EVALUATION OF THE IMMUNOMODULATORY EFFECT OF *Emblica officinalis* (AMLA) FRUIT PULP EXTRACTS IN MICE

SUJA RANI. S.

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Department of Pharmacology and Toxicology
COLLEGE OF VETERINARY AND ANIMAL SCIENCES
MANNUTHY, THRISSUR - 680651
KERALA, INDIA

DECLARATION

I hereby declare that the thesis entitled **"EVALUATION OF THE IMMUNOMODULATORY EFFECT OF *Emblica officinalis* (AMLA) FRUIT PULP EXTRACTS IN MICE"** is a bonafide record of research work done by me during the course of research and that this thesis has not previously formed the basis for the award to me of any degree, diploma, associateship, fellowship or other similar title, of any other University or Society.

Mannuthy

SUJA RANI. S

CERTIFICATE

Certified that this thesis, entitled **"EVALUATION OF THE IMMUNOMODULATORY EFFECT OF *Emblica officinalis* (AMLA) FRUIT PULP EXTRACTS IN MICE"** is a record of research work done independently by **Dr. Suja Rani. S.,** under my guidance and supervision and that it has not previously formed the basis for the award of any degree, diploma, associateship or fellowship to her.

Mannuthy

Dr. A.M. Chandrasekharan Nair,
(Chairman, Advisory Committee)
Associate Professor,
Department of Pharmacology and Toxicology,
College of Veterinary and Animal Sciences,
Mannuthy.

CERTIFICATE

We, the undersigned, members of the Advisory Committee of **Dr. Suja Rani. S.**, a candidate for the degree of **Master of Veterinary Science in Pharmacology and Toxicology,** agree that the thesis entitled **"EVALUATION OF THE IMMUNOMODULATORY EFFECT OF *Emblica officinalis* (AMLA) FRUIT PULP EXTRACTS IN MICE"** may be submitted by **Dr. Suja Rani. S.,** in partial fulfilment of the requirement for the degree.

Dr. A.M. Chandrasekharan Nair,
(Chairman, Advisory Committee)
Associate Professor,
Department of Pharmacology and Toxicology,
College of Veterinary and Animal Sciences,
Mannuthy.

Dr. N.Gopakumar,
Associate Professor and Head,
Department of Pharmacology and Toxicology
(Member)

Dr. V.Jayaprakasan,
Associate Professor and Head,
Department of Microbiology.
(Member)

Dr. P.T.A. Usha,
Assistant Professor,
Department of Pharmacology and Toxicology.
(Member)

3/12/03

Dr. K.V. Venkateswaran

EXTERNAL EXAMINER

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Introduction

1. INTRODUCTION

Research on medicinal plants is one of the most rapidly developing areas of biomedical research in India. Several herbal preparations used in the indigenous system of medicine can cure various ailments in humans and animals (Brekhman and Dardimov, 1969). The modulation of immune response as a possible therapeutic measure by using medicinal plant products has become a subject of active scientific investigations. The basic concept has, however, existed in the ancient vedic scripture, the Ayurveda and has been practiced in Indian traditional medicine for many centuries. The two main approaches to illness in Ayurveda are 'preventive' and 'curative', the major approach is one which emphasizes prevention. Thus it aims to increase body's natural resistance to the disease causing agent rather than directly neutralizing the agent itself (Atal, 1985). In practice, this is achieved by using extracts of various plant material called 'rasayanas' (Upadhyay, 1997). In terms of Charaka, by using rasayana therapy, one obtains longevity, regains youth, freedom from disease etc. (Charaka, 1949).

