Scrutinizing the therapeutic response of *Phyllanthus emblica*’s different doses to restore the immunomodulation potential in immunosuppressed female albino rats

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Abstract. – OBJECTIVE: Immunosuppression and microbial resistance are the major drawbacks in conventional pharmaceutics. The present research work was planned to screen and characterize phytochemical constituents present in *Phyllanthus emblica* and to explore the immunomodulation potential of *P. emblica* by evaluating stress markers and different biochemical parameters in animals.

MATERIALS AND METHODS: The phytochemical analysis explored the presence of antioxidant profiles and revealed the radical scavenging activities. In the second phase, an animal trial was performed using female albino rats. Female rats (n=18) were administered three different doses of *P. emblica* (low dose 100 mg/kg, intermediate 200 mg/kg, and high dose 300 mg/kg) for three weeks. After a significant change (p<0.05) in antioxidant status i.e., TOS and TAS, hematological, biochemical parameters, and immunoregulation i.e., IgM and IgG were elevated. Statistical analysis (ANOVA) illustrates that these selected plants have a great impact on microbial resistance and immunosuppression and have shown highly significant results.

RESULTS: The results of all *in vitro* and *in vivo* assays conducted as part of the recent research work offer considerable evidence that the chosen medicinal plant has the ability to induce specific hormone release and boost the immune system.

CONCLUSIONS: Based on our findings, it is proposed that medicinal herbs may be isolated using cutting-edge approaches to tackle the issues of immunosuppression and microbial resistance.

Key Words: Medicinal plant, Phytoconstituents, Therapeutic response, Antioxidant potential, Immunomodulation.

Introduction

Medicinal plants are used against diseases to maintain the physical and mental health of humans. Plants maintain the body’s health and cure diseases because of their bioactive compounds. According to the WHO, 80% of the population depends upon herbal medicine for health care¹. Many herbs are used to purify the blood as they release metabolic toxins from the body and improve immunity as well. Many aromatic plants reduce blood toxins and destroy infections as well. About half a million plants possess therapeutic properties and are used against various chronic diseases¹. Many biological compounds such as carotenoids, anthocyanins, phenolic compounds, proteins, enzymes, and vitamins are present in plants and are responsible for various therapeutic properties.

*Phyllanthus emblica*, also known as “amla”, has long been used in traditional healthcare in South-East Asia and has shown antioxidative and immunoregulatory properties. Various parts of plants are being used for therapeutic reasons, especially fruit, which has been used in herbal remedies as a strong ayurvedic medicine and in
traditional medicine to treat jaundice and diarrhea. The fruit has been used to cure a variety of ailments, including fever and the common cold; as a laxative, diuretic, antipyretic, alterative, hair tonic, anti-inflammatory; and to protect from dyspepsia and peptic ulcer. Furthermore, some parts of the plant have antioxidant, antidiabetic, antibacterial, gastroprotective, hepatoprotective, and chemopreventive characteristics. It has been confirmed that *P. emblica* fruits have a greater concentration of ascorbic acid as well as significantly elevated concentrations of most proteins, minerals, and amino acids. It includes proline, glutamic acid, alanine, aspartic acid, lysine and cystine. Vitamin C content is higher than that of lemons, tangerines, or oranges.

In immunosuppression, individuals lack the ability to fight against pathogenic microbes, infections, and other disorders. Certain disorders or situations, such as cancer, acquired immunodeficiency syndrome (AIDS), diabetes, genetic abnormalities, and malnutrition, may contribute to this. Immunosuppression is a decline in immune system activity or effectiveness. Some aspects of the immune system inhibit other sections of the immune response, and immunosuppression can arise as an unfavorable response to therapy for other illnesses. In short, immunosuppression is intentionally produced to protect the body from refusing organ transplantation. Therapeutic medicines could improve the immunosuppression. Different synthetic medicines i.e., immunomodulating medicines, are used, like pomalidomide (Pomalyst), thalidomide (Thalomid), and lenalidomide (Revlimid), but synthetic pharmaceutics could improve the immunosuppression. 80% of the population worldwide and more than 30% of pharmaceutical formulations are dependent upon medicinal plants, reported by WHO.

The main objectives of the present research work were to determine the phytochemical constituents of *Phyllanthus emblica*, the therapeutic response on immunomodulation potential, and hepatoprotective potential in dexamethasone-intoxicated female albino rats.

