen radicals, which are formed naturally in our body, which results in oxidative stress causing deleterious effects on genetic material. Such effects can be minimized by dietary antioxidants containing polyphenol compounds[45]. Balance between oxidation and antioxidation mechanism, which helps to maintain healthy biological system[46]. Consequently, the antioxidant properties of gallic acid facilitated in the modulation of immune function under both in vivo and in vitro conditions as an active component of plants or as an herbal product[47-52]. Also, antiinflammatory properties of gallic acid prevent the expression of inflammatory chemicals including cytokines and histamines and hence it can be used to treat inflammatory allergic diseases[53].

Since there are very few reports are available on the immunomodulatory effects of gallic acid as a
pure compound, the present study was undertaken to investigate the in vivo immunostimulatory/ immunomodulatory effects of gallic acid against cyclophosphamide- and cisplatin-induced immunosuppression by using haematological and haemagglutination titre assays as test parameters.

MATERIALS AND METHODS

Gallic acid (CAS No.: 5995-86-8), Sigma Aldrich (Lot No: MKBP6646V) was used. Levamisole 50 mg/kg (Khandelwal Laboratories Pvt. Ltd., Mumbai) was used as reference standard immunomodulatory drug. Cyclophosphamide (CAS No.-6055-19-2), Endoxan-N Baxter Oncology, Germany (Batch No-JN1045) and cisplatin (CAS: 15663-27-1), Sigma Life Science (Lot No.: MKBN7276V) were used as positive immunosuppressant drugs. All other chemicals were obtained from Merck, SRL and Hi-media, India. Alsevier’s solution was prepared by dissolving the following reagents: dextrose- 2.05 g, sodium citrate-0.80 g, sodium chloride- 0.42 g, distilled water-100 ml; pH of the solution was adjusted to 6 by using 0.1 N HCl or NaOH. Sheep red blood cells (SRBC’s) was collected in Alsevier’s solution (1:1), washed in equal volumes of sterile normal saline solution thrice and adjusted to a concentration of 1×10⁷ cells/0.1 ml were used for immunization and challenge.

Experimental animals:

Swiss albino mice belonging to Mus musculus species, bred and maintained in the institutional animal house, were used for the experiment. They were housed in polypropylene shoe box type cages, bedded with rice husk and kept in air-conditioned room, at 23° (±2°) and RH 50±5 %, were fed with a pelleted diet (Amruth Feeds, India) and water ad libitum. A 12:12, light:dark cycle was followed. Racks were positioned in a room so as to optimise air exchange. Eight to ten week old animals with average body weight of 25±2 g were used for the experiments. Five animals (3 females+2 males) were used for each treatment and control group. Care and experimental procedures were conducted as per the guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals, India. All groups of animals were kept under an absolute hygienic condition as per the recommended procedures by fulfilling the necessary ethical standards. In vivo animal studies were conducted after obtaining the prior approval from Institutional Animal Ethics Committee (IAEC) of Mangalore University (MU/AZ/99/2013-14/IAEC dt: 2.04.2013).

Dose and treatment schedule:

Scheme of an appropriate dosing schedule and regimen should be based on clinical use, exposure pattern, pharmacokinetics and practical consideration. If the substance is genotoxic, highest dose level used will show the evidence of adverse effects and maximum tolerated dose is normally used to set this dose level. Accordingly, doses of gallic acid selected for this study were 100, 200 and 400 mg/kg. The LD₅₀ value of gallic acid has been reported as 5000 mg/kg in rats (Nair and Nair, 2013)⁴². Experimental animals were immunized by injecting 0.1 ml of 20 % SRBC (1×10⁶ cells) intraperitoneal (i.p.), prepared in normal saline on day 0. Three different doses of 0.2 ml of GA i.e., 100, 200 and 400 mg/kg were administered orally for 7 consecutive days. Cyclophosphamide (50 mg/kg) and cisplatin (10 mg/kg) were dissolved in distilled water and 0.9 % saline, respectively and were administered i.p., in 0.1 ml quantity as a single dose. On the 7th d, 2 h after last treatment, the animals were euthanized and about 1-1.5 ml of blood was collected by heart puncture. Levamisole 50 mg/kg was used as standard immunomodulatory drug. It was centrifuged at 3000 rpm at (–20°) and serum was collected from supernatant fraction to measure the antibody titre value. About 0.5 % carboxymethyl cellulose (CMC), distilled water and 0.9 % saline administered groups were maintained separately, which formed as negative controls for gallic acid, cyclophosphamide and cisplatin, respectively.