The immune system, which plays an important role in biological adaptation, is contributing to the maintenance of homeostasis and to the establishment of body integrity. Moreover, it is known to be involved in the etiological as well as pathophysiological mechanism of many diseases. The recovery from any infective process is mainly influenced by the host defence mechanism, and there are a lot of factors that exert negative influence on the body resistance, posing challenge to the immune system. To counteract this, certain compounds have been tried and tested with varying degrees of success. Compounds that cause pharmacological manipulation of immune function are the "immunomodulators". Depending upon the capability of stimulating or suppressing the immune response, immunomodulators are classified as "immunostimulants" or immunosuppressants". The function and efficacy of immune system may be influenced by many exogenous and endogenous factors like food, pharmaceuticals, physical and psychological stress, hormones etc. resulting in either immunosupression or immunostimulation. The healthy state is

believed to be based on a sophisticated fine tuning of immune regulatory mechanism (Patwardhan *et al.*, 1990). The development of immunomodulatory agents, natural or synthetic, to stimulate natural defence mechanism of the body and restore the original immune functions is a focus of intense study.

The rationale for the development of immunostimulants is to provide benefits to immunocompromised or immunodeficient patients. These agents are indicated for conditions like chronic infections, lymphatic disorders and immune deficiency states. On the other hand, conditions like organ transplant, tissue grafting and treatment of auto immune diseases call for the use of immunosuppressants. Apart from being specifically stimulatory or suppressive, certain agents have been shown to possess activity to normalize or modulate pathophysiological process and hence called as true immunomodulatory agents (Wagner, 1983). The concept of immunomodulation relates to specific and nonspecific activation of immune system primarily a nonspecific stimulation (antigen independent) of the function and efficacy of macrophages, granulocytes, complement, NK cells, lymphocytes and also the production of various effector molecules by activated cells. These agents can produce an effect either through cellular, humoral or both the systems. Being non specific, it is expected to give protection against different pathogens including bacteria, fungi, virus etc. and constitutes an alternative or adjunct to conventional chemotherapy (Saraf and Bhide, 1983).

In recent years , a number of traditional medicinal plants have been identified as promising source of immunomodulators that will have a great deal of interest in enhancing the immune system and a few of them are *Ocimum sanctum, Picrorrhiza kurroa, Tinospora cordifolia, Nyctanthes arbor-tristis, Mangifera indica, Azadirachta indica, Withania somnifera, Boerhaavia diffusa* etc. (Atal,1985; Atal *et al.*, 1986; Godhwani *et al.*, 1988; Puri *et al.*, 1994; Sharma *et al.*, 1994; Ziauddin *et al.*, 1996). Compared to synthetic drugs, herbal drugs are less expensive and easily available. In the case of synthetic drugs, their adverse side effects and occasional toxicities overshadow their potency and thereby limit their wide usage.

Though the concept of using immunomodulators in human medicine for augmenting the immune function is being accepted in practice in many quarters, in veterinary medicine, it is still at a very preliminary stage. The dose, timing, route of administration and presence of concurrently administered compounds are important factors that may influence the action of a drug–host defence mechanism. In addition, animal factors such as age, degree of passive immunity, plane of nutrition and the disease process itself, may have an impact on the efficacy of immunomodulator.

Fruits of *Emblica officinalis*, commonly known as "Amla" or the "Indian gooseberry" a member of genus Emblica (family Euphorbiacea), is extensively used in Indian Ayurvedic and Sidha system of traditional medicine for the treatment of wide spectrum of diseases (Tiwari *et al.*, 1968; Thakur *et al.*, 1988). It is considered as a panacea in Indian system of medicine. Several experimental works have been done in laboratory animals in which *Emblica officinalis* was used either as its alcoholic or aqueous extract or as its preparations like Chyavanaprash, Rasayana, Triphala, Septilin etc. Some of the properties of fruit experimentally proved include hepatoprotective, antioxidant, antidiabetic, anticlastogenic, antibacterial, antipyretic, antifungal etc. (Dhir *et al.*, 1991; Dhir *et al.*, 1993; Gulati *et al.*, 1995; Vormisto *et al.*, 1997; Nandi *et al.*, 1997; Ahmed *et al.*, 1998).

Despite its extensive medicinal use, limited knowledge is available regarding the role in modulation of immune system. Therefore, the present study was designed to evaluate the role of Emblica on immunomodulation in mice, immunized with sheep red blood cell. The active principles present in the extracts of *Emblica officinalis* fruit pulp were also tested in the study.

