Evaluation of Immunomodulatory Potential of Phyllanthus Emblica Fruit Extract in Wistar Rats

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ABSTRACT: In the present study, the aqueous fruit extract of Phyllanthus emblica was evaluated for its immunomodulatory effect on the male wistar rats. The rats were divided into three groups. The first group received normal saline and served as control whereas the groups II and III received aqueous fruit extract of Phyllanthus emblica at a dose of 100 and 200mg/kg body weight/day respectively for 21 days. A significant increase was observed in the total leucocyte count, lymphocyte %, serum total protein, serum globulin and serum albumin in the treated rats in a dose-dependent manner when compared to the control rats. The treated group also exhibited an increase in the relative weight of the spleen. Further, Phyllanthus emblica treated rats (group II and III) also showed significant antibody titre values and DTPH reaction when compared to the control group thus indicating its ability to stimulate the humoral as well as cell mediated immunity.

Key words: Phyllanthus emblica, Rats, Immunomodulatory, humoral immunity, cell-mediated immunity.

Introduction:

In the last decades herbal medicine has grown increasingly as an imperative branch of complementary and alternative medicine[1]. The role of different plant extracts in maintaining the balance of life as well as in treating various diseases is well documented. In depth research is focussed more on the phytochemical analysis of plant extracts and their effects on several disease conditions in vitro. The plant extracts have proven to be more significant for reestablishing the body's equilibrium and providing the resistance against infection. The plant extracts also possess the restorative and rejuvenating powers as they act upon the immune system thus affecting the response of the body towards infection [2].

Immunomodulation is a process which alters the host immune system resulting either in immunomodulation or immunosuppression thus regulating or neutralizing it. Hence the immunomodulators which are referred to as biological response modifiers improve the host defense mechanism against various diseases by striking a balance between regulatory and effector cells [3,4].

Taking this quality of biological indicators, several alternative ayurvedic formulations have been developed for various diseases where they either activate the host defense mechanism or suppress it. Such immunomodulatory properties of various medicinal plants provide an alternative to the conventional synthetic drug therapy which causes the side effects, allergic reactions, tolerance to drugs and increased resistance of the micro-organisms to antibiotics.

Many medicinal herbs have been utilized as traditional therapies to curve several immune-related disorders. Research to discover the natural products as candidate drugs for the development of immunomodulatory agents have gained momentum as they offer safer alternatives to the conventional therapies [5]. The medicinal herbs with their active metabolites deliver alternative potential to the ongoing therapy for varied immunological disorders by modulating the immune response [5]. The active components present in the medicinal plants regulate the immune system by interacting with various immune cells and regulating their effector mechanism. Apart from this, the active components play a crucial role in enhancing body's resistance towards various diseases, memory and energy which ultimately balances the health of the individual as a whole.

Phyllanthus species are extensively studied for their immunomodulatory effects due to its enormous use in treating immune related diseases in indigineous medicine. The application of extracts of various Phyllanthus species with their bioactive components or metabolites as immunomodulators need to be studied to prove that their traditional uses are effective and safe and also allow the clinical trials to be pursued for their development as therapeutic agents to treat various immune related disorders.

Emblica officinalis or Phyllanthus emblica is a small to medium sized deciduous tree belonging to family Euphorbiaceae [6].It is commonly called as Indian gooseberry or Amla. It is considered to be an important medicinal herb in Ayurveda and Unani systems of medicine and WHO has approved it efficacy. It is one of the richest source of vitamin C with 200-900 mg/100gm of edible portion [7,8,9]. Almost all the parts of Amla bear medicinal properties, but the fruit is of immense use in traditional system of medicine. The fruit is reported to possess the phenolic constituents like gallic acid and its derivatives, mucic acid and its derivatives, corillagin, chebulagic acid, putrajivain A [10,11]. It also possess high amount of tannins like emblicanin A and B, punigluconin and pedunculagin [12], flavanoids like quercetin [13,14] and alkaloids like phyllantin and phyllantidin [15]. Several authors also reported high amount of vitamin C [16,17,18,19,20] and high amount of minerals, proteins and amino acids like proline, alanine, cysteine, glutamic acid, aspartic acid and lysine. The fruit also possesses fibers, glucose, phosphorus, iron and calcium [21,22].

The chemical constituents and the composition of Amla fruit pulp are summarized in Table-I and II respectively [23]. Keeping in view all the above facts as reported by several authors, the present work has been investigated to discuss the immunoodulatory activity of Phyllanthus emblica . The study was performed to evaluate the effect of aqueous fruit extract of Phyllanthus emblica on the humoral and cellular immune responses in normal and healthy male wistar rats.