**Materials and Methods**

**Plants Collection and Preparation of Bioactive Extract**

A medicinal plant (*Phyllanthus emblica*) was collected from various areas of Faisalabad-Pakistan. The valuable part i.e., leaves of the selected plant, was poised, desiccated, grounded into powder, and taken out on ethanol solvent. Aqueous Ethanolic (30:70) plant extract was prepared, and dry leaves were sonicated twice with 10 times higher volume of hydro alcohol for 48 hours at room temperature. Then, the solution was filtered by Whatman filter paper number 1 and evaporated using a rotary evaporator under reduced pressure. The percentage of extract (g/100g of dry plant) was calculated from the weighed concentrated extracts by using the formula given below.

Percentage Yield (%) = \( \frac{\text{Dried Extract Weight}}{\text{Dried Plant Material Weight}} \times 100 \)

**Phytochemical Analysis**

**Qualitative analysis**

Numerous phytochemicals such as flavonoids, alkaloids, tannins or saponins were identified in plant extract through conventional methods. The plant extract was studied for the detection of cardiac glycosides, steroids, triterpenoids etc.

**In Vivo Experimentation**

**Animal grouping and different doses**

In the *in vivo* study, only female albino rats weighing about 160 - 169 grams were used. Only female albino rats is used in this because of research benefits from the use of female rats because they may be more susceptible to certain cardiac or hepatic conditions and may respond differently to treatments. All the animals (n=30) were divided into different 6 groups, with 5 an-
imals (n=5) in each group as follows: Group 1: Healthy Control (no drug administered to five female rats); Group 2: Intoxicated control [0.2 mg Dexamethasone/kg body weight (b.w) orally and daily to five female rats]; Group 3: Positive control [0.2 mg Dexamethasone/kg body weight (b.w) and 30 mg Surbex Z/kg body weight (b.w.) orally and daily to five female rats]; Group 4: Treated group (Phyllanthus emblica low dose) namely PE (LD) [in which 0.2 mg Dexamethasone/kg body weight (b.w) orally and daily and low dose of Phyllanthus emblica 100 mg/kg orally and daily to five female rats (n=5)]; Group 5: Treated group (Phyllanthus emblica Intermediate dose) namely PE (ID) [in which 0.2 mg Dexamethasone/kg body weight (b.w) and intermediate dose of Phyllanthus emblica 200 mg/kg were given orally and daily to five female rats (n=5)]; Group 6: Treated group (Phyllanthus emblica high dose), namely PE (HD) [in which 0.2 mg Dexamethasone/kg body weight (b.w) orally and daily and a high dose of Phyllanthus emblica 300 mg/kg orally daily to five female rats (n=5)].

A normal husbandry environment was provided to all the study animals. After the approval by the Institutional Ethical Review Committee, all experimental animal groups were kept at the animal house of the Department of Physiology at Government College University in Faisalabad, Pakistan, for six weeks with a normal diet and 12-hour light/dark cycle. The Ethical approval was granted by PCSIR Lab Lahore under ref. No. LLC/FBRC/12/1567 dated 02/11/2022.

Collection of Blood Samples

On the final day of the experiment, to determine the therapeutic response of the plant extract, rats in each group were evaluated biochemically. For the determination of biochemical parameters, the heart puncture technique from all study subjects after 42 days was performed to collect blood samples in an EDTA-coated tube for a hematological study. Until analysis, the non-anticoagulated blood sample was centrifuged, clotted, and frozen until analysis.

Hematological Determination

The Cameron and Watson’ approach was used to determine the hematological parameters. Blood samples were obtained and treated in EDTA as an anticoagulant. The samples were then processed to identify the impact of plant extracts on the selected hematological parameters, including hemoglobin (Hb), platelet counts, white blood cell counts (WBCs), differential WBC counts, and red cell indices.

Biochemical Determination

The rats provided blood samples, which were analyzed to determine the parameters like liver enzymes, like transaminases [alanine transaminases (ALT) and aspartate transaminases (AST)] and metabolic profiles, total protein, albumin, urea, creatinine, and oxidative stress marker were determined by the automated photometric method.

Immunoglobulin

IgG and IgM antibodies were identified using photometric techniques. Bio-test Anti-Human Globulin reagents were used to confirm the in-vivo coating of RBC antibodies by analyzing the presence and absence of unexpected results.

