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# Evaluation of immunomodulatory effects of methanol extract of *Elaeocarpus ganitrus* seeds

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**Abstract**

The seeds of Rudraksha (*Elaeocarpus ganitrus*) have been traditionally used in India and other Asian countries. The methanolic extract of *Elaeocarpus ganitrus* (EGM) seeds were evaluated for immunomodulatory activity using *in vitro* and *in vivo* methodologies. Effect of the extract at various concentrations (832 - 6.5 µg/ml) on secretion of mediators like nitric oxide, superoxide and lysosomal enzyme from isolated murine peritoneal macrophages were evaluated. The extract showed significant stimulation of release of nitric oxide (at 416, 104, 52 µg/ml), superoxide (at 416, 208 µg/ml) and lysosomal enzyme (at 208, 104 µg/ml). The extract was also evaluated for*in vivo* phagocytic activity by carbon clearance assay in mice and it showed significant increase in the phagocytic index at 100 and 200mg/kg dose. The effect of the extract in delayed type hypersensitivity (DTH) and antibody titre assay were evaluated in ovalbumin immunized mice. The extract showed significant stimulation of DTH response at 200 mg/kg dose and antibody titer at 100 and 200 mg/kg dose. The effect of EGM extract in cyclophosphamide induced myelosuppressed mice was not significant. The results suggest that EGM influences both non-specific (phagocytosis) and specific (cell mediated and humoral) arms of immune system.

**Key words:** *Elaeocarpus ganitrus*; immunomodulation; nitric oxide; superoxide; carbon clearance; delayed hypersensitivity; phagocytosis; ovalbumin; antibody

## 1. Introduction

The immune system evolved to protect the host from potentially pathogenic agents including microorganisms (viruses and bacteria), parasites, and fungi; to eliminate neoplastic cells; and to reject non-self components. The structural and functional alterations of the immune system may lead to immunodepression/ immunosuppression, which may modify the host defense mechanisms against infection, cancer, and induction of abnormal immune responses resulting in allergy and autoimmunity [1]. Immunostimulant drugs are required to overcome the immunosuppression induced by drugs or environmental factors and immunosuppressants are required where there is undesired immunopotentiation. There is strong requirement of the drugs which can boost immune system to combat the immunosupp-

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ressive consequences caused by stress, chronic diseases like tuberculosis, conditions of impaired immune responsiveness (e.g. AIDS) etc [2].

*Elaeocarpus ganitrus* Roxb. commonly known as Rudraksha in India belongs to the Elaeocarpaceae family and grows in the Himalayan region. It is popular for its attractive fruit stones and for medicinal uses [3]. The seed of Rudraksha has been given a very special place and it is credited with mystical and divine properties. Rudraksha beads have been traditionally used in India and other Asian countries. This species name is synonymous with *E. sphaericus,* whose fruits are used in Ayurveda for psycho-somatic diseases, epilepsy, asthma, hypertension, arthritis and liver diseases. Previous studies shows that *E. ganitrus* possesses sedative, hypnotic, tranquillizing, anticonvulsive, antiepileptic and antihypertensive activities. *E. ganitrus* contains quercetin, gallic and ellagic acids, (-) elaeocarpine, (-) iso-elaeocarpine and rudrakine [4, 5]. Till date no systematic scientific immunomodulation studies were documented. Therefore the objective of the present study was to preliminarily evaluate immunomodulatory potential of *E. ganitrus* using its methanol extract which would contain maximum chemical constituents.

## 2. Materials and Methods

### *2.1 Plant material and Preparation of extract*

Authenticated dried seeds of *Elaeocarpus ganitrus* Roxb of forest area near Katmandu, Nepal origin were obtained from Rudraksha Research and Testing Laboratory (RRTL), Mumbai, India. The seeds were collected in July 2008. The voucher specimen was deposited at the Herbarium of department. The seeds were coarse powdered in cutter and grinding mill. The powdered seeds of *Elaeocarpus ganitrus* (1 kg) were extracted twice with methanol (5 liters) by using soxhlet extraction assembly. The extract was then concentrated (450 ml) and then evaporated to dryness at 50°C in hot air oven.