The modulatory effect of *Emblica officinalis* on the immune system was assessed by observing the following parameters.

a) Changes in absolute and differential leukocyte count.

b) Total serum protein and globulin.

c) Qualitative changes in the specific antibody activity of the serum.

d) Alteration in the migration property of macrophage.

e) Qualitative change in the oxidative property of macrophage.

f) Delayed hypersensitivity reaction.

g) Changes in body weight and relative organ weights.

Review of Literature

2. REVIEW OF LITERATURE

2.1 PLANT IN GENERAL

Emblica officinalis (*Phyllanthus emblica*) is a perennial plant widely used in many of the indigenous medicinal preparations against a variety of conditions (Thakur *et al.*, 1988).

Xia *et al.* (1997) quoted that there are 17 countries in the world using various parts of *Phyllanthus emblica* in their medical treatment. The plant is found good in the regulation of gastric function and possess hepatoprotective, anticancer and antiinflammatory activities. It is regarded as a traditional immunomodulator and a natural adaptogen .

Emblica officinalis is a constituent of several widely used preparations such as Chyavanaprash, Brahma Rasayana, Triphala, Septilin etc. (Khandelwal *et al.*, 2002).

2.2 PHYTOCHEMICAL STUDIES ON *Emblica officinalis*

Pillay and Iyer (1988) reported that the components present in *Emblica officinalis* included ascorbic acid, tannins, flavonoids etc. The ascorbic acid present in the plant was associated with polyphenols like ellagic acid and phyllaemblic acid and its reducing activity was heat stable.

Ghosal *et al.* (1996) observed that L-ascorbic acid was absent in the fruits of *Emblica officinalis* and the potent Vitamin C like activity had been located in the low molecular weight hydrolysable tannins which included emblicanin A, emblicanin B, punigluconin and pedunculagin.

Emblica officnalis contained several polyphenolic compounds including tannins. These polyphenols and their glycosides were responsible for the antioxidant and anticarcinogenic activity of *Emblica officinalis.* Although *E. officinalis* had been reported to contain ascorbic acid, it had only a minor role in the biological activity of its fruit (Jose *et al.*, 1997).

The pericarp of both bigger and smaller varieties and freeze dried powder of amla fruit were analysed for Vitamin C content by O-phenylene diamine fluorimetric method and was found to contain 2.915(±0.1), 3.775(±0.15) and 23.24(±0.18) mg of Vitamin C per gram of pulp/ powder respectively (Shishoo *et al.*, 1997).

Bhattacharya *et al.* (1999) reported that amla fruits had ascorbic acid conjugated to gallic acid and reducing sugars, forming a tannoid complex which was more stable. It had been suggested that due to an internal mechanism tannoid complex liberated the nascent ascorbic acid into the body and these tannoids were responsible for the antioxidant activity of *E. officinalis*.

Zhang *et al.* (2000) isolated three ester glycosides named phyllemblicins A, B and C and a methyl ester from the roots of *Phyllanthus emblica* along with 15 tannins and related compounds.

Ascorbic acid and other polyphenols present in the extracts of amla showed much superior antioxidant activity, compared to their equivalent amounts in pure isolated forms (Khopde *et al.*, 2001).

Rajeshkumar and Kuttan (2001) observed that the methanol fraction of 50 g *Emblica officinalis* fruit powder gave 17g concentrated extract and vacuum evaporation of ethyl acetate phase yielded 3.2 g of poly phenol fraction.

Six new phenolic constituents , L-malic acid, 2-O-(1) mucic acid, 2-O-(5) mucic acid, 1,4- lactone2-O-(6), 5-O-(8), 3-O-(10) and 3,5-di-O-(11) gallates and its methyl esters were isolated from the fresh juice of *Phyllanthus emblica* (Zhang *et al.*, 2001).

Zhang *et al.* (2001) isolated six new ellagitannins, Phyllanemblins A-F(1-6) from *Phyllanthus emblica*, along with 30 known tannins and related compounds. Their structures were also determined by spectral and chemical methods.