Haemagglutination antibody titre assay:

Haemagglutination test was performed by following the standard method⁴⁴. Two fold serial dilutions of serum samples were made in 96 well U bottomed haemagglutination microtitre plates containing 100 μl of phosphate-buffered saline. Then, 50 μl of 1 % SRBC suspension is added to each well. The plates were shaken gently and incubated at room temperature for 2 h and examined visually for agglutination. The value of the highest serum dilution causing visible haemagglutination was considered as the antibody titre⁴¹. Thymus weight was determined immediately after the animals were euthanized. The weight was measured in milligrams and expressed as relative weight using the formula; relative weight= weight of thymus in milligrams/weight of the animals in grams×100.

Haematological studies:

Haematological parameters such as total RBC, WBC, platelet and Hb count was found to be vital constituents
of the immune system and were analysed using haematology analyser (Unitron Bio-Medicals, India). For differential count of WBCs, Wright’s staining method was followed.

Statistical analysis:
Statistical significance of the results was tested by comparing gallic acid and levamisole treated groups with negative control 0.5 % CMC treated groups. Gallic acid combined treatment groups was compared with the respective cyclophosphamide and cisplatin treated groups by employing one way ANOVA and Dunnett’s post hoc tests using GraphPad Prism 5 (GraphPad Software, Inc., CA, USA). Differences with a p-value of 0.05 or lower were considered to be statistically significant.

RESULTS AND DISCUSSION
Animals administered with cyclophosphamide and cisplatin showed adverse effects on body weight and reduced food consumption. Whereas gallic acid combined treatment groups increased the food consumption rate thereby increased animal body weight, which is comparable with vehicle treated groups.

In antibody titre assay levamisole induced significant antibody production compared to the 0.5 % CMC control, which indicated the stimulatory activity of the positive immunomodulatory agent (Table 1). The relative weight of thymus was also increased in levamisole treated animals. In gallic acid treated animals, the antibody titre values were increased. These results are comparable with positive control levamisole treated group. Augmentation in antibody titre values in gallic acid treated groups indicates the immunostimulatory activity of gallic acid. Whereas in cyclophosphamide and cisplatin alone treated groups, there was a reduction in the antibody titre values when compared with distilled water and saline controls, respectively. This result indicates the immunosuppressive property of cyclophosphamide and cisplatin resulting in decrease in the antibody production. The production of antibody was increased in levamisole and gallic acid treated groups by increasing the antibody titre values. Gallic acid enhanced antibody titre values showing similar effect to that of positive immunomodulatory drug levamisole. In gallic acid+cyclophosphamide and gallic acid+cisplatin combined treatment groups, there is a significant improvement in the antibody titre values and moreover there was significant increase in relative thymus weight at all three doses tested; compared with cyclophosphamide and cisplatin alone treated groups (Tables 2 and 3). Increase in the thymus weight indicates that there was significant stimulation of immune response with gallic acid treated groups. This may confirm the immunostimulant action of gallic acid is cell mediated immunity. But, the maximum effect of gallic acid was observed at lower dose of 100 mg/kg. At higher doses gallic acid may acts as prooxidant.

The effect of gallic acid on haematological parameters was studied. The parameters studied in this were differential and total leucocyte count. There was significant reduction in Hb concentration, total RBC, WBC and platelet counts in cyclophosphamide and cisplatin treated groups (Table 2). Levamisole, a standard reference drug, significantly increased all these parameters when compared with negative control groups. Similarly, gallic acid alone treated groups significantly increased WBC counts compared to 0.5 % CMC control group. However, there was no much variation in the RBC and platelet counts and Hb concentration (Table 4).