### *2.2 Experimental animals*

Swiss albino mice were obtained from Haffkine Bio-pharmaceuticals Ltd., Mumbai. The animals were acclimatized for 10 days before being used for the experiments. They were housed in a room with controlled temperature (23 ± 2°C) and a 12-h light/ 12-h dark cycle. The animals were fed with standard pellet diet (Amrut brand, Sangli, India) and water *ad libitum*. The experimental protocols were approved by the Institutional Animal Ethics Committee of institute and conducted according to the guidelines of Committee for the Purpose of Control and Supervision on Experiments on Animals (CPCSEA), India. Animal experiments were carried out in CPCSEA registered animal house (87/1999/CPCSEA).

### *2.3 Chemicals*

Ovalbumin, Freund's complete adjuvant and Tetra Methyl Benzedrine/Hydrogen peroxide (TMB/$H_2O_2$) were procured from Bangalore Genei, India. Streptomycin, penicillin, Roswell Park Memorial Institute (RPMI) 1640 medium and HEPES buffer were procured from Himedia Pvt. Ltd. India. Fetal bovine serum (FBS) and PHA (Phytohemagglutinin-M) were procured from Sigma Aldrich (St. Louis, MO, USA). All other chemicals used were of analytical grade.

### *2.4 Isolation of peritoneal macrophage and culture conditions*

Peritoneal macrophages were isolated from mice which were injected intraperitoneally (i.p.) with 2 ml of 4% (w/v) fluid thioglycollate medium 3 days prior to peritoneal lavage with 10 ml of RPMI 1640 medium. The collected cells were washed with RPMI 1640 and cultured in RPMI 1640 supplemented with 10% FBS, 2 mML-glutamine, 100 U/ml penicillin, and

100 mg/ml streptomycin (complete RPMI). The macrophage count was determined by using hemocytometer and cell viability was tested by trypan-blue dye exclusion technique. Then the cells were adjusted to required cell count per ml and plated on a 96-well flat-bottom culture plate (Tarsons Products Pvt. Ltd., India) and then incubated for 2 h at 37°C in a 5% $CO_2$ humidified incubator. After removing the non-adherent cells, the mono-layered macrophages were treated with EGM extract dissolved in complete RPMI medium containing 0.1% DMSO and maintained for 24 h at 37°C in a 5% $CO_2$ humidified incubator [6].

*2.5 Nitrite assay*

Nitrite accumulation was used as an indicator of nitric oxide (NO) production in the medium as previously described by Lee *et al*. Peritoneal macrophages ($5x10^5$ cells/ml) prepared as above were incubated with different concentrations (832 – 6.5 µg/ml) of EGM extract for 24 h at 37°C in 5% $CO_2$ atmosphere. Cell-free supernatant (50 µl) was mixed with 50 µl of Griess reagent (1% sulfanilamide, 0.1% napthylethylenediamine dihydrochloride, and 2% phosphoric acid) and incubated at room temperature for 10 min. The optical density (OD) was measured at 540 nm with a microplate reader (ELX800MS, Bio-tek Instruments Inc., USA). PHA (100 µg/ml) was used as a positive control. Nitrite concentrations were determined from standard curve of sodium nitrite in culture conditions. Stimulation index (SI) was calculated as the nitrite concentrations ratio of the treated and control macrophages [7].

*2.6 In vitro phagocytic assay on nitroblue tetrazolium (NBT) dye reduction*

The NBT dye reduction assay was carried out as described previously. Briefly, 20 µl of the adherent macrophages ($1\times10^6$ cells/ml) and 40 µl of complete RPMI were put in a flat bottom 96-well plate. The EGM extract (20 µl) was added in different concentrations (832 – 6.5 µg/ml). After incubation for 24h at 37°C in humidified 5% $CO_2$, 50 µl of 0.3% NBT solution in PBS (phosphate buffered saline, pH = 7.4) were added and the mixture was further incubated at the same condition. After incubation for 1h, the adherent macrophages were rinsed vigorously with complete RPMI medium, and washed four times with 200 µl methanol. After air-dried, formazan-deposits were solubilized in 120 µl of 2M KOH and 140 µl of DMSO. After homogenization of the contents of the wells, the OD was read at 630 nm by using a microplate reader. Stimulation index (SI) was calculated as the OD ratio of the treated and control macrophages [8].

