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

A. RIASAT[1], M. JAHANGEER[2], A. SARWAR[2], Y. SALEEM[2], K. SHAHZAD[2], S. UR RAHMAN[3], R. AZIZ[3], T. AZIZ[4], M. ALHARBI[5], T.H. ALBAKEIRI[5], A.F. ALASMARI[5]

[1]School of Biological Sciences, University of the Punjab, Quaid-e-Azam Campus, Lahore, Pakistan
[2]Food and Biotechnology Research Center, PCSIR Laboratories Complex Ferozepur Road, Lahore, Pakistan
[3]Department of Environmental Sciences Shaheed Benazir Bhutto University, Sheringal Dir Upper, Pakistan
[4]Department of Agriculture, University of Ioannina, Arta, Greece
[5]Department of Pharmacology and Toxicology, College of Pharmacy, King Saud University, Riyadh, Saudi Arabia

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 care[1]. 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 diseases[1]. 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

Corresponding Author: Tariq Aziz, MD; e-mail: iwockd@gmail.com

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[2]. It has been confirmed[3] 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[4]. 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 have various side effects. It includes constipation, drowsiness, blood clots, diarrhea, nausea, rashes, and kidney and liver damage. Thalidomide may induce hand and foot discomfort, tingling, numbness and muscular weakness. Immunosuppressive medications also interact with other medications, affecting their absorption and effectiveness.

Therefore, we want to use *Phyllanthus emblica* against microbial resistance and immunosuppression as it contains antioxidant properties and a variety of active constituents that are responsible for various bio functional activities. These compounds help to support the immune system, and cardiovascular system, boost resistant capacity, and reduce hypertension, osteoarthritis, heart disease, viral diseases of the heart, and HIV/AIDS. The herbs seem to improve natural killer (NK) cell activity and antibody-dependent cellular cytotoxicity. Moreover, it was reported that 80% of the population worldwide and more than 30% of pharmaceutical formulations are dependent upon medicinal plants, reported by WHO[5]. 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.

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

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[6].

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[7] 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)][8] and metabolic profiles, total protein, albumin, urea[9], 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[10]. 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[11]. 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 ± 0.1^{BC}, as compared to the normal control group of female rats, 7.8 ± 0.2^{B}, and the positive control group, 8.3 ± 0.3^{A}, 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 ± 0.2^{B}, 7.5 ± 0.1^{BC}, and 8.6 ± 0.1^{C}, 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., $1{,}411\pm2.0^{B}$, $1{,}256\pm2.0^{C}$, and $1{,}600\pm2.0^{A}$, respectively, compared to the normal control group, 700 ± 2.0^{E}.

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 ± 0.1^{F} compared to the control group of female rats, which was 9.8 ± 0.1^{E}. The total WBC count increased significantly ($p<0.05$) dose-dependently in rats' groups that were treated with plant extract as 20.4 ± 0.2^{C}, 23.6 ± 0.1^{A}, and 21.8 ± 0.1^{B} as compared to the Surbex z-treated group, which was 19.1 ± 0.2^{D}. 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 ± 0.2^{A}, 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 ± 0.1^{C}, 13.1 ± 0.2^{D}, and 14.7 ± 0.2^{B}), compared to standard normal rats (15.2 ± 0.2^{A}). The positive control group dramatically restored normal hemoglobin levels from 14.0 ± 0.1^{C} to 15.0 ± 0.1^{A}, 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 blood[12]. The breakdown of toxic phytoconstituents found in plants and the waste products the liver excretes may be connected to

Table I. Influence of studied plants extract and control treatment on blood parameters in female rats.