Statistical Analysis

The acquired data were reported as Mean±SD and analyzed statistically using a one-way ANOVA test. The difference among groups pairwise research was assessed by Tukey’s test and Fisher’s test by using the statistical software Minitab 17 (trial version, PA, USA). Using the Tukey multiple range test, significant differences between treatment means were identified at a level of significance of p<0.05. Different letters in superscripts in the table row indicate significant group mean differences. The p<0.05 is considered statistically significant, while p<0.01 indicates high significance.

Results

Phytochemical Constituents

Medicinal plants serve an important role in society and individual healthcare. Because of the existence of particular chemical components as bioactive molecules, such as alkaloids, flavonoids, tannins, and saponins, certain plant species are employed for medicinal purposes. The selected part of the studied medicinal plant (Phyllanthus emblica roots) was screened for qualitative phytochemical constituents (as described in the methodology). Our results demonstrated that very high concentrations of phenolics, flavonoids, tannins and saponins were present in P. emblica as compared to the alkaloids, glycosides, steroids, and triterpenoids that were present in low concentrations.
Ethanolic Extract Doses Response in the Form of Blood Biochemical Parameters, Oxidative Stress Markers and Immunomodulatory System

Hematological parameters

The efficacy of the methanolic extract of chosen herbal medicine on blood parameters was investigated in the present study and their Mean±SD values are shown in Table I, respectively. Values are expressed as mean±SE (standard error). Different letters in superscripts in the same row indicate significant group mean differences, p<0.05 is considered statistically significant, while p<0.01 indicates high significance. Total RBCs were observed to be significantly (p<0.05) lower in dexamethasone (intoxicated) treated rats, 7.5±0.1BC, as compared to the normal control group of female rats, 7.8±0.2B, and the positive control group, 8.3±0.3A, respectively. The total RBC count rose considerably (p<0.05) in female rats when the RBC count of the rats given plant extract (P. emblica) was compared to the rats in the positive control group as 7.7±0.2B, 7.5±0.1BC, and 8.6±0.1C, respectively. Platelet count varied significantly (p<0.01) between test group rats and corresponding control group rats. Platelet count was observed to be enhanced in female rats given 100 mg, 200 mg, and 300 mg per Kg b.w. of methanolic extract of P. emblica leaves, and this elevation was dosage-dependent, i.e., 1,411±2.0E, 1,256±2.0C, and 1,600±2.0F, respectively, compared to the normal control group, 700±2.0B.

The treatment using P. emblica extract at different doses of 100 mg, 200 mg, and 300 mg per kg b.w. of rats considerably enhanced the total white blood cell count of female rats (p<0.01). Dexamethasone injection in female rats lowered the total WBC count by 7.8±0.1F compared to the control group of female rats, which was 9.8±0.1E. The total WBC count increased significantly (p<0.05) dose-dependently in rats’ groups that were treated with plant extract as 20.4±0.2C, 23.6±0.1A, and 21.8±0.1B as compared to the Surbex z-treated group, which was 19.1±0.2B. When rats were given different doses of P. emblica leaves (100, 200, and 300 mg/Kg b.w.), compared to animals administered with dexamethasone orally, the total WBCs count rose considerably (p<0.05). When the hemoglobin concentration of the group treated with the plant extract was compared to that of the corresponding normal rats’ group, 15.2±0.2A, it was discovered that the Hb concentration differed significantly (p<0.01) in distinct examined group rats. The results of the current study demonstrated that rats that were given the plant extract had substantially (p<0.05) reduced Hb concentrations (14.2±0.1F, 13.1±0.2B, and 14.7±0.2B), compared to standard normal rats (15.2±0.2A). The positive control group dramatically restored normal hemoglobin levels from 14.0±0.1C to 15.0±0.1A, respectively. Hb levels in plants methanolic extract-treated female rats did not rise significantly as compared to the dexamethasone-administered female rats’ group.

Hepatoprotective Activities of Phyllanthus Emblica Extract

Enzymatic activity analysis in blood samples, tissue specimens, and other body fluids is an essential tool for illness diagnosis, research, and therapy. Enzymes are often produced from metabolic activities, cell breakdown, and cellular turnover in tissue and blood13. The breakdown of toxic phytoconstituents found in plants and the waste products the liver excretes may be connected to