### TABLE 1: BODY WEIGHT, RELATIVE WEIGHT OF THYMUS AND ANTIBODY TITRE VALUES IN VARIOUS CONTROL GROUPS

<table>
<thead>
<tr>
<th>Treatment (mg/kg)</th>
<th>Initial body weight (g)±SEM</th>
<th>Final body weight (g)±SEM</th>
<th>Relative weight of thymus (g)±SEM</th>
<th>Antibody titre ±SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dist. water</td>
<td>25.80±0.20</td>
<td>26.66±0.20</td>
<td>1.50±0.02</td>
<td>7.40±0.20</td>
</tr>
<tr>
<td>Saline</td>
<td>24.72±0.14</td>
<td>26.08±0.22</td>
<td>1.54±0.01</td>
<td>7.40±0.24</td>
</tr>
<tr>
<td>CMC (0.5 %)</td>
<td>25.96±0.04</td>
<td>26.98±0.34</td>
<td>1.53±0.02</td>
<td>7.40±0.24</td>
</tr>
<tr>
<td>Levamisole</td>
<td>25.99±0.12</td>
<td>27.07±0.14</td>
<td>1.86±0.02</td>
<td>16.0±0.31</td>
</tr>
<tr>
<td>Gallic acid (100)</td>
<td>26.86±0.21</td>
<td>27.80±0.24</td>
<td>1.56±0.02</td>
<td>12.6±0.24</td>
</tr>
<tr>
<td>Gallic acid (200)</td>
<td>26.14±0.20</td>
<td>27.18±0.25</td>
<td>1.55±0.02</td>
<td>12.4±0.24</td>
</tr>
<tr>
<td>Gallic acid (400)</td>
<td>26.20±0.33</td>
<td>27.02±0.36</td>
<td>1.58±0.01</td>
<td>11.6±0.24</td>
</tr>
</tbody>
</table>

*5 animals/group
Further, in both combined treatment groups, levamisole increased all the above mentioned blood parameters significantly. Similarly, all three doses of gallic acid tested improved the blood parameters. Among them, lower dose of gallic acid (100 mg/kg) was found to be more effective in ameliorating the haematosuppressive effect induced by cyclophosphamide and cisplatin (figs. 2 and 3).

There was significant reduction in lymphocytes and monocytes percent in both cyclophosphamide and cisplatin treated groups, compared with distilled water control. Similarly, all the above mentioned parameters were significantly decreased in cisplatin treated groups, compared with control groups. In both combined treatment groups levamisole and gallic acid (200 mg/kg) showed significant amelioration in all the above mentioned parameters. Among the test samples, gallic acid (200 mg/kg) was most effective in improving all the above mentioned parameters.

**TABLE 2: BODY WEIGHT, RELATIVE WEIGHT OF THYMUS AND ANTIBODY TITRE VALUES IN GROUPS TREATED WITH CYCLOPHOSPHAMIDE AND GALLIC ACID**

<table>
<thead>
<tr>
<th><em>Treatment</em> (mg/kg)</th>
<th>Initial body weight (g)±SEM</th>
<th>Final body weight (g)±SEM</th>
<th>Relative weight of thymus (g)±SEM</th>
<th>Antibody titre ±SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cyclophosphamide (50)</td>
<td>26.13±0.18</td>
<td>24.33±0.17</td>
<td>1.30±0.01</td>
<td>3.00±0.00</td>
</tr>
<tr>
<td>CMC+cyclophosphamide (50)</td>
<td>26.34±0.34</td>
<td>24.84±0.33</td>
<td>1.30±0.01</td>
<td>3.20±0.20</td>
</tr>
<tr>
<td>Levamisole+cyclophosphamide (50)</td>
<td>25.30±0.31</td>
<td>23.50±0.31</td>
<td>1.97±0.02</td>
<td>5.60±0.24</td>
</tr>
<tr>
<td>Gallic acid (100)+cyclophosphamide (50)</td>
<td>24.39±0.29</td>
<td>23.90±0.50</td>
<td>1.69±0.04a</td>
<td>4.00±0.00</td>
</tr>
<tr>
<td>Gallic acid (200)+cyclophosphamide (50)</td>
<td>24.43±0.43</td>
<td>23.90±0.50</td>
<td>1.69±0.04a</td>
<td>4.00±0.00</td>
</tr>
</tbody>
</table>

*5 animals/group; *p<0.05 when compared to cyclophosphamide treated group

**TABLE 3: BODY WEIGHT, RELATIVE WEIGHT OF THYMUS AND ANTIBODY TITRE VALUES IN GROUPS TREATED WITH CISPLATIN AND GALLIC ACID**