Perimeters	Normal Group	Intoxicated Group	Positive Control	PE (LD)	PE (ID)	PE (HD)	*p*-value
Red blood cell (RBC 10^3/µL)	7.8 ± 0.2^{B}	7.5 ± 0.1^{BC}	8.3 ± 0.3^{A}	7.5 ± 0.1^{BC}	8.6 ± 0.1^{C}	7.7 ± 0.2^{B}	<0.01
White blood cell (WBC 10^3/µL)	9.8 ± 0.1^{E}	7.8 ± 0.1^{F}	19.1 ± 0.2^{D}	20.4 ± 0.2^{C}	23.6 ± 0.1^{A}	21.8 ± 0.1^{B}	<0.01
Hemoglobin (HB g/dL)	15.2 ± 0.2^{A}	14.0 ± 0.1^{C}	15.0 ± 0.1^{A}	14.2 ± 0.1^{C}	13.1 ± 0.2^{D}	14.7 ± 0.2^{B}	<0.01
Platelet (10^3/µL)	700 ± 2.0^{E}	390 ± 2.0^{F}	$1{,}215\pm2.0^{D}$	$1{,}411\pm2.0^{B}$	$12{,}56\pm2.0^{C}$	$1{,}600\pm2.0^{A}$	<0.01

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.

Perimeters	Normal Group	Intoxicated Group	Positive Control	PE (LD)	PE (ID)	PE (HD)	*p*-value
ALT (IU/L)	105.0 ± 1.0^{D}	179.0 ± 1.0^{A}	156.0 ± 1.0^{B}	114.0 ± 1.0^{C}	71.0 ± 1.01^{F}	97.0 ± 1.0^{E}	<0.01
AST (IU/L)	122.0 ± 1.0^{D}	165.0 ± 1.0^{A}	128.0 ± 1.0^{C}	145.0 ± 1.0^{B}	95.0 ± 1.0^{F}	103.0 ± 1.0^{E}	<0.01
Urea (mg/dl)	22 ± 1.0^{C}	37 ± 1.0^{A}	24 ± 1.0^{B}	25.0 ± 1.0^{B}	21 ± 1.0^{CD}	20 ± 1.0^{D}	<0.01
CRP (mg/dl)	3.1 ± 0.1^{E}	5.4 ± 0.1^{A}	4.4 ± 0.1^{B}	4.2 ± 0.1^{C}	3.7 ± 0.1^{D}	3.8 ± 0.1^{D}	<0.01
Creatinine (mg/dl)	0.5 ± 0.1^{BC}	0.6 ± 0.1^{AB}	0.6 ± 0.1^{AB}	0.7 ± 0.1^{A}	0.6 ± 0.1^{AB}	0.4 ± 0.1^{C}	<0.05

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[13]. 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[14].

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 ± 1.0^{A} as compared to the control rats' group as 105.0 ± 1.0^{D}. On the other hand, ALT levels reverted to normal after the administration of different doses of *P. emblica* methanolic extract as 114.0 ± 1.0^{C}, 71.0 ± 1.01^{F}, and 97.0 ± 1.0^{E}, 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 ± 1.0^{C} and 165.0 ± 1.0^{A}, as compared to the respective control rats, which was 122.0 ± 1.0^{D}. 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 ± 1.0^{D}. 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., $95.01.0^{F}$ and $103.01.0^{E}$, respectively, when compared to intoxicated supplied female rats, i.e., 165.0 ± 1.0^{A}.

The current study results demonstrated considerably ($p<0.05$) decreased levels of urea in the *P. emblica*-treated group rats as 25 ± 1.0^{B}, 21 ± 1.0^{CD}, and 20 ± 1.0^{D} compared to the intoxicated group (37 ± 1.0^{A}). According to data analysis, dexamethasone intoxication substantially elevated the CRP level ($p<0.01$) in female rats, i.e., 5.4 ± 0.1^{A} compared to the normal range in the control group rats as 3.1 ± 0.1^{E}. CRP levels reverted to normal after administering varied doses of *P. emblica* methanolic leaf extract in groups of female rats at 4.2 ± 0.1^{C}, 3.7 ± 0.1^{D}, and 3.8 ± 0.1^{D}, 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 ± 0.1^{C}), as compared to the high concentration in the dexamethasone treatment group (0.6 ± 0.1^{AB}), and the positive control group (0.6 ± 0.1^{AB}), 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[15]. 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