Three novel sesquiterpenoids, phyllaemblic acids B and C and phyllaemblicin D together with two new phenolic glycosides were isolated from the roots of *Phyllanthus emblica* (Zhang *et al.*, 2001).

The flavonoids isolated from *Emblica officinalis* effectively reduced lipid levels in serum and tissues of rats having hyperlipidaemia. The hepatic Hydroxy Methyl Glutaryl CoA reductase activity was also inhibited (Anila and Vijayalakshmi, 2002).

Gas chromatographic / mass spectrometric analysis identified pyrogallol, both in unfractionated and n-butanol fraction of *Emblica officinalis* extracts. This active principle showed antiproliferative effects on human tumor cell lines. (Khan *et al.*, 2002).

Zhang *et al.* (2002) isolated two new acylated flavonone glycosides from the leaves and branches of *Phyllanthus emblica* together with a new phenolic glycoside as well as 22 known compounds. Their structures were determined by spectral and chemical methods.

2.3 IMMUNOMODULATORS OF PLANT ORIGIN

2.3.1 Positive Immunomodulators

Godhwani *et al.* (1988) reported that methanolic and aqueous extract of *Ocimum sanctum* leaves stimulated humoral immunity as evidenced by an increase in antibody titre and cellular immunity showed by E-rosette formation and lymphocytosis.

Azadirachta indica at á dose of 100mg/kg enhanced the humoral antibody response in rats immunized with Sheep Red Blood Cells (SRBC). It facilitated foot pad thickness response to SRBC in sensitized mice and also enhanced leucocyte migration in immunized rats (Sen *et al.*, 1992).

The extract of *Viscum album* and *Dentropthia falcata* was found to increase total WBC count and polymorphonuclear cells in mice. It also enhanced antibody

forming cells and the antibody titre indicating immunostimulant activity (Kuttan and Kuttan, 1992; Mary *et al.*, 1993).

Puri *et al.* (1993) reported that ethanolic extract and purified diterpene andrographolide of *Andrographis paniculata* induced significant stimulation of antibody and delayed hypersensitivity response to SRBC in mice. They stimulated non specific immune response also, showed by an increase in macrophage migration index.

Pippali rasayana, an Ayurvedic herbal medicine, prepared from *Piper longum* and *Butea monosperma* induced significant activation of macrophages as evidenced by increased macrophage migration index and phagocytic activity in mice infected with *Giardia lamblia* trophozoites (Agarwal *et al.*, 1994).

Effect of Rasayanas on cellular immune responses were studied by Kumar *et al.* (1994;1999) and found that administration of Brahma Rasayana , Amrutha Prasham and Narasimha Rasayana enhanced the proliferation of lymphocytes in response to mitogen induced proliferation of bone marrow cells in culture and enhanced NK cell activity.

Puri *et al.* (1994) evaluated the immunostimulatory potential of *Nyctanthes arbor-tristis* in mice fed with 50 percent ethanolic extracts of seeds, flowers and leaves of this plant. An increase in humoral and delayed hypersensitivity response to SRBC and macrophage migration index were noticed.

Fifty percent ethanolic extract of *Picrorrhiza kurroa* leaves stimulated the cell mediated and humoral components of the immune system as well as phagocytosis in mice (Sharma *et al.*, 1994).

Subramaniam *et al.* (1996) found that *Janakia arayalpathra* root suspension (JARS) stimulated immune system in mice at a dose rate of 500mg/kg. It elicited an increase in humoral antibody titre and antibody secreting spleen cells. It also enhanced SRBC induced DTH reaction in mice.

The extracts of Ashwagandha (*Withania somnifera*) in mice showed an increase in WBC count, platelet count and haemagglutinating antibody response towards SRBC. It provided protection from cyclophosphamide induced immunosupression. (Ziauddin *et al.*, 1996; Agarwal *et al.*, 1999).

Kapil and Sharma (1997) reported that the active principles of *Tinospora cordifolia* like syringin and cordiol significantly increased Ig G antibodies in serum. The other active principles like cordioside, cordiofolioside A and cordiol were found to activate macrophages.