<table>
<thead>
<tr>
<th><em>Treatment</em> (mg/kg)</th>
<th>Initial body weight (g)±SEM</th>
<th>Final body weight (g)±SEM</th>
<th>Relative weight of thymus (g)±SEM</th>
<th>Antibody titre ±SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cisplatin (10)</td>
<td>26.28±0.15</td>
<td>24.02±0.09</td>
<td>1.24±0.02</td>
<td>2.80±0.20</td>
</tr>
<tr>
<td>CMC+cisplatin (10)</td>
<td>26.50±0.20</td>
<td>24.60±0.22</td>
<td>1.27±0.01</td>
<td>3.00±0.00</td>
</tr>
<tr>
<td>Levamisole+cisplatin (10)</td>
<td>24.14±0.19</td>
<td>24.14±0.19</td>
<td>1.98±0.04</td>
<td>5.60±0.24</td>
</tr>
<tr>
<td>Gallic acid (100)+cisplatin (10)</td>
<td>25.34±0.09</td>
<td>25.34±0.09</td>
<td>1.66±0.01a</td>
<td>6.60±0.24</td>
</tr>
<tr>
<td>Gallic acid (200)+cisplatin (10)</td>
<td>25.00±0.29</td>
<td>25.00±0.29</td>
<td>1.58±0.03a</td>
<td>4.20±0.20</td>
</tr>
<tr>
<td>Gallic acid (400)+cisplatin (10)</td>
<td>25.08±0.09</td>
<td>25.08±0.09</td>
<td>1.50±0.03a</td>
<td>4.00±0.00</td>
</tr>
</tbody>
</table>

*5 animals/group; *p<0.05 when compared to cisplatin treated group

**TABLE 4: HAEMATOLOGICAL PARAMETERS OF VARIOUS TREATMENT AND CONTROL GROUPS**

<table>
<thead>
<tr>
<th><em>Treatment</em> (mg/kg)</th>
<th>Hb concentration (g/dl)</th>
<th>Total WBC×10^3/mm^3</th>
<th>RBC×10^6/mm^3</th>
<th>Platelet×10^4/mm^3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Distilled water</td>
<td>13.42±0.05</td>
<td>4.72±0.20</td>
<td>9.01±0.07</td>
<td>2.48±0.58</td>
</tr>
<tr>
<td>CMC</td>
<td>13.32±0.10</td>
<td>4.70±0.23</td>
<td>9.25±0.08</td>
<td>2.42±0.05</td>
</tr>
<tr>
<td>Levamisole</td>
<td>14.64±0.10</td>
<td>5.18±0.12</td>
<td>9.80±0.22</td>
<td>2.86±0.07</td>
</tr>
<tr>
<td>Gallic acid (100)</td>
<td>13.40±0.07</td>
<td>5.26±0.05</td>
<td>9.38±0.12</td>
<td>2.47±0.03</td>
</tr>
<tr>
<td>Gallic acid (200)</td>
<td>13.16±0.10</td>
<td>5.34±0.13</td>
<td>9.14±0.12</td>
<td>2.36±0.03</td>
</tr>
<tr>
<td>Gallic acid (400)</td>
<td>13.26±0.25</td>
<td>5.18±0.12</td>
<td>8.95±0.06</td>
<td>2.35±0.02</td>
</tr>
<tr>
<td>Cyclophosphamide (50)</td>
<td>9.96±0.27</td>
<td>3.56±0.10</td>
<td>6.64±0.11</td>
<td>2.16±0.03</td>
</tr>
<tr>
<td>CMC+cyclophosphamide (50)</td>
<td>10.24±0.14</td>
<td>3.60±0.10</td>
<td>6.74±0.08</td>
<td>2.20±0.04</td>
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<tr>
<td>Levamisole+cyclophosphamide (50)</td>
<td>13.99±0.11</td>
<td>6.61±0.20</td>
<td>8.99±0.12</td>
<td>2.87±0.01</td>
</tr>
<tr>
<td>Gallic acid (100)+cyclophosphamide (50)</td>
<td>6.70±0.31</td>
<td>8.38±0.12</td>
<td>2.38±0.02</td>
<td></td>
</tr>
<tr>
<td>Gallic acid (200)+cyclophosphamide (50)</td>
<td>6.70±0.23</td>
<td>8.23±0.12</td>
<td>2.33±0.11</td>
<td></td>
</tr>
<tr>
<td>Gallic acid (400)+cyclophosphamide (50)</td>
<td>6.40±0.13</td>
<td>7.64±0.39</td>
<td>2.33±0.02</td>
<td></td>
</tr>
<tr>
<td>Cisplatin (10)</td>
<td>9.82±0.10</td>
<td>3.44±0.10</td>
<td>6.38±0.05</td>
<td>2.15±0.00</td>
</tr>
<tr>
<td>CMC+cisplatin (10)</td>
<td>10.04±0.05</td>
<td>3.56±0.08</td>
<td>6.36±0.02</td>
<td>2.15±0.01</td>
</tr>
<tr>
<td>Levamisole+cisplatin (10)</td>
<td>14.22±0.27</td>
<td>6.00±0.20</td>
<td>9.19±0.14</td>
<td>2.65±0.02</td>
</tr>
<tr>
<td>Gallic acid (100)+cisplatin (10)</td>
<td>5.50±0.15</td>
<td>7.65±0.16</td>
<td>2.81±0.01</td>
<td></td>
</tr>
<tr>
<td>Gallic acid (200)+cisplatin (10)</td>
<td>5.00±0.10</td>
<td>7.01±0.06</td>
<td>2.27±0.02</td>
<td></td>
</tr>
<tr>
<td>Gallic acid (400)+cisplatin (10)</td>
<td>4.68±0.11a</td>
<td>6.70±0.03a</td>
<td>2.26±0.00</td>
<td></td>
</tr>
</tbody>
</table>