Materials and Methods:

Preparation of Aqueous fruit extract of Phyllanthus emblica:

Fresh fruits of Phyllanthus emblica were purchased from local market. The fruits were then washed, deseeded, air dried and powdered with a mechanical grinder passing through a sieve and stored in a tight container. Then about 25gms of the air dried fruit powder is refluxed with ethanol at 45°C for 3 hours using the Soxhlet apparatus. The mixture is then filtered and the filtrate is evaporated using the vacuum evaporator and air dried at 40°C. The stock solution of the crude ethanolic extract (aqueous extract) was prepared by diluting the dried extract with 0.25% dimethyl sulphoxide (DMSO) solution to obtain a final concentration of 100mg/ml.

SRBC-Antigen:

Fresh blood was collected from sheep sacrificed in the local slaughter house.Sheep red blood cells(SRBC's) were washed three times in large volumes of pyrogen-free in 0.9% normal saline and adjusted to a concentration of 1x 10^{8} cells for immunization and challenge.

Experimental Design:

Healthy male wistar rats weighing about 25-35gms were used for the study. The rats were then divided into three groups (I,II and III) with each group consisting of 6 rats. The group I rats received the normal saline and served as the control group whereas group II and group III rats received aqueous fruit extract of Phyllanthus emblica at a dose of 100 and 200mg/kg body weight/day respectively for about 21 days. Further the rats were fed with basal diet and water ad libitum during the experimental period. The experiment was performed for 21 days and the day before the commencement of the experiment was considered as the zeroth day. The rats from all the groups i.e. (I, III and III) were sensitized intraperitoneally with 0.1 ml of sheep red blood cells (SRBC) antigen containing 1×10^{8} cells on the 7th day. The immunomodulatory effect of the aqueous fruit extract of Phyllanthus emblica was then evaluated on the 14th and 21st day of the experiment.

The blood was collected from the wistar rats on Zeroth, 7th, 14th and 21st day of the experiment. The total WBC count, Lymphocyte %, Serum total protein, Serum albumin and Serum globulin were estimated following the method of Gomall et al [24], Doumas [25]. The body weights of the experimental rats were measured before and after the treatment and also the relative weight of the spleen was recorded after sacrifice on the 14th and 21st day of the experiment. The blood was also analysed for haemagglutination antibody [HA] titer on the Zeroth, 14th, and 21st day of the experiment following the method of Puri et al [26]. Delayed type of hypersensitivity (DTH) was also performed following the method of Saraf et al[27] on 14th and 21st day of experiment.The antibody levels were determined by haemagglutination technique. Briefly equal volumes of individual serum samples of each group were pooled.To serial two fold dilutions of pooled serum samples made in 25µl volumes of normal saline in microtitration plates was added 25µl of 1% suspension of SRBC's in saline. After mixing, the plates were incubated at 37° C for 1 hour and examined for agglutination. The reciprocal of the highest dilution of the test serum giving agglutination was taken as the antibody titer.For the analysis of Delayed type of hypersensity(DTH), the thickness of the right hand foot pad was measured using a vernier caliper. The mice were then challenged by injecting SRBC's in the right hand foot pad.The thickness was measured 24hrs after the challenge and the difference between the pre- and post- challenge foot thickness(expressed in mm) was taken as a measure of delayed type hypersensitivity.

Statistical Analysis:

The Statistical Analysis was performed using one way ANOVA. All the values are expressed as Mean±SEM (n=6). The statistical significance was set at $p<0.05$ following the method of Snedecor and Cochran [28].

Results and Discussion:

The results of the effect of Phyllanthus emblica on the total leucocyte count, lymphocyte %, serum total protein, serum albumin, serum globulin, Haemagglutination antibody (HA) titre and DTH are presented in Table III and Table-IV respectively.It was observed that there was a significant increase in the total leucocyte count, lymphocyte %, serum total protein, serum globulin and serum albumin in treated rats. i.e. (Group-II and Group III) on the 14th and 21st day of experiment in a dose dependent manner when compared to the control group (Group-I). The treated group also showed significant antibody titre values and DTH reaction when compared to the control. Further, the treated group also showed an increase in the relative weight of the spleen as indicated in (Table-V).

In the present study, the aqueous fruit extract of Phyllanthus emblica has shown promising immunomodulatory activity. The concept of immunomodulation advocated by Jayalalitha and Mishra[29], relates to the activation of function and efficacy of macrophages, granulocytes, complement, natural killer cells (NK Cells)), lymphocytes and also to the production of several effector molecules generated by activated cells.

The significant increase in the total leucocyte count and lymphocyte percentage in the treated rats in the present study can be correlated to its ability to stimulate the haemolymphopoietic system [30]. When compared to the control rats, the treated rats produced higher total serum proteins. especially serum globulin which plays a crucial role in maintaining homeostasis thus regulating inflammatory response and providing resistance to infection [31]. Spleen considered as a secondary lymphoid organ contains many phagocytes and lymphocytes. Thus, increase in the weight of the spleen after administration of Phyllanthus emblica fruit extract can be viewed as an increased immuno competence in the treated rats [31]. Further, increase in the haemagglutination antibody (HA) titre and DTH activity in the treated rats may be due its stimulant effect on the humoral and cell mediated immune responses respectively.

