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EVALUATION OF IMMUNOMODULATORY POTENTIAL OF *EMBLICA OFFICINALIS* FRUIT PULP EXTRACT IN MICE

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ABSTRACT

Aqueous extract of dried *Emblica officinalis* Gaertn. (Amla) fruit pulp powder was evaluated for immunomodulatory effect on male Swiss Albino mice. The mice were divided into three groups. The first group received vehicle alone to serve as control. The second and third groups received the extract orally at 100 and 200 mg/ kg body weight dose levels respectively per day for a period of 19 days. There was significant dose dependent increase in haemagglutination antibody titre, sheep red blood cells induced delayed type of hypersensitivity reaction, macrophage migration index, respiratory burst activity of the peritoneal macrophages, total leukocyte count, percentage lymphocyte distribution, serum globulin and relative lymphoid organ weight in Emblica treated mice indicating its ability to stimulate humoral as well as cell mediated immunity along with macrophage phagocyte.

Key words: Immunomodulation, *Emblica officinalis*, Mice

INTRODUCTION

The fruits of *Emblica officinalis* (Amla or Indian Gooseberry) is extensively used in Indian Ayurveda and Sidha system of traditional medicines for the treatment of wide spectrum of diseases and possesses hypolipidaemic, antiviral, hepatoprotective, antioxidant, antidiabetic, anticlastogenic and anti-inflammatory properties (Thakur *et al.*, 1988; Elizabeth, 2002). Though Amla is widely accepted as an immune booster among the people, the supportive scientific proof available is limited. Therefore, the effect of aqueous extract of dried *Emblica officinalis* fruit pulp powder on humoral and cellular immune responses was studied in normal healthy male Swiss albino mice.

MATERIAL AND METHODS

Healthy male Swiss Albino mice weighing 20-25 g were used for the study, after taking approval from the institutional animal ethics committee. Fresh fruits of *Emblica officinalis* were collected, cleaned, deseeded, dried and powdered. The aqueous extract from the powdered fruit was obtained as per the standard procedure (Harborne, 1991). The mice were divided into three groups (I, II and III) of eight each. The group I animals received normal saline (vehicle) and constituted control group, whereas group II and III animals received aqueous extract of Emblica at dose levels of 100 and 200 mg / kg body weight/ day respectively for 19 days. The mice were provided with basal diet and water ad libitum during the experiment. The day before the commencement of experiment was taken as zero day. Mice from all groups were sensitized with 0.1 ml of Sheep red blood cells (SRBC) antigen containing $1x10^8$ cells intraperitoneally on 5th day of experiment and the immunomodulatory effect of the extract was evaluated seven and 14 days after sensitization (i.e. 12th and 19th day of experiment).

Blood was collected from retro orbital plexus of mice on zero, 5th, 12th and 19th day of the experiment and total WBC count, percentage lymphocyte distribution, serum total protein, albumin and globulin were recorded (Gomall *et al.*, 1949; Doumas, 1971). The body weights of the experimental animals were recorded before and after drug treatment and relative weight of spleen was recorded after sacrifice on 12th and 19th day of experiment. The blood was analysed for Haemagglutination [HA] antibody titre on zero, 12th and 19th day of experiment (Puri *et al.*, 1994) and Delayed type of Hypersensitivity [DTH] (Saraf *et al.*, 1989) on 12th and 19th day of experiment.

The peritoneal macrophages collected from mice of the three groups after sacrifice, using procedure of Benencia *et al.* (1996) on 12th and 19th day, were washed three times in fresh Hanks Balanced Salt Solution. Macrophage activation was determined by Macrophage Migration test by method of Saxena *et al.* (1991) and Nitro Blue Tetrazolium (NBT) dye reduction test as per Sairam *et al.* (1997). The ratio of migration area of peritoneal macrophages obtained from extract treated groups to the control group was expressed as Macrophage Migration Index [MMI] (Sairam *et al.*, 1997).