*5 animals/group; *p<0.05 when compared to cisplatin treated group
water and saline control groups. Levamisole and gallic acid alone treated groups significantly enhanced these values (fig 1). In combined treatment groups levamisole and gallic acid resulted in increase in the percent lymphocytes and monocytes significantly.

The effect of gallic acid at a dose of 100 mg/kg in combination of cyclophosphamide and cisplatin was almost with that of standard drug levamisole (figs. 2 and 3).

Cyclophosphamide and cisplatin are potent chemotherapeutic drugs, which supresses the growth of cancerous cells by their alkylating action. In addition to their chemotherapeutic properties most chemotherapeutic drugs are known to cause toxic effects on normal cells by damaging the respiratory, cardiovascular, renal, hepatic, nervous, gastrointestinal and haematopoietic systems. Cyclophosphamide and cisplatin were considered as most effective immunosuppressive drugs.

In this study, we evaluated the immunomodulating effects of gallic acid against cyclophosphamide and cisplatin-induced immunosuppression in Swiss albino mice. Immunosuppressive/chemotherapeutic agents, cyclophosphamide and cisplatin were administered to experimental animals through intraperitoneal route. Whereas, i.p. route is an alternative route to the most conventional routes used for chemotherapy and used to deliver the drugs to target site within peritoneal cavity. Bioavailability of i.p. route is almost similar to intravenous (i.v.) and is greater than the oral and this is the most commonly used route in small laboratory animals as it is difficult to trace the vein. Moreover, i.p. route of cyclophosphamide and cisplatin administration is to avoid the drugs which are response of the host. Increase in the WBC counts indicates the immunostimulant activity. Macrophages are phagocytic in function and play a fundamental role in the cellular nonspecific defence mechanism. Gallic acid is immunostimulatory in function by enhancing the phagocytes and lymphocytes, the major innate immune cells.
degraded by gastric juices and acids, if given by oral. Also, cyclophosphamide requires metabolic activation by oxidase enzyme, which is present in the liver. By contrast, compounds absorbed from the mouth diffuse into the bloodstream and are not transported directly to the liver and their biotransformation is thereby delayed\(^{[59]}\).

Gallic acid is the major bioactive phenolic compounds present in the plants\(^{[39,60]}\). Dietary polyphenols are derived from plants and are consumed in the forms of fruits, vegetables, species and herbs. Polyphenols are agents targeted for disease prevention and have oral bioavailability. Gallic acid is nontoxic to mammals at pharmacological doses is generally absorbed in the intestine\(^{[62]}\). Oral route of administration is the only viable route and hence gallic acid was administered to animals through oral route\(^{[61]}\).