The alkaloidal fraction of *Boerhaavia diffusa* possessed immunostimulatory activity in vivo without an in vitro effect. It enhanced responsiveness of macrophages and B-lymphocyte subsets involved in antibody synthesis leading to increased antibody responsiveness to SRBC (Mungantiwar *et al.*, 1997; 1999).

Sairam *et al.* (1997) found that a volatile fraction of neem oil, NIM-76 increased the polymorphonuclear leukocyte count with a concomitant decrease in lymphocyte count. It enhanced macrophage activity while the humoral component of immunity was unaffected.

Jayadev *et al.* (1998) tested the immunostimulant activity of *Centella asiatica* in rats and found that the extract at the dose rate of 100mg/kg/day orally for seven consecutive days resulted in an increased haemagglutination titre and SRBC induced delayed hypersensitivity response.

Curcumin, an active ingredient present in *Curcuma longa* increased the total WBC count, circulating antibody titre, plaque forming cells in the spleen and macrophage phagocytic activity in mice after immunization with SRBC. Bone marrow cellularity and α esterase positive cells were also enhanced (Antony *et al.*, 1999).

The water and ethanol extracts of stems of *Tinospora cordifolia* and *Tinospora sinensis* inhibited immunosuppression produced by cyclophosphamide. Plaque forming cells in the spleen and circulating antibody titre were increased along

with enhanced macrophage activation upon *T. cordifolia* administration (Mathew and Kuttan, 1999; Manjrekar *et al.*, 2000).

Dikshit *et al.* (2000) revealed that Imunocin® (a herbal preparation containing *Withania somnifera, Ocimum sanctum, Piper longum, Azadirachta indica, Curcuma longa, Tinospora cordifolia* and *Boerhaavia diffusa*) significantly increased SRBC induced delayed type hypersensitivity and humoral antibody titre and thus stimulated both cellular and humoral immunity.

Muruganandan *et al.* (2000) showed that the ethanolic extract of *Asparagus racemosus* stimulated both humoral and cell mediated immunity as evidenced by the increased haemagglutination titre and increased delayed type of hypersensitivity .

Qiu *et al.* (2000) noted that modified *Aloe barbadensis* polysaccharide derived from Aloe gel had high immunostimulatory activity as evidenced by the increased Tumor necrosis factor-α (TNFα) release from mouse peritoneal macrophage. It showed higher upregulation of immune function gene expression.

Immunomodulatory potential of the aqueous extract of *Plantago ovata* was evaluated by Rezaeipoor *et al.* (2000) and found that the oral administration and intraperitoneal injection of the extract at a dose of 0.25 and 0.5 g/kg body weight resulted in significant increase in White Blood Cells. The spleen leukocyte count and spleen weight were also increased.

Administration of alcoholic extract of *Mangifera indica* stem bark produced increase in humoral antibody titre and delayed hypersensitivity in mice and concluded that the extract was a promising immunostimulant (Makare *et al.*, 2001).

Colic *et al.* (2002) showed that the aqueous and ethanolic extract of garlic modulated lymphocyte proliferation, triggered by the potent T cell mitogen, depending on the type and dilutions of extracts and concentrations of Concavalin A.

Abrin, a lectin obtained from *Abrus precatorious* increased total WBC count, weight of spleen and thymus, circulating antibody titre, antibody forming cells, bone

marrow cellularity and α esterase positive bone marrow cells when administered at a nontoxic dose of 1.25microgram/kg body weight consecutively for 5 days in normal mice (Ramnath *et al.*, 2002; Tripathi and Maiti, 2003).

Hafeez *et al.* (2003) studied the immunomodulatory activity of aqueous extract of *Trigonella foenum graecum* L and found that administration of 50 and 100mg/kg of extract increased relative organ weight of thymus and liver, cellularity of thymus and bone marrow, delayed hypersensitivity response, plaque forming cells, haemagglutination titre and phagocytic index of macrophages.