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

Perimeters	Normal Group	Intoxicated Group	Positive Control	PE (LD)	PE (ID)	PE (HD)	*p*-value
TOS (µ Mol equiv. /L)	11.1 ± 0.1^{C}	14.1 ± 0.1^{A}	9.1 ± 0.1^{D}	12.1 ± 0.1^{B}	8.1 ± 0.1^{E}	7.0 ± 0.2^{F}	<0.01
TAS (µ Mol equiv. /L)	0.6 ± 0.1^{BC}	0.5 ± 0.1^{C}	0.7 ± 0.1^{B}	0.5 ± 0.1^{C}	0.9 ± 0.1^{A}	0.9 ± 0.1^{A}	<0.01

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 $p<0.05$ is considered statistically significant, while $p<0.01$ indicates high significance.

as 12.1 ± 0.1^{B}, 8.1 ± 0.1^{E}, and 7.0 ± 0.2^{F} 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^{C}, 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, TOS increased while TAS 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^{D}. 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^{D}) and the positive control group (373.0 ± 1.0^{A}). 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^{E}, 340.0 ± 1.0^{C}, and 351.0 ± 1.0^{B}, 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.

Perimeters	Normal Group	Intoxicated Group	Positive Control	PE (LD)	PE (ID)	PE (HD)	*p*-value
Immunoglobulin M (IgM) (mg/dL)	250.0 ± 1.0^{D}	192.0 ± 1.0^{F}	373.0 ± 1.0^{A}	245.0 ± 1.0^{E}	340.0 ± 1.0^{C}	351.0 ± 1.0^{B}	<0.01
Immunoglobulin G (IgG) (mg/dL)	611.0 ± 1.0^{A}	450.0 ± 1.0^{F}	560.0 ± 1.0^{D}	456.0 ± 1.0^{E}	566.0 ± 1.0^{C}	604.0 ± 1.0^{B}	<0.01

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.

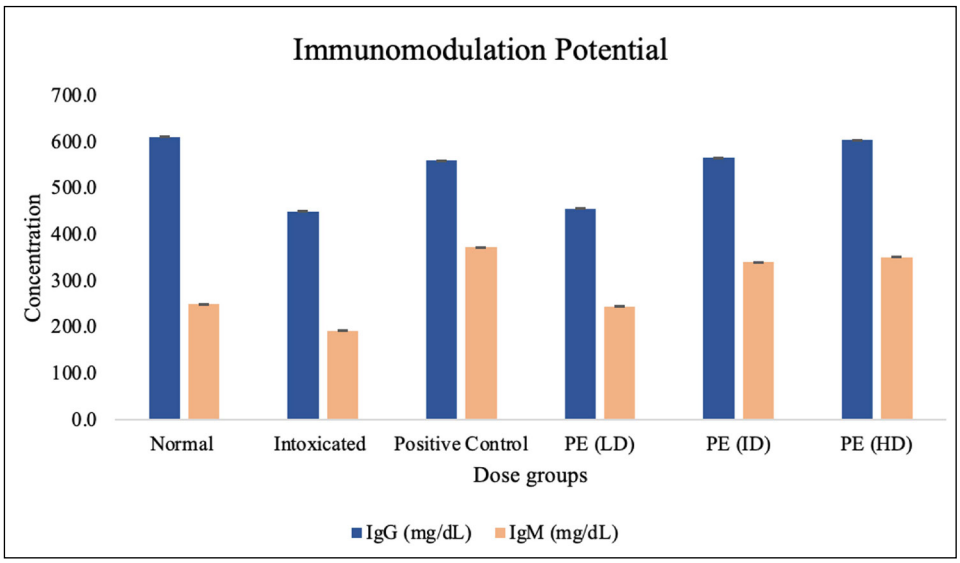

Figure 1. Bar graph depicting the potential of plant extracts control treatments on female rats' immune response (IgG and IgM). The findings are shown as Means (bars)±SD (lines). $p<0.05$ is regarded as statistically significant, whereas $p<0.01$ is considered very significant.