*2.7 In vitro Phagocytic assay on cellular lysosomal enzyme activity*

The cellular lysosomal enzyme activity of macrophages was evaluated by measuring acid phosphatase activity as described earlier. Briefly, 20 µl of the adherent macrophages ($1\times10^6$ cells/ml) and 40 µl of complete RPMI were put in a flat bottom 96-well plate. The EGM extract (20 µl) was added in different concentrations (832 – 6.5 µg/ml). After incubation for 24h at 37°C in humidified 5% $CO_2$, the medium was removed by aspiration and 20 µl of 0.1% Triton X-100 (Himedia, India) were added to each well. After 15 minutes incubation, 100 ml of 10 mm *p*-nitrophenyl phosphate (*p*NPP) and 50 ml of 0.1 M citrate buffer (pH 5.0) were added. Further the plates were incubated for 1h and 0.2 M borate buffer (150 µl, pH 9.8) was added. The OD was measured at 405 nm by using a microplate reader. The Phagocytic stimulation index (SI) was calculated as the OD ratio of the treated and control macrophages [8].

**Table 1.** *In vivo* effects of EGM extract on phagocytosis (phagocytic index), DTH reaction and antibody titre in ovalbumin immunized mice.

| Treatment group (dose mg/kg) | Phagocytic index (K) | DTH reaction (x $10^{-2}$ mm) | Ovalbumin specific antibody titre (log2 of reciprocal) |
|---|---|---|---|
| Control | 0.062 ± 0.008 | 21.34 ± 1.78 | 11.64 ± 0.36 |
| EGM (50) | 0.073 ± 0.006 | 20.80 ± 1.45 | 11.98 ± 0.56 |
| EGM (100) | 0.092 ± 0.007* | 25.04 ± 1.98 | 13.48 ± 0.48* |
| EGM (200) | 0.097 ± 0.011* | 28.17 ± 2.18* | 15.64 ± 0.36** |

The data is expressed as Mean ± S.E.M; n=6. EGM - methanol extract of *Elaeocarpus ganitrus* seeds; *P<0.05 and **P<0.01 vs control group