<table>
<thead>
<tr>
<th>Perimeters</th>
<th>Normal Group</th>
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<th>PE (ID)</th>
<th>PE (HD)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Red blood cell (RBC 10^6/µL)</td>
<td>7.8±0.2B</td>
<td>7.5±0.1BC</td>
<td>8.3±0.3A</td>
<td>7.5±0.1BC</td>
<td>8.6±0.1C</td>
<td>7.7±0.2B</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>White blood cell (WBC 10^9/µL)</td>
<td>9.8±0.1E</td>
<td>7.8±0.1F</td>
<td>19.1±0.2B</td>
<td>20.4±0.2C</td>
<td>23.6±0.1A</td>
<td>21.8±0.1B</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Hemoglobin (HB g/dL)</td>
<td>15.2±0.2A</td>
<td>14.0±0.1C</td>
<td>15.0±0.1A</td>
<td>14.2±0.1C</td>
<td>13.1±0.2B</td>
<td>14.7±0.2B</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Platelet (10^3/µL)</td>
<td>700±2.0F</td>
<td>390±2.0B</td>
<td>1,215±2.0D</td>
<td>1,410±2.0B</td>
<td>12,56±2.0E</td>
<td>1,600±2.0A</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

PE (LD)=(Phyllanthus emblica low dose), PE (ID)=(Phyllanthus emblica Intermediate dose), PE (HD)=(Phyllanthus emblica high dose). Values are expressed as mean±SE (standard error). This is a Tukey pairwise comparison and values that do not share a letter are significantly different. The p<0.05 is considered statistically significant, while p<0.01 indicates high significance.
Table II. Potential of plant extract on liver enzymes of control and experimental groups of female rats.

<table>
<thead>
<tr>
<th>Perimeters</th>
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<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALT (IU/L)</td>
<td>105.0±1.0⁰</td>
<td>179.0±1.0⁴</td>
<td>156.0±1.0⁰</td>
<td>114.0±1.0⁴</td>
<td>71.0±1.0¹</td>
<td>97.0±1.0⁶</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>AST (IU/L)</td>
<td>122.0±1.0⁰</td>
<td>165.0±1.0⁴</td>
<td>128.0±1.0⁰</td>
<td>145.0±1.0⁴</td>
<td>95.0±1.0⁶</td>
<td>103.0±1.0⁴</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Urea (mg/dl)</td>
<td>22±1.0⁰</td>
<td>37±1.0¹</td>
<td>24±1.0⁰</td>
<td>25±1.0⁰</td>
<td>21±1.0⁰</td>
<td>20±1.0⁰</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>CRP (mg/dl)</td>
<td>3.1±0.1⁰</td>
<td>5.4±0.1¹</td>
<td>4.4±0.1⁰</td>
<td>4.2±0.1⁰</td>
<td>3.7±0.1⁰</td>
<td>3.8±0.1⁰</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Creatinine (mg/dl)</td>
<td>0.5±0.1⁰⁹</td>
<td>0.6±0.1¹⁰</td>
<td>0.6±0.1¹⁰</td>
<td>0.7±0.1¹⁰</td>
<td>0.6±0.1¹⁰</td>
<td>0.4±0.1⁰</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

ALT=Alanine transaminase, AST=Aspartate transaminase, CRP=C-Reactive Protein. This is a Tukey pairwise comparison and values that do not share a letter are significantly different. The p<0.05 is considered statistically significant, while p>0.01 indicates high significance.

abnormal liver function, leading to increased levels of serum liver enzyme in blood flow. A ‘liver function test’ is a measurement of the liver’s proper functioning, and an increase in blood aminotransferases, like ALT and AST activity, indicates dysfunctional liver cells. Elevated ALT and AST values in the blood suggest impaired hepatocyte damage.

The results of liver function tests like ALT, AST, and serum proteins, including total C-Reactive protein and urea concentration, were represented as Mean±SEM in Table II, respectively, for comparing the significant group mean differences of the studied animals. According to data analysis, dexamethasone intoxication considerably elevated the ALT level (p<0.01) in female rats, i.e., 179.0±1.0⁴ as compared to the control rats’ group as 105.0±1.0⁰. On the other hand, ALT levels reverted to normal after the administration of different doses of P. emblica methanolic extract as 114.0±1.0⁴, 71.0±1.0¹, and 97.0±1.0⁶, respectively. The reduction in ALT levels in rats given plant extract is dose-dependent. A significant (p<0.01) difference in ALT level was discovered in the P. emblica-treated group as compared to the control group.