Discussion

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[16] to have immunomodulatory properties, such as increasing antibody against antigens and increasing IL levels.

According to reports[17], 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[18]. 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[19] to be effective in the treatment of autoimmune disorders and malignancies. In another research[20], 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[21] evaluated the effect of *Equisetum arvense* on total RBC count and found that total RBC count showed significant ($p<0.05$) 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 ($p>0.05$) results of total RBC count in plant-treated rats when compared to normal rats; however, total RBCs were found to increase significantly ($p<0.01$) 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 ($p<0.05$). Our findings were consistent with those of Badole and Kotwal[21], who showed an increment in platelet count in plant-treated female rats as compared to normal control female rats. Sarvaiya et al[22] 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 ($p<0.05$). White blood cells (WBCs) or leuco-

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[23] 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[23]. Badole and Kotwal[21] 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 Abbas[26] 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[29]. Shanmugarajan et al[30] 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[31] 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[32] 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 studies[34-56] 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, Shafiq.; formal analysis, Riffat 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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ORCID ID

Abid Sarwar: 0000-0003-2105-7201
Tariq Aziz: 0000-0003-0905-8076

References

1) Khan MSA, Ahmad I. Herbal medicine: current trends and future prospects. In New look to phytomedicine. 2019; 3-13
2) Renuka R, Devi KR, Sivakami M, Thilagavathi T, Uthrakumar R, Kaviyarasu K. Biosynthesis of silver nanoparticles using Phyllanthus emblica fruit extract for antimicrobial application. Biocatal Agric Biotechnol 2020; 24: 101567.
3) Ilić M, Samardžić S, Kotur-Stevuljević J, Ušjak D, Milenković M, Kovačević N, Drobac M. Polyphenol rich extracts of Geranium L. species as potential natural antioxidant and antimicrobial agents. Eur Rev Med Pharmacol Sci 2021; 25: 6283-6294.
4) Jabs DA. Immunosuppression for the uveitides. Ophthalmology 2018; 125: 193-202.
5) Aslam MS, Ahmad MS. Worldwide importance of medicinal plants: Current and historical perspectives. Recent Adv Biol Med 2016; 2: 909.
6) Zhang A, Sun H, Yan G, Wang X. Recent developments and emerging trends of mass spectrometry for herbal ingredients analysis. TrAC 2017; 94: 70-76.
7) Cameron DG, Watson GM. The blood counts of the adult albino rat. Blood 1949; 4: 816-818.
8) Bergmeyer HU, Herder M, Ref R. International federation of clinical chemistry (IFCC). J Clin Chem Clin Biochem 1986; 24: 497-510.
9) Burtis CA, Ashwood ER. Tietz textbook of clinical chemistry. Amer Assn Clin Chem 1994.
10) Montgomery DC. Design and analysis of experiments. John Wiley & Sons 2009.
11) Ajuru MG, Williams LF, Ajuru G. Qualitative and quantitative phytochemical screening of some plants used in ethnomedicine in the Niger Delta region of Nigeria. J Food Nutr Sci 2017; 5: 198-205.
12) Emery PW. Basic metabolism: protein. Surgery (Oxford) 2015; 33: 143-147.
13) Nweze CC, Mustapha AA, Alkali IM. Aqueous leaf extracts of Tobacco plant (Nicotiana tabaccum) cause hepatotoxicity in male Wistar albino rats. Asian J Pharmacol Toxicol 2015; 3: 27-30.