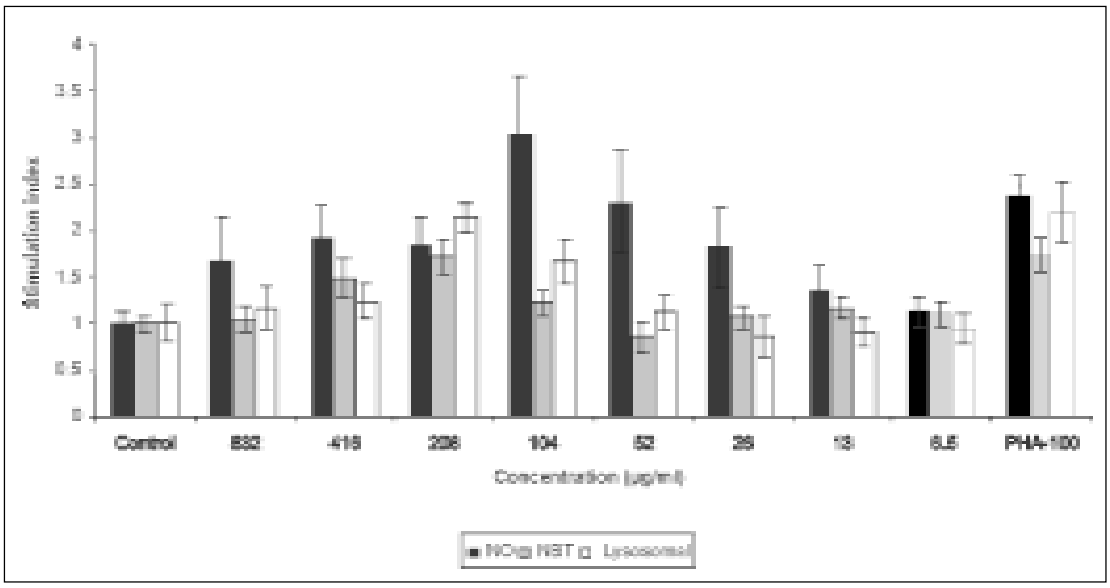


**Fig. 1:** The data plotted represent Mean ± SEM. n=3. * *P*<0.05 and ** *P*<0.01 vs control group.

### *2.8 In vivo phagocytic activity by carbon clearance assay*

Phagocytic activity of EGM extract was determined as per the method reported earlier [9]. Mice were divided into four groups, of six animals each. The control group received vehicle (0.5% NaCMC - sodium carboxy methyl cellulose). Mice in the treatment groups were administered with EGM extract (50, 100 and 200 mg/kg, per oral - p.o.) suspended in vehicle daily for 20 days. Colloidal carbon solution, Rotring ink® (Hamburg, Germany) diluted with normal saline (1:8). Diluted carbon solution was injected (0.01 ml/gm body weight) via tail vein to each mouse 24 h after last dose. Blood samples were drawn from retro-orbital plexus under ether anesthesia at 2 and 15 min after injection. Blood (25 µl) was mixed with 0.1 % sodium carbonate (2 ml) for the lysis of erythrocytes and subjected for determination of

optical densities (OD) at 660 nm. The phagocytic index K, was calculated by using following equation:

$$K= (\ln OD1-\ln OD2) / (T_2-T_1)$$

where OD1 and OD2 are the optical densities at times $T_1$ and $T_2$, respectively.

*2.9 Immunization and treatment*

Mice were divided into 4 groups of six each. The control group received vehicle (0.5% NaCMC) as vehicle; while mice in the treatment groups were administered with the EGM extract (50, 100 and 200 mg/kg, p.o.) in vehicle daily for 20 days. On 14$^{th}$ the animals were immunized subcutaneously (s.c.) with ovalbumin (3 mg) dissolved in normal saline emulsified with equal volume of Freund's complete adjuvant (Bangalore Genei).

*2.10 Measurement of DTH response*

To assess the delayed type hypersensitivity (DTH) response, mice were challenged (s.c.) with 25 µg ovalbumin in 25 µl normal saline in the left hind footpad 7 days after the immunization. The right hind footpad was injected with 25 µl vehicle and served as control. The increase in footpad thickness was measured 24 h after the challenge with the help of a digimatic caliper (Mitutoyo Corporation, Japan) [10].

*2.11 Detection of serum antibody response*

Blood were collected from mice through retro-orbital plexus after 7 days of immunization and serum were separated under centrifugation. Serum antibody titers for quantification of serum IgG to ovalbumin were estimated by using Enzyme Linked Immunosorbent Assay (ELISA) as described earlier [10, 11].

Flat bottom polystyrene plates were coated with 12.5 µg of ovalbumin dissolved in 100 µl of sodium carbonate buffer (pH 9.6) at 4°C for 12h. The coated plates were washed three times with phosphate buffer saline (0.15M, pH 7.2) containing 0.05% TWEEN-20 (PBS-Tw). The wells were incubated with 100 µl of 1% BSA (Bovine serum albumin) in sodium carbonate buffer at 37°C for 1 h. Serial dilutions of mouse serum samples in PBS-Tw were prepared and 100 µl was incubated with coated wells for 1 h at 37°C. After washing, diluted (1:2000) antimouse IgG conjugated with peroxidase (100 µl) was added and the plates were incubated at 37°C for 1 h. The enzyme activity was determined by addition of TMB/$H_2O_2$. The enzyme reaction was stopped by addition of 50 µl, 8N sulphuric acid and the absorbance was measured at 450 nm. Endpoint antibody titers were expressed as log2 of the reciprocal of the highest dilution of the test serum samples showing three times more optical density (OD) as compared with control samples.