The data were analyzed using one way ANOVA and Dunnet's T3 method as the Post-hoc test. All values were expressed as mean ± SEM (n=8). Statistical significance was set at $P< 0.05$ (Snedecor and Cochran, 1976).

RESULTS AND DISCUSSION

There was significant increase in total WBC count, percentage lymphocyte distribution, relative weight of spleen, serum total protein and serum globulin in group II and III compared to group I on 12th and 19th day of experiment (Table 1, Fig. 1). The Amla treated mice also showed significantly higher anti SRBC titre and DTH reaction compared to the control (Table 2, Fig. 2).

Amla is highly nutritious and could be an important dietary source of Vitamin C, minerals and aminoacids. It also contains tannins, phyllaemblic acid, phyllaemblin, rutin and phenolic compounds (Zhang *et al.*, 2000). In the present study, aqueous extract of *Emblica officinalis* has shown promising immunomodulatory activity. The concept of immunomodulation relates to nonspecific activation of the function and efficacy of macrophages,

Table 1: Effect of *Emblica officinalis* on total leukocyte count (x10^3 /cu.mm.), percentage lymphocyte distribution (%), serum total protein (g/dl) serum albumin (g/dl) and serum globulin (g/dl)

Parameters	Groups	Day of experiment			
		0	5	12	19
TLC (x10^3 /cu.mm)	I	6.53 ± 0.87	6.57 ± 0.60	6.80 ± 0.70	7.18 ± 0.72
	II	6.97 ± 0.38	6.68 ± 0.70	9.06 ± 0.43*	11.37 ± 0.42*
	III	6.60 ± 0.70	6.73 ± 0.59	11.67 ± 0.43*	13.90 ± 0.75*
Lymphocyte%	I	72.5 ± 1.15	72.67 ± 1.36	74.33 ± 1.38	75.33 ± 1.19
	II	73.38 ± 1.35	73.67 ± 1.52	81.67 ± 1.51*	83.83 ± 1.42*
	III	73.5 ± 1.24	74.33 ± 1.45	83.83 ± 1.28*	89.33 ± 1.36*
Serum total protein (g/dl)	I	4.04 ±1.62	4.13 ±0.05	4.16±0.06	4.26 ± 0.07
	II	4.22±0.05	4.26 ±0.04	4.32± 0.08	5.48 ±0.09*
	III	4.23±0.07	4.33±0.08	5.09 ±0.06*	6.26±0.07*
Serum albumin (g/dl)	I	2.78 ± 0.09	2.88 ± 0.12	2.89 ± 0.14	2.98± 0.08
	II	2.93 ± 0.08	2.96 ± 0.06	2.98 ± 0.09	3.74± 0.12
	III	2.94 ± 0.05	3.0 ± 0.09	3.53 ± 0.07	4.29± 0.09*
Serum globulin (g/dl)	I	1.26 ±0.06	1.26 ±0.05	1.28± 0.06	1.26±0.07
	II	1.29±0.05	1.28 ±0.04	1.33± 0.08	1.74 ±0.06*
	III	1.29± 0.07	1.27±0.08	1.56±0.06*	1.97± 0.07*

Values are expressed as mean ± S.E.M, n = 8, * $P< 0.05$ compared to control group

Table 2: Effect of *Emblica officinalis* on delayed type of hypersensitivity (mm), macrophage migration area (mm^2) and NBT reduction test

Day of Experiment	Groups	Parameters		
		DTH	Macrophage Migration Area	NBT
12th day	I	0.43 ± 0.02	5.76±1.25	0.31 ± 0.02
	II	1.43 ± 0.05*	10.51± 2.3*	0.33 ± 0.03*
	III	1.83 ± 0.04*	11.86 ± 2.07*	0.42 ± 0.03*
19th day	I	0.56 ± 0.02	6.50± 1.36	0.33 ± 0.05
	II	1.67 ± 0.05*	12.09± 1.95*	0.39 ± 0.02*
	III	1.98 ± 0.06*	15.69± 1.58*	0.48 ±0.04*