14) Pastori D, Pani A, Di Rocco A, Menichelli D, Gazzaniga G, Farcomeni A, Baratta F. Statin liver safety in non-alcoholic fatty liver disease: A systematic review and metanalysis. Br J Clin Pharmacol 2022; 88: 441-451.
15) Nascimento-Souza MA, Paiva PG, Martino HSD, Ribeiro AQ. Dietary total antioxidant capacity as a tool in health outcomes in middle-aged and older adults: a systematic review. Crit. Rev Food Sci Nutr 2018; 58: 905-912.
16) Da Silva Mesquita R, Kyrylchuk A, Costa de Oliveira R, Costa Sá IS, Coutinho Borges Camargo G, Soares Pontes G, Grafov A. Alkaloids of Abuta panurensis Eichler: In silico and in vitro study of acetylcholinesterase inhibition, cytotoxic and immunomodulatory activities. PLoS One 2020; 15: e0239364.
17) Bribi N. Pharmacological activity of alkaloids: a review. AJB 2018; 1: 1-6.
18) Elfalleh W, Kirkan B, Sarikurkcu C. Antioxidant potential and phenolic composition of extracts from Stachys tmolea: An endemic plant from Turkey. Ind Crops Prod 2019; 127: 212-216.
19) Raina R, Afroze N, Sundaram MK, Haque S, Bajbouj K, Hamad M, Hussain A. Chrysin inhibits propagation of HeLa cells by attenuating cell survival and inducing apoptotic pathways. Eur Rev Med Pharmacol Sci 2021; 25: 2206-2220.
20) Chaphalkar R, Apte KG, Talekar Y, Ojha SK, Nandave M. Antioxidants of Phyllanthus emblica L. Bark extract provide hepatoprotection against ethanol-induced hepatic damage: a comparison with silymarin. Oxid Med Cell Longev 2017; 2017: 3876040.
21) Badole S, Kotwal S. Biochemical, hematological and histological changes in response to graded dose of extract of Equisetum arvense in adult female wistar rats. Int J Pharm Sci Res 2015; 6: 3321.
22) Sarvaiya VN, Sadariya KA, Pancha PG, Thaker AM, Patel AC, Prajapati AS. Evaluation of antigout activity of Phyllanthus emblica fruit extracts on potassium oxonate-induced gout rat model. Vet World 2015; 8: 1230.
23) Montejo JF, Mondonedo JAB, Lee MGA, Ples MB, Vitor II RJS. Hematological effects of Ipomoea batatas (camote) and Phyllanthus niruri (sampa-sampalukan) from Philippines in the ICR mice (Mus musculus). Asian Pac J Trop Biomed 2015; 5: 29-33.
24) Mehdi MAH, Alarabi FY, Omar GMN, Pradhan V. Effect of extracts on haematological parameters in albino rats Tamarindus indica infected with parasite Entamoeba histolytica. AJPP 2019; 5: 889-894.
25) Yin K, Li X, Luo X, Sha Y, Gong P, Gu J, Tan R. Hepatoprotective effect and potential mechanism of aqueous extract from Phyllanthus emblica on carbon-tetrachloride-induced liver fibrosis in rats. eCAM 2021; 2021: 5345821.
26) Naz M, Abbas N. Study of Hepatoprotective effect produced by the fruits of Phyllanthus emblicus and silymarin against cisplatin induced hepatotoxicity. J Pharm Res 2016; 10: 1-7.
27) Anto EJ, Syahputra RA, Silitonga HA, Situmorang PC, Nugaraha SE. Oral acute toxicity study extract ethanol of balakka fruit (Phyllanthus emblica). Pharmacia 2022; 9: 187-194.
28) Usharani P, Merugu PL, Nutalapati C. Evaluation of the effects of a standardized aqueous extract of Phyllanthus emblica fruits on endothelial dysfunction, oxidative stress, systemic inflammation and lipid profile in subjects with metabolic syndrome: A randomised, double blind, placebo controlled clinical study. BMC Complement Altern Med 2019; 19: 1-8.
29) Chaudhary P, Pandey A, Azad CS, Tia N, Singh M, Gambhir IS. Association of oxidative stress and endothelial dysfunction in hypertension. Anal Biochem 2020; 590: 113535.
30) Shanmugarajan D, Girish C, Harivenkatesh N, Chanaveerappa B, Prasanna Lakshmi NC. Antihypertensive and pleiotropic effects of Phyllanthus emblica extract as an add-on therapy in patients with essential hypertension – A randomized double-blind placebo-controlled trial. Phytother Res 2021; 35: 3275-3285.
31) Tasanarong A, Kongkham S, Itharat A. Antioxidant effect of Phyllanthus emblica extract prevents contrast-induced acute kidney injury. BMC Complement Altern Med 2014; 14: 1-11.