*2.12 Cyclophosphamide induced Myelosuppression*

This method was studied according to the procedure described earlier [12] with some modifications. Mice were divided into five groups of six each. The control groups (vehicle and negative) received vehicle (0.5% NaCMC); while animals in treatment groups were given the EGM extract (50, 100 and 200 mg/kg, p.o.) in vehicle daily for 19 days. On days 17, 18, 19 all the animals except in the vehicle control group were injected with cyclophosphamide (30mg/kg, i.p.) 1 h after administration of the extract or vehicle. Blood samples were collected on day 20 and total white blood cell (WBC) count was determined using hematology analyzer (Arcus, Diatron, Wien Austria).

*2.13 Statistical Analysis*

Results are expressed as Mean ± SEM. Data were analyzed by one way ANOVA followed by Dunnet's test for multiple comparisons with $P<0.05$ as the criteria for significance.

## 3. Results

### *3.1 Extraction efficiency*

The extraction efficiency of *Elaeocarpus ganitrus seeds* in methanol using soxhlation method was approximately 20.5% w/w. The methanol extract of *Elaeocarpus ganitrus* (EGM) was stored at 4°C till further use.

### *3.2 Nitrite assay on isolated peritoneal macrophages*

The ability of macrophages to induce the release of nitric oxide (NO) was tested, measured through nitrite, which is a stable breakdown product of NO. The nitrite level (nitric oxide) produced in cell culture supernatants was measured at 24 h of treatment, showing that EGM extract induced nitrite production in statistically significant higher amounts at 416 (SI=1.94), 104 (SI=3.04) and 52 µg/ml (SI= 2.31) concentrations than controls in all three experiments performed. PHA (positive control) also showed significant increase in nitrite release (SI=2.36). High NO production is an indication of macrophage activation (Fig. 1).

### *3.3 In vitro phagocytic assay on nitroblue tetrazolium (NBT) dye reduction and cellular lysosomal enzyme activity*

The *in vitro* phagocytic effects of different concentrations of EGM on the reduction of NBT dye and cellular lysosomal enzyme activity of macrophages are presented in Fig. 1. Significant stimulation of NBT reduction was observed at 416 (SI=1.48) and 208 µg/ml (SI=1.71) of EGM extract with respect to control (SI=1). Release of cellular lysosomal enzyme was found to be significant at 208 (SI=2.14) and 104 µg/ml (SI=1.67) for EGM with respect to control (SI=1). Positive control, PHA also showed significant stimulation of NBT reduction (SI=1.73) and lysosomal enzyme release (SI=2.19).

### *3.4 In vivo phagocytic activity by carbon clearance assay*

Macrophages accomplish nonspecific immune function through phagocytosis. *In vivo* phagocytic activity of EGM extract was determined by the carbon clearance assay in mice. The results of this assay are presented in Table 1. The phagocytic index (K) for the EGM extract was significantly higher ($P$<0.05) at 100 mg/kg (48.39%) and 200 mg/kg (56.45%) dose levels as compared to control group.

### *3.5 DTH response in ovalbumin immunized mice*

To examine effect of EGM on cellular immune system, its activity was investigated as DTH reaction to ovalbumin immunized mice. The effect of EGM on T cell mediated DTH response i.e. difference in footpad thickness of mice is shown in Table 1. The EGM extract has shown significant increase ($P$<0.05) in the DTH response to ovalbumin 200 mg/kg (59.69%).

### *3.6 Detection of serum antibody response to ovalbumin*

Humoral response to ovalbumin was studied by ELISA antibody titer assay. Mice treated with different doses of the EGM extract showed an increase in the antibody titer in a dose dependent manner. There was significant increase in serum antibody titer at 100 (16%) ($P$<0.05) and 200mg/kg (34%) ($P$<0.001) dose as compared to control group values (Table 1).