Significantly (p<0.05) elevated AST levels were reported in positive control and intoxicated groups i.e., 128.0±1.0⁰ and 165.0±1.0⁴, as compared to the respective control rats, which was 122.0±1.0⁰. In contrast, there was a significant difference in all of the P. emblica-treated groups of rats compared to the normal rats in the healthy group, i.e., 122.0±1.0⁴. Significant (p<0.01) dose-dependent reductions in AST levels were found in rats given 200 mg and 300 mg of P. emblica leaves extract per kg b.w. of rats, i.e., 95.0±1.0² and 103.0±1.0³, respectively, when compared to intoxicated supplied female rats, i.e., 165.0±1.0⁴.

The current study results demonstrated considerably (p<0.05) decreased levels of urea in the P. emblica-treated group rats as 25±1.0⁰, 21±1.0⁰, and 20±1.0⁰ compared to the intoxicated group (37±1.0⁴). According to data analysis, dexamethasone intoxication substantially elevated the CRP level (p<0.01) in female rats, i.e., 5.4±0.1⁴ compared to the normal range in the control group rats as 3.1±0.1⁴. CRP levels reverted to normal after administering varied doses of P. emblica methanolic leaf extract in groups of female rats at 4.2±0.1⁴, 3.7±0.1³, and 3.8±0.1³, respectively. The reduction of CRP levels in rats given plant extract is dose-dependent. Creatinine levels were observed to be lower in female rats given high-dosage concentration of plant extract (0.4±0.1⁴), as compared to the high concentration in the dexamethasone treatment group (0.6±0.1⁴), and the positive control group (0.6±0.1⁴), respectively. Creatinine counts increased considerably in the dexamethasone-administered group, but creatinine levels returned to normal in the extract-treated group of female rats.

**Stress Markers Examination**

Oxidants are agents that alter the chemical and physical properties of macromolecules. Enzymatic and non-enzymatic antioxidants are both components of the body’s natural defensive mechanism. Total antioxidant status (TAS) provides the necessary biological knowledge to define the body’s equilibrium state between antioxidant defense and pro-oxidants. TAS and TOS concentrations were determined in the serum of all female rats in the current investigation to evaluate the P. emblica methanolic leaf extract. Variations in Mean±SD values of TAS and TOS in female rats are given in Table III. Table III illustrates that the level of TOS in female groups of rats decreased significantly (p<0.01) after the treatment with P. emblica methanolic leave extract.
Scrutinizing the therapeutic response of *Phyllanthus emblica*’s different

Table III. Effect of selected medicinal plant extract and control treatments on stress markers in female rats.

<table>
<thead>
<tr>
<th>Perimeters</th>
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<th>p-value</th>
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</thead>
<tbody>
<tr>
<td>TOS (μ Mol equiv./L)</td>
<td>11.1±0.1^A</td>
<td>14.1±0.1^A</td>
<td>9.1±0.1^B</td>
<td>12.1±0.1^B</td>
<td>8.1±0.1^E</td>
<td>7.0±0.2^D</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>TAS (μ Mol equiv./L)</td>
<td>0.6±0.1^Bc</td>
<td>0.5±0.1^Bc</td>
<td>0.7±0.1^Bc</td>
<td>0.5±0.1^Bc</td>
<td>0.9±0.1^A</td>
<td>0.9±0.1^A</td>
<td>&lt;0.01</td>
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</table>

TOS=Total Oxidant Status, TAS=Total Antioxidant Status. This is a Tukey pairwise comparison and values that do not share a letter are significantly different. The *p*-value *<0.05* is considered statistically significant, while *<0.01* indicates high significance.

As 12.1±0.1^B, 8.1±0.1^E, and 7.0±0.2^D as compared to the intoxicated (administered dexamethasone orally) group of female rats. Dexamethasone intoxication considerably (*p*<0.01) enhanced the TOS level in female rats, 14.1±0.1^A, as compared to the normal control group, 11.1±0.1^C.

The level of TAS was significantly (*p*<0.01) reduced in rats administered with dexamethasone, i.e., 0.5±0.1^Bc, whereas the intermediate and high doses of selected plant extract administration in dexamethasone intoxicated rats dramatically improved the TAS as 0.9±0.1^A in comparison to the normal control group, i.e., 0.6±0.1^Bc. *P. emblica* extract reduces TOS in female rats in a dose-dependent manner. In the intoxicated group rats’ serum, TAS increased while TOS dropped. The treatment with the selected medicinal plant extract (*P. emblica*) considerably reduced the TOS while increasing the TAS (*p*<0.01).