Conclusion:

The study concludes that the use of Phyllanthus emblica with its constituents prove to be a better and potential alternative for allopathic immunomodulation.

Further, the presence of different active compounds like gallic acid, chebulagic acid, ellagic acid, flavonoids, tannins and phenols are responsible for its effective immunomodulatory property making it a strong contender as a plant based ayurvedic immunomodulator.

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TABLE - I

Chemical constituents of fruit of Phyllanthus emblica

Type	Chemical constituents
Hydrolysable tannins	Emblicanin A and B,Punigluconin,Pedunculagin,Chebulinic acid(Ellagitannin),Chebulagic acid(Benzopyran tannin),corillagin(Ellagitannin), Geranin(Dehydroellagitannin),Ellagotannin.
Alkaloids	Phyllantine, Phyllembein, Phyllantidine.
Phenolic compounds	Gallic acid,Methyl gallate,Ellagic acid, Trigallayl glucose.
Aminoacids	Glutamic acid,Proline, Aspartic acid, Alanine, Cystine,Lysine.
Carbohydrates	Pectin.
Vitamins	Ascorbic acid.
Flavonoids	Quercetin, Kaempferol.
Organic acids	Citric acid.

TABLE-II

Average percentage composition of fruit pulp of Phyllanthus emblica

Component	Percentage
Moisture	81.2
Protein	0.5
Fat	0.1
Mineral matter	0.7
Fibre	3.4
Carbohydrate	14.1
Calcium	0.05
Phosphorus	0.02
Iron	1.2mg/100gm
Nicotinic acid	0.2mg/100gm
Vitamin c	600mg/100gm

TABLE-III

Effect of Phyllanthus emblica on Total leucocyte count,Lymphocyte %, Serum total protein,Serum albumin and Serum globulin in Rats.

Parameter	Group	0^{th} Day of Experiment	7^{th} Day of Experiment	14^{th} Day of Experiment	21^{st} Day of Experiment
TLC($x10^3$ /cu.mm	I	6.55±0.97	6.58±0.60	6.80±0.71	7.28±0.73
	II	6.80±0.70	6.69±0.72	9.12±0.41*	11.57±0.41*
	III	6.98±0.38	6.83±0.58	11.87±0.43*	14.01±0.72*
Lymphocyte %	I	72.70±1.15	74.32±1.38	77.38±1.19	78.33±1.21
	II	73.18±1.12	76.17±1.05	81.62±1.50*	84.83±1.41*
	III	74.0±1.22	77.33±1.45	84.13±1.26*	88.33±1.26*
Serum total protein(g/dl)	I	4.14±1.52	4.23±0.05	4.26±0.06	4.28±0.07
	II	4.22±0.07	4.28±0.08	4.39±0.09	5.38±0.09*
	III	4.25±0.05	4.37±0.04	5.10±0.08*	6.16±0.07*
Serum albumin(g/dl)	I	2.76±0.07	2.88±0.14	2.91±0.14	2.98±0.06
	II	2.94±0.09	2.97±0.02	2.98±0.07	3.75±0.13
	III	2.96±0.04	3.10±0.07	3.64±0.06	4.32±0.08*
Serum globulin(g/dl)	I	1.26±0.05	1.27±0.07	1.28±0.04	1.29±0.07
	II	1.29±0.07	1.28±0.05	1.34±0.08	1.75±0.06*
	III	1.30±0.06	1.29±0.08	1.57±0.06*	1.98±0.07*

Values are expressed as Mean±S.E.M; n=6 Rats; *p<0.05 compared to control group.

TABLE-IV

Effect of Phyllanthus emblica on Delayed type of Hypersensitivity(DTH) and Haemagglutination antibody titer (HA)of Rats

Parameter	Group	14th Day of Experiment	21st Day of Experiment
DTH(mm)	I	0.45±0.02	0.58±0.03
	II	1.56±0.05*	1.69±0.05*
	III	1.85±0.07*	1.97±0.06*
HA Titer	I	26.91±8.9	28.96±10.23
	II	64.12±9.40*	125.50±7.70*
	III	75.80±6.12*	182.30±9.10*

Values are expressed as Mean±S.E.M; n=6 Rats; * p<0.05 compared to control.

TABLE- V

Effect of Phyllanthus emblica on Relative body weight of spleen(gm/100gm of Body weight)of Rats

Group	Weight of spleen 14th Day of Experiment	Weight of spleen 21st Day of Experiment
I	0.32±0.01	0.35±0.03
II	0.37±0.02	0.40±0.01*
III	0.42±0.05*	0.58±0.03*

Values are Mean±S.E.M; n= 6 Rats; *p<0.05 compared to control group.