### *3.7 Cyclophosphamide induced Myelosuppression*

There was significant reduction ($P$<0.01) in total WBC count of cyclophosphamide (CYP) (30 mg/kg) treated mice (6.02 $x10^3$ cells/cmm) as compared to vehicle control group (14.43$x10^3$ cells/cmm). No significant increase in total WBC count were observed with EGM (50, 100 or 200 mg/kg) and CYP treated groups. The values of total WBC count for these three groups are

$6.94x10^3$; $8.21x10^3$ and $9.38x10^3$ cells/cmm respectively.

## 4. Discussion

Immunomodulating activity refers to biological or pharmacological effects of compounds on humoral or cellular aspects of the immune response. The human response is a highly complex and extraordinarily sophisticated system involving both innate and adaptive mechanisms. Studies of how plant substances affect immune response employ mechanistic bioassay methodologies [13]. Modulation of the immune response through stimulation or suppression may help in maintaining a disease-free state. Agents that activate host defense mechanisms in the presence of an impaired immune responsiveness can provide supportive therapy to conventional chemotherapy [14]. There is a growing interest in identifying herbal immunomodulators ever since their possible use in modern medicine has been suggested [15]. The main objective of the study was to investigate the preliminary immunomodulatory effects of seeds of *Elaeocarpus ganitrus* Roxb (EGM) using its methanol extract. The results mentioned above have demonstrated that EGM possesses potent immunostimulant property.

Macrophages play an important role in innate and acquired immunity. They are the first cells to participate in the immune response, and can be activated by a variety of stimuli. Their principal functions include the phagocytosis of foreign particles and the production of cytokines and reactive oxygen species (ROS) and nitrogen species (RNS) involved in the destruction of pathogens [16]. Nitric oxide (NO) has been shown to be the principal effector molecule produced in macrophages by inducible nitric oxide synthase (iNOS) for cytotoxic activity and can be used as a quantitative index of macrophage activation [17, 18]. Agents that modulate the activity of NO may be of considerable therapeutic value [19]. NO mediates diverse functions, including vasodilatation, neurotransmission, antithrombotic and inflammation [20, 21]. The methanol extract of *E. ganitrus* (EGM) has shown significant increase in release of nitric oxide from macrophages. This effect of EGM was not dose-proportionate. The released NO could be toxic to immune and inflammatory cells as well as microorganisms and tumour cells. Thus, NO might be regarded as both immune effector and regulatory molecules [22]. Excess production of NO is associated with various diseases such as diabetes, arthritis and other chronic inflammatory diseases, autoimmune diseases, septic shock, and atherosclerosis [23]. Nitric oxide has been reported as an important mechanism for macrophages against microorganisms [24].

It is well known that macrophages play an important role in defense mechanism against host infection and in killing tumour cells. A higher reduction in NBT dye represents a higher activity of oxidase enzyme, reflecting stimulation of phagocytes in proportion to intracellular killing. For lysosomal enzyme activity, the transformation of *p*-NPP to coloured compound by the acid phosphatase of the stimulated macrophages correlates to the extent of degranulation in phagocytisis [25]. Phagocytosis of particles by macrophages is usually accompanied by a burst of oxidative metabolism allowing the generation of reactive oxygen species which can be detected through an assay based on the reduction of NBT [26]. The effects of various concentrations of methanol extract of *E. ganitrus* (EGM) on the reduction of NBT dye and lysosomal enzyme activity response of macrophages were studied for phagocytic assay. EGM extract appeared to produce phagocytic modulation without dose response relationship in these experiments.

Murine isolated peritoneal macrophages incubated with the EGM extract at different concentrations ranging between 832-6.5 µg/ml for 24h, showed a significant activation of macrophages by modulating the secretion of various mediators including oxide (NO), lysosomal enzyme and superoxide ($O^-$) ions. This suggests that EGM can effectively strengthen innate immunity against foreign particles [27].