Among various naturally occurring polyphenols, gallic acid is one of the triphenolic compounds found in a variety of foods and herbs, which has been considered as effective antioxidant agent\(^{[62]}\). Antioxidants were deliberated as major immunomodulators, which results in the increase or decrease in the immune cells by playing a dual role on the immune system. Higher concentrations of antioxidants were found in immune cells than the other cells; hence, immune cells provide protection against various oxidative stress\(^{[63]}\). In this point, we have selected gallic acid an antioxidant as a test compound, to study the in vivo immunostimulating effects on cyclophosphamide and cisplatin-induced immunosupression in mice. In view of evaluating the possible protective effects of gallic acid on immune system, thymus weight was considered as one of the study parameters. Also, we focused on variation in the haematological parameters, if any variation in this may indicates the modulation in the immune responses. Therefore, the assessment of haematological parameters is also useful to determine the extent of deleterious/favourable effects of foreign compounds on immune response related cells. A rise or fall in the haematological parameters may vary the immune constitution of the body, thereby affecting the health\(^{[64,65]}\). Differential count of WBCs; total count of WBCs and erythrocytes and also platelet counts were done from the blood samples collected from various control and treated groups. The level of Hb was also determined.

In the present study, pre-treatment with gallic acid has effectively reduced the immune suppression induced by two anticancer drugs i.e cyclophosphamid and cisplatin in Swiss albino mice. Further, gallic acid improved the antibody titer effects indicating that, it facilitates humoral immune response.

Several investigators have reported the immunomodulatory properties of some plants containing gallic acid as major bioactive molecule. Pretreatment with alcoholic extract of *Terminalia chebula* shown increase in counts of neutrophils and lymphocytes and further it significantly ameliorated the damage on immune cells induced by cyclophosphamide by increasing the phagocytic activity in male Wistar rats\(^{[48]}\). Experimental evidences has demonstrated the immunomodulatory and wound healing properties of methanolic extract of *Schinus terebinthifolius* against cyclophosphamide-induced damage in male Swiss albino mice\(^{[51]}\). These immunostimulating properties of this extract is due to the presence of phenolic compounds; where gallic acid is also one of the major phytochemical constituents\(^{[50]}\), reported the immunomodulatory properties of ethanolic extract of *Luffa acutangula*, using carbon clearance and neutrophil adhesion test in vivo. Concurrently they stated that this effect is due to the presence of plant phenolics such as gallic acid and p-hydroxybenzoic acid as major polyphenolics. In Swiss albino mice, pre-treatment with hydroalcoholic extraction of *Dendrophthoe falcata* (L.f) Ettingsh stimulated the macrophages for phagocytosis and also increased the humoral immune response in a dose-dependent manner. Further this study demonstrated the presence of gallic acid as one of the chemical constituents in this plant is responsible to attribute the immunostimulatory effect\(^{[28]}\). Similarly, *triphala*, an Ayurvedic herbal product a rich source of vitamin C, ellagic acid, gallic acid, chebulinic acid, bellericanin, sitosterol and flavonoids has been reported as good immunomodulator\(^{[47,52]}\).

In the present study, gallic acid improved the antibody titre effects indicating that, it may facilitate humoral immune response. The humoral immunity involves interaction of B cells with the antigen and their subsequent proliferation and differentiation into antibody-secreting plasma cells. Antibody functions as the effector of the humoral response by binding to antigen and neutralizing it or facilitating its elimination by cross-linking to form clusters that are more readily ingested by phagocytic cells\(^{[53,66]}\). In our study, anticancer drugs cyclophosphamid and cisplatin-induced significant inhibition of antibody titre response whereas; gallic acid counteracted the suppression
of humoral response induced by cyclophosphamide and cisplatin. Furthermore, gallic acid significantly increased the weight of thymus a primary lymphoid organ, which involved in the adaptive immune response in both gallic acid alone and combined treatment groups. This observation shows its direct effect on the immune cells as well as its immunoprotective effects when administered along with immunosuppressive agents. Thereby, gallic acid ameliorated the immune suppressive effects induced by cyclophosphamide and cisplatin efficiently. Cyclophosphamide and cisplatin also induced significant decrease in total leukocyte count, RBC count and Hb level. Whereas, administration with gallic acid improved blood parameters, which is suppressed by anticancer drugs cyclophosphamide and cisplatin. Results indicate that gallic acid restored effects induced by immunosuppressive drugs. These results revealed the role of gallic acid in the immunomodulation process. The results are correlating with the antibody titre assays.