Pereza *et al.* (2003) evaluated the immunomodulatory activity of *Ginkgo biloba* extract (EGb 761) and found that it had stress alleviating property through its modulation of immune system. It possessed antioxidant activity which contributed to its immunostimulatory property.

2.3.2 Negative Immunomodulators

Cannabinol and delta-8-tetrahydro cannabinol, two cannabinoid marihuana compounds suppressed delayed hypersensitivity response in a slight to moderate level (Levy and Heppner, 1978).

Benencia *et al.* (1995) observed that human peripheral blood monocytes and polymorphonuclear leukocyte treated with aqueous extract of *Cedrela tubiflora* leaves showed a diminution of both phagocytic and respiratory burst activities. Inhibition of proliferation of Concavalin A stimulated lymphocytes and decrease in haemolytic capacity of the human complement were also noticed.

Albezzia lebbeck extract administration in mice at a dose of 25mg/kg body weight for 7 days resulted in a mitogenic unresponsive cell mediated immunity showing immunosuppressive property of the drug in mice (Baruah, 1999).

Rezaeipoor *et al.* (2000) observed a decrease in haemagglutination antibody titre in mice when *Plantago ovata* was administered orally, indicating suppression of humoral immunity.

Diethyl ether extract of the rhizomes of *Picrorrhiza scrophulariflora* showed potent inhibitory activity towards the classical pathway of the complement system, the respiratory burst of activated polymorphonuclear leukocytes and mitogen induced proliferation of T lymphocytes, thus indicated its immunosuppressive activity (Smit *et al.*, 2000).

Ganguli *et al.* (2001) reported that Tylophora alkaloids inhibited the proliferation of splenocytes at higher concentrations and augmented the same at lower concentrations and thus showed a biphasic effect. It inhibited delayed hypersensitivity of SRBC and contact sensitivity to Dinitrochlorobenzene (DNCB).

Lakshmana *et al.* (2001) observed that E-721 B, an indigenous herbal combination inhibited mast cell degranulation induced by antigen and compound 48/80. It suppressed the production of reagenic antibody (Ig E) and thus showed therapeutic effect in allergic reactions.

Oral administration of *Solanum melongena* aqueous extract significantly inhibited passive cutaneous anaphylactic reaction and histamine release when induced. It had significant inhibitory effect on Ig E induced Tumor Necrosis factor secretion from rat peritoneal mast cells (Mi-Lee *et al.*, 2001).

Oral administration of water soluble fraction of *Terminalia chebula* inhibited passive cutaneous anaphylaxis. It also significantly inhibited histamine release from rat peritoneal mast cells by compound 48/80 and thus exhibited strong antianaphylactic action (Shina *et al.*, 2001).

Jiang and Xu (2003) observed that the aqueous extract from the rhizome of *Smilax glabra* acted as a therapeutic agent for immunoinflammatory diseases through selectively suppressing and modulating cellular immune response involved in inflammation. The agent down regulated the function of over activated macrophages and upregulated the dysfunctional T Lymphocytes from adjuvant arthritic rats.

Buck wheat grain extract (BGE) showed antiallergic action by the inhibition of histamine release and cytokine gene expression in the mast cells (Kima *et al.*, 2003).

The ethanolic extract of *Acorus calamus* rhizome inhibited proliferation of mitogen and antigen stimulated human peripheral mononuclear cells. It also inhibited production of nitric oxide (NO), Interleukin-2 (IL-2) and tumor necrosis factor-α (TNFα) (Mehrotraa *et al.*, 2003).

2.4 IMMUNOMODULATORS OTHER THAN PLANT ORIGIN

Bovine serum albumin when covalently conjugated with dodecanoic acid, stimulated Delayed Type of Hypersensitivity (DTH) and suppressed the antibody production. Stimulation of DTH was correlated with the covalent conjugation of a large number of hydrophobic group and suppression of antibody stimulation was correlated with covering of antibody combining sites (Coon and Hunter, 1975).

Machida *et al.* (1977) found that delayed hypersensitivity induction was suppressed by an intravenous injection of 100 microgram of Concavalin A when administered one day before or simultaneously with immunization.