**Immunomodulation Potential of Phyllanthus Emblica Extract**

Table IV and Figure 1 show the immunoregulatory efficiency of *P. emblica* aqueous extracts in immunosuppressive female rats for antibodies i.e., immunoglobulin M (IgM). IgM is found primarily in lymph fluid and blood, and it is the first antibody produced by the body when fighting a new infection. Immunoglobulin G (IgG), the most frequent form of antibody commonly found in blood circulation, accounts for around 70-80% of immunoglobulins in the blood and plasma B cells produce and release IgG antibodies.

According to statistical data, dexamethasone intoxication considerably (*p*<0.01) lowered the IgM quantity in female rats, i.e., 450.0±1.0^F in contrast to the normal control in the female healthy group of rats, 611.0±1.0^A. While IgM levels returned to normal after the administration of different doses of *P. emblica* methanolic leaves in groups of female rats i.e., 456.0±1.0^E, 566.0±1.0^C, 604.0±1.0^B, a substantial improvement is seen in the high dose of *P. emblica* i.e., 604.0±1.0^B in contrast to the positive group as 560.0±1.0^P. The enhancement in IgM level in rats given plant extract is dose-dependent. A significant (*p*<0.01) difference in IgM levels was identified in the plant extract treatment group as compared to the control group of female rats.

IgG levels differed considerably (*p*<0.05) across research groups of female rats and their corresponding control rats. IgG levels in dexamethasone-treated rats were found to be considerably (*p*<0.05) lower than in the normal group of female rats (250.0±1.0^A) and the positive control group (373.0±1.0^B). When the IgG level in plants treated groups compared to the positive control group female rats, it was discovered that the IgG count significantly increased (*p*<0.05) in female rats treated with different doses of methanolic roots extract of *P. emblica*, namely 245.0±1.0^B, 340.0±1.0^B, and 351.0±1.0^D, respectively. The increment not only in IgG but also in IgM levels in *P. emblica*-treated groups of female rats suggests that the *P. emblica* extract has immunomodulatory potential.

Table IV. Effect of selected medicinal plant extract and control treatments on immunomodulation potential of in female rats.

<table>
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<tr>
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</tr>
</thead>
<tbody>
<tr>
<td>Immunoglobulin M (IgM) (mg/dL)</td>
<td>250.0±1.0^D</td>
<td>192.0±1.0^D</td>
<td>373.0±1.0^A</td>
<td>245.0±1.0^E</td>
<td>340.0±1.0^C</td>
<td>351.0±1.0^D</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Immunoglobulin G (IgG) (mg/dL)</td>
<td>611.0±1.0^A</td>
<td>450.0±1.0^F</td>
<td>560.0±1.0^P</td>
<td>456.0±1.0^E</td>
<td>566.0±1.0^C</td>
<td>604.0±1.0^B</td>
<td>&lt;0.01</td>
</tr>
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</table>

Values are expressed as mean±SE (standard error). This is a Tukey pairwise comparison and values that do not share a letter are significantly different. The *p*-value *<0.05* is considered statistically significant, while *<0.01* indicates high significance.
Discusssion

Phytochemical Constituents

A wide range of medicinal plants are being used for their therapeutic potential due to the presence of phytochemical constituents, including alkaloids, flavonoids, steroids, glycosides, tannins and saponins. Tannins and saponins were present in *P. emblica* as compared to the alkaloids, glycosides, steroids and triterpenoids that were present in low concentration. Alkaloids found in *Phyllanthus emblica* and *Eucalyptus globulus* have been shown to have immunomodulatory properties, such as increasing antibody against antigens and increasing IL levels. According to reports, native alkaloids and their synthesized derivatives contain pharmacological actions such as analgesic, antispasmodic and antibacterial properties. Due to their various antioxidant properties, phenolics found in medicinal plants have been widely studied. Flavonoids, which seem to be arguably the most significant natural phenolics, are one of the most ubiquitous and diversified categories of natural goods. Different flavonoids were shown to be effective in the treatment of autoimmune disorders and malignancies. In another research, phytochemical screening, and characterization of *P. emblica* indicated the occurrence of phenols, flavonoids, and tannins, that revealed the beneficial impact of the hydroalcoholic extract of the bark of *Phyllanthus emblica* (PEE) in an ethanol-induced hepatotoxicity in rat models.

Effect on Hematological Parameters

The complete blood count (CBC) is indeed a major blood calculation that evaluates total red blood cells count (RBCs), hemoglobin (Hb) concentration, total white blood cell count (WBCs), and platelet count. Badole and Kotwal evaluated the effect of *Equisetum arvense* on total RBC count and found that total RBC count showed significant differences in rats treated with plants in comparison to respective normal control rats. So, their results supported the findings of our current research work but with different plant extract treatments. We also found non-significant results of total RBC count in plant-treated rats when compared to normal rats; however, total RBCs were found to increase significantly compared to dexamethasone-treated female rats.