32) Uddin MS, Al Mamun A, Hossain MS, Akter F, Iqbal MA, Asaduzzaman M. Exploring the effect of Phyllanthus emblica L. on cognitive performance, brain antioxidant markers and acetylcholinesterase activity in rats: promising natural gift for the mitigation of Alzheimer's disease. Ann Neurosci 2016; 23: 218-229.
33) Prisingkorn W, Rinthong PO, Yuangsoi B, Doolgindachbaporn S, Wiriyapattanasub P, Doan HV, Wongmaneeprateep S. The effects of Terminalia chebula, Terminalia belerica, Phyllanthus emblica and Triphala on the growth performance and immune response in Nile tilapia (Oreochromis niloticus). Aquac Res 2022; 53: 625-632.
34) Ahmad B, Muhammad Yousafzai A, Maria H, Khan AA, Aziz T, Alharbi M, Alsahammari A, Alasmari AF. Curative Effects of Dianthus orientalis against Paracetamol Triggered Oxidative Stress, Hepatic and Renal Injuries in Rabbit as an Experimental Model. Separations 2023; 10: 182.
35) Ahmad E, Jahangeer M, Mahmood Akhtar Z, Aziz T, Alharbi M, Alshammari A, Alasmari AF, Irfan Bukhari N. Characterization and gastroprotective effects of Rosa brunonii Lindl. fruit on gastric mucosal injury in experimental rats - A preliminary study. Acta Biochim Pol 2023; 18: 633-641.
36) Ammara A, Sobia A, Nureen Z, Sohail A, Abid S, Aziz T, Nahaa MA, Rewaa SJ, Ahellah MJ, Nouf SAA, Nehad AS, Manal YS, Amnah AA, Majid A, Abdulhakeem SA, Anas SD, Saad A. Revolutionizing the effect of Azadirachta indica extracts on edema induced changes in C-reactive protein and interleukin-6 in albino rats: in silico and in vivo approach. Eur Rev Med Pharmacol Sci 2023; 27: 5951-5963.
37) Ayesha S, Muhmmad A, Muhammad N, Syeda IM, Modasrah M, Tariq A, Ayaz AK, Muhammad S, Metab A, Abdulrahman A. HPLC and GC-MS Quantification of Phytoconstituents from T. vulgaris eliciting the potential of bioactive compounds by executing multiple in-vitro and in vivo biological activities inducing functionalized capabilities on COX-1, COX-2 and gastric cancer genes computationally. Molecules 2022; 27: 8512.
38) Aziz T, Ihsan F, Ali Khan A, Ur Rahman S, Zamani GY, Alharbi M, Alshammari A, Alasmari AF. Assessing the pharmacological and biochemical effects of Salvia hispanica (Chia seed) against oxidized Helianthus annuus (sunflower) oil in selected animals. Acta Biochim Pol 2023; 27: 211-218.
39) Zawar H, Muhammad J, Abid S, Najeeb U, Tariq A, Metab A, Abdulrahman Al. Synthesis of silver nanoparticles by aqueous extract of Zingiber officinale and their antibacterial activities against selected species. Polish J. Chem Tech 2023; 25: 23-30.
40) Aziz T, Nadeem AA, Sarwar A, Perveen I, Hussain N, Khan AA, Daudzai Z, Cui H, Lin L. Particle Nanoarchitectonics for Nanomedicine and Nanotherapeutic Drugs with Special Emphasis on Nasal Drugs and Aging. Biomedicines 2023; 11: 354.
41) Ejaz A, Muhammad J, Nadeem IB, Abid S, Tariq A, Metab A, Abdulrahman A, Abdullah FA. Isolation, Structure Elucidation & Antidiabetic Potential of Rosa brunonii L. fruit – Fight Diabetes with Natural Remedies. J Chil Chem Soc 2023; 68: 5887-5894.