Phagocytosis is the process by which certain body cells, collectively known as phagocytes, ingest and removes microorganisms, malignant cells, inorganic particles and tissue debris [28]. Phagocytosis and killing of invading microorganisms by macrophages constitute body's primary line of defense. Macrophages are an integral part of the immune system, acting as phagocytic, microbicidal and tumoricidal effector cells [29]. Through interaction with lymphocytes, macrophages play an important role in the initiation and regulation of immune response. Macrophages also present antigen to lymphocytes during the development of specific immunity and serve as supportive accessory cells to lymphocytes. When activated, macrophages increase the phagocytic activity and release various materials such as cytokines and reactive intermediates and then carry out nonspecific immune responses [30]. In view of the pivotal role played by the macrophages, EGM extract was also evaluated for its *in vivo* effect on macrophage phagocytic activity. The methanol extract of *E. ganitrus* (EGM) was treated to the experimental animals. The increase in carbon clearance i.e. phagocytic index by EGM extract (100 and 200 mg/kg) reflects the enhancement of phagocytic function of mononuclear macrophage and thus non-specific immunity. This indicates that EGM was able to activate murine peritoneal macrophages, and hence phagocytic assays *in vitro* and *in vivo.*

Cell-mediated immunity (CMI) involves effector mechanisms carried out by T lymphocytes and their products (lymphokines). CMI responses are critical to defense against infectious organisms, infection of foreign grafts, tumour immunity and delayed-type hypersensitivity reactions (DTH) [12, 28]. DTH requires the specific recognition of a given antigen by memory Th1 cells, which subsequently proliferate and release cytokines. In turn, the cytokines increase vascular permeability, induce vasodilatation and accumulation of inflammatory cells (neutrophils, macrophages) [10]. The significant increase in DTH response in response to ovalbumin, T cell dependent antigen revealed the stimulatory effect of EGM extract (200mg/kg) on lymphocytes and on other accessory cell types required for the expression of the reaction.

Antibody titer was determined to establish the humoral response against ovalbumin, a T cell dependant antigen. It is well documented that T-B cognate interaction provides an optimal signal for B cell differentiation and antibody production towards T-dependent antigen [31]. The augmentation of the humoral immune response to ovalbumin by EGM (100 and 200 mg/kg), as evidenced by increase in the serum antibody titre (log 2 of the highest dilution) in mice indicated the enhanced responsiveness of T and B lymphocyte subsets, involved in the antibody synthesis.

Cyclophosphamide (CYP), extremely potent alkylating agent, is used in cancer chemotherapy. Upon cellular uptake, it is extensively metabolized to form the active compounds, phosphoramide mustard and acrolein [32]. Reaction of the phosphoramide mustard with DNA is considered to be the cytotoxic step. The cross-linking of phosphoramide with guanine bases in DNA double-helix strands makes the strands unable to uncoil and separate [33]. As this is necessary in DNA replication, the cells

can no longer divide and results disruption of DNA function and cell death. This confers cyclophosphamide, the ability to kill rapidly dividing cancerous cells, bone marrow cells and stimulated lymphocytes [34]. In the present study, administration of cyclophosphamide caused bone marrow suppression as evident by significant decrease in total WBC (leukocytes) counts as compared to vehicle control group. Concomitant treatment of EGM extract in CYP treated mice resulted in increase in total WBC count of mice in dose dependent manner till 400 mg/kg dose but EGM treatment was not able to restore the WBC levels to normal. This suggests that further studies at higher dose levels and/or therapeutic treatment studies needs to be carried out to investigate possible effects of EGM in myelosuppressive conditions.

The immunomodulatory activity of *Elaeocarpus ganitrus* could be attributed to various phytoconstituents present in it and as it contains mainly alkaloids, they might play major role.

## 5. Conclusion

The studies have demonstrated *in vitro* and *in vivo* immunostimulating properties of the methanol extract of *Elaeocarpus ganitrus seeds* (EGM). The results suggest that EGM influences both non-specific (phagocytosis) and specific (cell mediated and humoral) immune system. This suggests its therapeutic usefulness. Further detailed studies are required to identify the active constituents and their mechanism for this effect. This might establish its possible use in immunocompromised conditions.

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