Platelets, also known as thrombocytes, are blood cells that block the blood flow from an artery to the outside following an injury while simultaneously preserving the body’s homeostatic process. Female rats treated with dexamethasone have substantially lower platelet counts. Our findings were consistent with those of Badole and Kotwal, who showed an increment in platelet count in plant-treated female rats as compared to normal control female rats. Sarvaiya et al found a significant change in platelet count in *P. emblica* fruit extracts administered to gout rat models, which is consistent with our current findings, which revealed a favorable outcome.

![Bar graph depicting the potential of plant extracts control treatments on female rats immune response (IgG and IgM). The findings are shown as Means (bars)±SD (lines).](image_url)
cytes are blood-specialized cells that are primarily engaged in the body’s immunological defense process, which protects the body against external attacking agents. Montejo et al.²⁵ examined the blood parameters of female rats in their study and discovered a substantially ($p<0.05$) higher total WBC count in treated rats with Phyllanthus niruri extract, compared to normal control rats. As a consequence, our present study findings are corroborated by Montejo et al.²⁵. Badole and Kotwal²¹ investigated the impact of Equisetum arvense on total WBC count in rats and discovered that extract therapy significantly enhanced total WBC count. Mehdi et al.²⁴ examined the effect of plant extracts on hematological parameters in rats and reported the administration of Tamarindus indica to rats that were infected with the parasite Entamoeba histolytica. The results of Mehdi et al.²⁴ research indicate a substantial increase ($p<0.05$) in WBCs, RBCs, and mixed neutrophil, eosinophil, and basophil cells during treatment phases as compared to the metronidazole medication and the rat control group. From this, it is concluded that plant extract substantially ($p<0.05$) boosted WBCs in mice, which is consistent with our findings.

**Hepatoprotective Activity of Phyllanthus Emblica Extract**

The liver, as a chief organ in the body, plays vital roles in the body, like synthetic, detoxification, storage, excretion, and secretion functions, and the failure of any of these metabolic processes might be associated with liver abnormalities leading to increased serum liver enzymes levels in blood circulation. Yin et al.²⁵ studied the preventive effect of Phyllanthus emblica aqueous extract (AEPE) on liver fibrosis and discovered that AEPE substantially reduced concentrations of ALT, and AST which might confirm our current findings. Naz and Abbas²⁶ investigated the hepatoprotective activity of P. emblicus and the fruits of silymarin against cisplatin-induced hepatotoxicity. It was found that their fruit extract considerably lowered the levels of ALT and AST in a rat model, which agreed with the outcomes of our current investigation.

Sarvaiya et al.²² found a significant difference ($p<0.05$) in urea content in P. emblica fruit extracts administered to a gout rat model, which not only validated the findings of our present study’s control groups but with other plant extract treatments. Anto et al.²⁷ investigated the oral chronic toxicity of ethanol extract of Balakka fruit (Phyllanthus emblica) and determined that ethanol extract of P. emblica (EEPE) considerably reduced the amount of urea in female rat bodies, which was consistent with our observations. Usharani et al.²⁸ examined the impact of P. emblica fruit aqueous extract on oxidative stress, endothelial dysfunction, lipid profile and systemic inflammation. CRP levels were found to be considerably lower ($p<0.05$) in plant-administered groups, which is consistent with our findings. Anto et al.²⁷ investigated the acute oral toxicity against ethanol extract of Balakka fruit (Phyllanthus emblica) and reported that ethanol extract of Phyllanthus emblica (EEPE) considerably decreased the levels of creatinine levels in female rat bodies, which was consistent with our findings.