42) Gul R, Rahmatullah Q, Ali H, Bashir A, Ayaz AK, Tariq A, Metab A, Abdulrahman A, Abdullah FA. Phytochemical, Antimicrobial, Radical Scavenging andIn- vitro biological activities of Teucrium stocksianum leaves". J Chil Chem Soc 2023; 68: 5748-5754.
43) Hena Z, Mohsin S, Shafiq UR, Zafar I, Ayaz AK, Tariq A, Waqar A, Ghazala YZ, Saeed A, Muhammad S, Metab A, Abdulrahman A. Assessing the effect of walnut (juglans regia) and olive (olea europaea) oil against the bacterial strains found in Gut Microbiome. Progress in Nutrition 2022; 24: 1-13.
44) Hamid M, Salar U, Rashid Y, Azim MK, Khan KM, Naz S, Aziz T, Alharbi M, Alshammari A, Alasmari FA. Determining the 3-substituted Coumarins inhibitory potential against the HslV protease of E. coli. Eur Rev Med Pharmacol Sci 2023; 27: 9169-9182.
45) Naveed M, Bukhari B, Aziz T, Zaib S, Mansoor MA, Khan AA, Shahzad M, Dablool AS, Alruways MW, Almalki AA, Alamri AS, Alhomrani M. Green Synthesis of Silver Nanoparticles Using the Plant Extract of Acer oblongifolium and Study of Its Antibacterial and Antiproliferative Activity via Mathematical Approaches. Molecules 2022; 27: 4226.
46) Naveed M, Batool H, Rehman SU, Javed A, Makhdoom SI, Aziz T, Mohamed AA, Sameeh MY, Alruways MW, Dablool AS, Almalki AA, Alamri AS, Alhomrani M. Characterization and Evaluation of the Antioxidant, Antidiabetic, Anti-Inflammatory, and Cytotoxic Activities of Silver Nanoparticles Synthesized Using Brachychiton populneus Leaf Extract. Processes 2022; 10: 1521.
47) Naveed M, Makhdoom SI, Rehman SU, Aziz T, Bashir F, Ali U, Alharbi M, Alshammari A, Alasmari AF. Biosynthesis and Mathematical Interpretation of Zero-Valent Iron NPs Using Nigella sativa Seed Tincture for Indemnification of Carcinogenic Metals Present in Industrial Effluents. Molecules 2023; 7: 3299.
48) Nureen Z, Tahira F, Muhammad H, Basit Z, Abid S, Tariq A, Metab A, Abdulrahman A, Abdullah FA. In-Vivo and In-Silico analysis of Anti- Inflammatory, Analgesic, and Anti pyretic activities of Citrus paradisi Leaf Extract. J Chil Chem Soc 2023; 68: 5813-5821.
49) Hayat P, Khan I, Rehman A, Jamil T, Hayat A, Rehman MU, Ullah N, Sarwar A, Alharbi AA, Dablool AS, Daudzai Z, Alamri AS, Alhomrani M, Aziz T. Myogenesis and Analysis of Antimicrobial Potential of Sliver Nanoparticles (AgNPs) against Pathogenic Bacteria. Molecules 2023; 28: 637.
50) Rauf B, Alyasi S, Zahra N, Ahmad S, Sarwar A, Aziz T, Alharbi M, Alshammari A, Alasmari AF. Evaluating the influence of Aloe barbadensis extracts on edema induced changes in C-reactive protein and interleukin-6 in albino rats through in vivo and in silico approaches. Acta Biochim Pol 2023; 17: 425-433.
51) Saleem K, Aziz T, Ali Khan A, Muhammad A, Ur Rahman S, Alharbi M, Alshammari A, F Alasmari A. Evaluating the in-vivo effects of olive oil, soya bean oil, and vitamins against oxidized ghee toxicity. Acta Biochim Pol 2023; 10: 305-312.