In this study, levamisole was used as a standard reference immunomodulatory drug. Experimental evidences have been proved levamisole as a good immunomodulator in both human and animals and it has been used as standard immunomodulatory drug. The results obtained in the present study are comparable with positive control levamisole treated groups indicating the immunostimulatory effects of gallic acid.

Although there are some reports available on the immunomodulatory effects of gallic acid as a constituent or as a bioactive molecule of several plants or herbal products, but there is no much reports available on the individual immunomodulatory properties of gallic acid as a pure compound. Hence, in this study we evaluated the immunomodulatory effect of gallic acid as a pure compound against two widely used anticancer, immunosuppressive drugs, cyclophosphamide and cisplatin in Swiss albino mice. In the present study, the positive test agents cyclophosphamide and cisplatin significantly reduced WBC and RBC counts and also Hb concentration compared to the distilled water and saline controls. Pre-treatment with gallic acid at three different doses significantly increased the values of all the above mentioned parameters, indicating its protective effects on the blood cells.

There are several reports on the immunomodulatory effects of plants constituents against cyclophosphamide and cisplatin-induced myelosuppression in Swiss albino mice. The possible immunomodulatory effects of these plants may be due to the presence of flavonoids, phenols, saponins, phenols, proteins, terpenes, alkaloids and tannins as their major constituents. In view of this immunomodulatory properties of ethanolic extract of Glycosmis pentaphylla were reported. In their study, cyclophosphamide has shown its immunosuppressive effect by decreasing the antibody titre values whereas this plant extract stimulated the both the specific and nonspecific immune responses by enhancing the responsiveness of macrophages, T and B lymphocytes. Administration of albino mice with Abutilon indicum extract significantly ameliorated both the primary and secondary haemagglutination titre suppression induced by cyclophosphamide and also potentiated the delayed type of hypersensitivity and phagocytic reactions. Methanolic extract of Sesbania grandiflora flowers, enhanced the production of antibody in a dose-dependent manner against cyclophosphamide-induced myelosuppression in mice by potentiating the humoral as well as cell mediated immunity. In previous studies, effective immunomodulatory properties of Dalbergia latifolia extract against cyclophosphamide-induced myelosuppression were reported. Pretreatment with plant extract elevated the depleted levels of RBC, WBC counts and percent Hb and neutrophils in Swiss albino mice.

Some authors have correlated the immunomodulatory effects with the antioxidant status reported that higher antioxidant capacity of E. malaccensis L. and E. uniflora L. helped to reduce the inflammatory response in vivo. Plant extracts with antioxidant activity could also have immunomodulatory ability. Antioxidants commonly present in our diet improves different immune function exhibiting protection against various infectious agents. Brambilla et al. reviewed the beneficiary role of dietary antioxidant in the regulation of immune response. This type of mechanism can also be expected in the present study with gallic acid because it is one of the phenolic compounds with high antioxidant activity and hence, it may show immunomodulatory effects. Though all three doses of gallic acid tested, showed immunomodulatory effect but the highest effect was observed at lower dose (100 mg/kg). This may be due to under certain circumstances at higher dose some antioxidants may acts as prooxidant. High level of antioxidant supplements may disturb normal physiological balance between the ROS formation and neutralization. Gallic acid naturally occurring flavonoid in fruits,
vegetables and other plant parts and they have many favourable biological effects due to their antioxidant and free radical scavenging abilities. Investigation on bioactive polyphenolics shows the correlation between the antioxidant/prooxidant effects. According to this study, the most effective antioxidants are also the most cytotoxic and effective antiproliferative agents, may be due to the dual antioxidant/prooxidant effect of polyphenols.

In conclusion, our study revealed the immunostimulatory potential of gallic acid against two potent anticancer drugs, cyclophosphamide and cisplatin-induced immunosuppression in Swiss albino mice. Immunostimulants can be used as adjuvants to heighten the specific immune response. Hence, gallic acid can be used as an immunoadjuvant with immunosuppressive drugs to reduce their contrary effects on immune system.

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