Values are expressed as mean ± S.E.M., n = 8, * $P< 0.05$ compared to control group

Figure 1: Effect of *Emblica officinalis* on relative weight of spleen (g/ 100 g bodyweight)

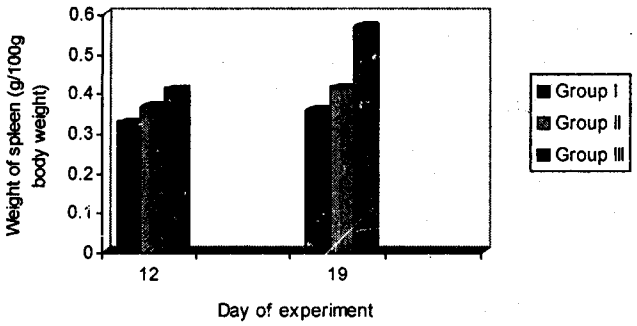
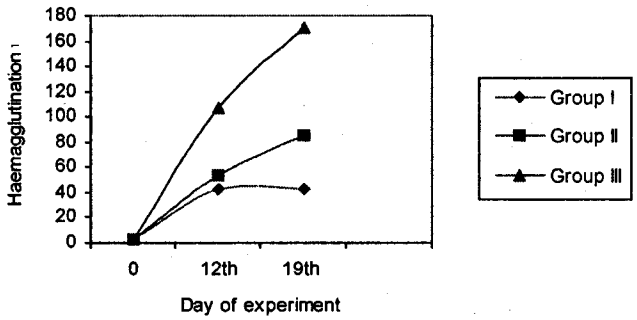


Figure 2: Effect of *Emblica officinalis* on haemagglutination antibody titre



granulocytes, complement, NK cells and lymphocytes and also to the production of various effector molecules generated by activated cells (Jayathirtha and Mishra, 2004). The significant increase in total WBC count and percentage lymphocyte distribution in mice after treatment of Amla can be correlated to its ability to stimulate haemolymphopoietic system. Compared to the control group, Emblica treated groups could produce higher serum protein especially serum globulin that plays a role in maintaining homeostasis, regulating inflammatory response and providing resistance to infection (Kaneko *et al.*, 1997). Spleen being a secondary lymphoid organ containing many phagocytes as well as T and B lymphocytes, increase in spleen weight after Emblica administration can be viewed as increased immunocompetence in the drug treated animals. Increase in DTH activity and circulating HA antibody titre by Emblica treatment in mice can be due to it stimulant effect on cell mediated and humoral immune responses respectively.

Macrophages play a major role in nonspecific and specific immune responses. In innate immunity, the phagocytosis of foreign bodies by macrophages render the first barricade against infection and in acquired immunity, they contribute to the regulation of both humoral and cellular immune responses (Kapil and Sharma, 1997). MMI appears to be a close correlate of macrophage activation and of the status of cell mediated response (Saxena *et al.*, 1991). *Emblica officinalis* caused a significant increase in migration area as well as NBT reduction of peritoneal macrophages in groups II and III compared to group I (Tables 2, 3), indicating macrophage activation upon Emblica administration. Phagocytosis of particles by macrophage is usually accompanied by a burst of the oxidative metabolism leading to the generation of reactive oxygen species (ROS) which can be detected through NBT assay and thus .confirming the intra cellular killing property of phagocytosing macrophage (Nores *et al.*, 1997). The increased NBT reduction of macrophages in drug treated groups also indicated enhanced functioning of macrophage system by Emblica.

Table 3: Effect of *Emblica officinalis* on MMI

Treatment Groups	12th day	19th day
Group II	1.76±0.07	1.82± 0.03
Group III	2.03± 0.09	2.48± 0.04

Values are expressed as mean ± S.E.M., n = 8

Thus, the results of the present study indicated the immunostimulatory activity of *Emblica officinalis* which might be due to its combined action on humoral and cell mediated immune responses along with macrophage phagocyte.

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