52) Sana, Ur Rahman S, Zahid M, Khan AA, Aziz T, Iqbal Z, Ali W, Khan FF, Jamil S, Shahzad M, Alharbi M, Alshammari A. Hepatoprotective effects of walnut oil and Caralluma tuberculata against paracetamol in experimentally induced liver toxicity in mice. Acta Biochim Pol 2022; 24: 871-878.
53) Shabbir MA, Naveed M, Rehman SU, Ain NU, Aziz T, Alharbi M, Alsahammari A, Alasmari AF. Synthesis of Iron Oxide Nanoparticles from Madhuca indica Plant Extract and Assessment of Their Cytotoxic, Antioxidant, Anti-Inflammatory, and Anti-Diabetic Properties via Different Nanoinformatics Approaches. ACS Omega 2023; 7: 33358-33366.
54) Wajid AS, Muhammad SA, Mujaddad UR, Azam H, Abid S, Tariq A, Alharbi M, Alsahammari A, Alasmari AF. In-Vitro Evaluation of Phytochemicals, Heavy Metals and Antimicrobial Activities of Leaf, Stem and Roots Extracts of Caltha palustris var. alba. J Chil Chem Soc 2023; 68: 5807-5812.
55) Waseem M, Naveed M, Rehman SU, Makhdoom SI, Aziz T, Alharbi M, Alsahammari A, Alasmari AF. Molecular Characterization of spa, hld, fmhA, and lukD Genes and Computational Modeling the Multidrug Resistance of Staphylococcus Species through Callindra harrisii Silver Nanoparticles. ACS Omega 2023; 5: 20920-20936.
56) Zawar H, Muhammad AR, Muhammad J, Abid S, Abad AN, Sumaira N, Tariq A, Metab A, Abdulrahman A, Abdullah FA. Green Synthesis of Silver Nanoparticles Prepared by Leaves Extract of Trigonila foenum-graecum and its Antibacterial Potential Against Escherichia coli and Pseudomonas aeruginosa. Biomass Conv Bioref 2023; 1-8.
57) Wang HMD, Fu L, Cheng CC, Gao R, Lin MY, Su HL, Hsieh LP. Inhibition of LPS-induced oxidative damages and potential anti-inflammatory effects of Phyllanthus emblica extract via down-regulating NF-κB, COX-2, and iNOS in RAW 264.7 cells. Antioxidants 2019; 8: 270.
58) Van Doan H, Lumsangkul C, Sringarm K, Hoseinifar SH, Dawood MA, El-Haroun E, Paolucci M. Impacts of Amla (Phyllanthus emblica) fruit extract on growth, skin mucosal and serum immunities, and disease resistance of Nile tilapia (Oreochromis niloticus) raised under biofloc system. Aquac Rep 2022; 22: 100953.
59) Bakr ESH, Naga ME. Immunomodulatory efficacy of Phyllanthus emblica and costus speciosus aqueous extracts for immunosuppressive rats. Egypt J Nutr. 2020; 35: 101-123.
60) Khan MSA, Ahmad I. Herbal medicine: current trends and future prospects. In New look to phytomedicine, 2019; 3-13.
61) Zawar H, Muhammad J, Abid S, Najeeb U, Tariq A, Metab A, Abdulrahman A. Synthesis and Characterization of Silver Nanoparticles mediated by the Mentha piperita Leaves Extract and Exploration of its Antimicrobial Activities. J Chil Chem Soc 2023; 68; 5865-5870.