**Effect On Stress Markers in Rat’s Blood**

Antioxidant systems, both enzymatic and non-enzymatic, act as the natural body defense mechanism to neutralize the oxidant molecules. Total antioxidant status in the body often reveals the dynamic equilibrium between the body’s antioxidant defense and pro-oxidants. The body produces enough antioxidants to cope with the creation of oxygen-free radicals. Reduction of oxygen enzymatically produces free radicals, which serve as an energy source. The orbitals of oxygen radicals contain unpaired electrons. Oxidative stress occurs when free radical generation surpasses the scavenging capability of the body’s antioxidant defense mechanism. Shanmugarajan et al.²⁹ studied the pleiotropic and antihypertensive effects of P. emblica extract as an adjunct treatment in hypertensive individuals and found that P. emblica considerably reduced TOS while increasing TAS ($p<0.01$). They also found considerably higher levels of non-enzymatic and antioxidants in rats given P. emblica extract, which confirmed our findings in this study. Tasanarong et al.³⁰ investigated the antioxidant impact of P. emblica extract on contrast-induced chronic renal damage and observed that it possesses radical scavenging capacity and dramatically boosted the TAS of the body, which is consistent with our findings. Uddin et al.³¹ found that ethanolic fruit extract of P. emblica (EEPE) at 200 mg/kg b.w. significantly ($p<0.05$) enhanced the levels of glutathione S-transferases (GST) and catalase (CAT), suggesting that P. emblica has antioxidant properties that decrease reactive oxygen species.
Immunomodulation Potential of Phyllanthus Emblica Extract

A secondary antibody reaction occurs when the IgG antibodies exhibit their peak activity. IgG antibodies possess a comparatively higher affinity and a longer half-life in the bloodstream than IgM antibodies. Antibodies like these can be detected for a period of time following infection, and they are created between seven and fourteen days after exposure. Specific IgG antibodies are created during the first few weeks of an illness or even other antigen response, peaking a few weeks later, then declining and stabilizing. Prisingkorn et al.33 explored the role of *Phyllanthus emblica*, *Terminalia belerica*, *Terminalia chebula* and *Triphala* on the growth and immunity of the body. Their findings indicated that they had a favorable influence on various plasma biochemical markers, considerably lowering triglycerides, total cholesterol (tChol), low-density lipoprotein (LDL), AST, and ALT (*p*<0.05). Some other research studies34-56 also imply that they have no observable negative side effects and can boost fish immunity and development. Wang et al.57 studied the role of *P. emblica* extract on RAW 264.7 cells by down-regulating inducible nitric oxide synthase (iNOS), cyclooxygenase-2 (COX-2), and nuclear factor-kappa B (NF-κB). The extract was shown to reduce all three targets considerably (*p*<0.01) and in a dose-dependent way. In summary, the powder of *P. emblica* fruit extract not only reduced the effects of oxidative stress but also prevented inflammatory responses, helping to boost the defense system. Van Doan et al.58 investigated the effects of Amla (*P. emblica*) extract of fruit (AEF) on serum immunities, skin mucosa, growth and food supplements, and found that 20 mg AFE kg⁻¹ might be employed as an immunostimulant and growth promoter in Nile tilapia aquaculture. Bakr and Naga.59 studied the immunomodulatory effectiveness of *P. emblica* and *costus speciosus* in immunocompromised rats. Their findings revealed that extracts of *costus speciosus* and *P. emblica* increased (*p*<0.05) both IgM and IgG levels in a dose-dependent way.60-61

Conclusions

Medicinal plants possess unique advantages in the regulation of the immune system and show a positive effect on microbial resistance. Here, we researched the medicinal plant *Phyllanthus emblica* based on ethno-medicinal properties and determined its effect on microbial resistance and immunoregulation. *Phyllanthus emblica* has a wide range of therapeutic applications, and these beneficial effects are due to the presence of various phytoconstituents that have been isolated. The results of the current study also revealed that *E. grandiflorum* revealed the presence of a wide range of phytochemical constituents, their significant in vitro and in vivo antioxidant potential, significant hepatoprotective, and immunomodulatory restoring capacity in female albino rats as therapeutic response directly proportional to doses of ethanolic extract administered. However, more research is required to isolate the novel compounds from this therapeutic plant to address the problems, particularly of immunosuppression.

Conflict of Interest

The authors declare no conflict of interest.

Availability of Data and Materials

All the data generated in this research study has been included in the manuscript.

Authors’ Contributions

Conceptualization: Areej Riasat and Muhammad Jahangeer methodology, Abid and Yasir.; software, Khurram.; validation, Shafaq.; formal analysis, Riflat investigation, Tariq.; resources, Metab; data curation, Abid and Tariq; writing—original draft preparation, Abdulrahman.; writing-review and editing, Abdullah; visualization, Abid supervision, Tariq.; project administration, Abid; funding acquisition, Tariq.

Ethics Approval

Ethical Approval for this study was granted by PCSIR Lahore under reference No. LLC/FBRC/12/1567 dated 02/11/2022.

Informed Consent

Not applicable.

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