Dextran sulphate delayed the onset or completely suppressed the expression of DTH to SRBC in mice when administered via a route different from that of eliciting antigen. The target for the action was found to be accessory cells (monocytes) and not the T effector cells (Stehr and Diamanstein, 1977).

Bicker (1978) found that BM 12.531, a 2-cyan substituted aziridine had therapeutic and immunomodulatory effects. A dose dependent increase in T lymphocytes was found in cancer patients after the administration of BM 12.531.

The effects of Cyclophosphamide, Methotrexate, Procarbazine and Azathioprine on the DTH response and antibody response to SRBC were studied in mice by Doherty (1981) and found that they inhibited antibody response and potentiated or had no effect on DTH.

Levamisole was reported to have immunomodulatory properties when half of its anthelminthic dose was administered in kids. Immunostimulation was characterised by lymphocytic and neutrophilic leukocytosis, enhancement in phagocytic activity of neutrophils and increase in concentration of gammaglobulin (Anilkumar and Rajan, 1986).

Kato *et al.* (1988) observed a suppression of DTH responses in female mice treated with Diethylstilbesterol (DES), a synthetic non steroidal compound possessing estrogenic activity, however in males there was no such suppression. It was suggested that testosterone inhibited the DES suppression of DTH response.

Navolotskaya *et al.* (1993) investigated the effect of 'immunocortin', a synthetic ACTH like decapeptide on the activity of immunocompetent cells in vitro. The results showed an inhibition of blast transformation of mouse thymocytes, spontaneous motility of mouse peritoneal macrophages and bactericidal activity against *Salmomella typhimurium* 415.

The water soluble derivative of Propolis (bee glue) showed anticomplement activity in a dose dependent manner which also varied according to the source of complement. It decreased splenocyte proliferation (Ivanovska *et al.*, 1995).

Rodgers *et al* (1996) found that Malathion when administered at noncholinergic doses, enhanced the humoral immune response to SRBC and macrophage function.

Administration of ascorbic acid (Celin tab) orally for 21 days at a dose rate of 500mg thrice daily in humans and 200 mg/kg /day in rabbits resulted in an increased serum immunoglobulin in human subjects and raised antibody titre in rabbits. But the effect on cell mediated immunity was found nil in both the cases (Ansari *et al.*, 1998).

Miller (2001) observed that Cephalosporins altered the host's reaction to an infection, by its actions upon phagocytic function, chemotaxis and lymphocytic

activities. Among cephalosporins, cefodizime showed the greatest immunostimulant activity while cefotaxime showed an immunodepressing effect in vitro.

Metals altered the immune homeostasis by excessive production of inflammatory cytokines. They modified the response by influencing expression of new antigen, which resulted in chronic inflammatory process and autoimmune diseases (Lawrencea and Mc Cabe, 2002).

Nghiem *et al.* (2002) reported that a hallmark of UV induced immune suppression was the generation of antigen specific suppressor T cells. The suppressor cells suppressed anti tumor immunity as well as other cell mediated responses such as delayed type of hypersensitivity reaction.

Ponkshe and Indap (2002) showed that the whole body ether extracts of a marine prawn *Nematopaleamon tencipes* and two gastropods viz, *Euchelus asper* and *Hemifuscus pugilinus* possessed concentration dependent switch from immunostimulation to immunosuppression in in vitro systems ie, they acted as immunostimulant at low doses.

de Waard *et al.* (2002) investigated the effects of Lactobacillus strains on T cell mediated immunological memory responses and demonstrated that long term daily oral administration enhanced acquired cellular resistance towards Listeria reinfection while short term daily ingestion decreased DTH expression.

2.5 IMMUNOMODULATORY STUDIES ON *Emblica Officinalis*

Septilin, a poly herbal formulation containing *Emblica officinalis* when administered orally at a dose rate of 500mg/kg enhanced both primary and secondary immune response in mice immunized with SRBC. Septilin increased the total counts of leukocytes as well as preferential increase in the polymorphonuclear cells (Kumar *et al.*, 1992; Sharma and Ray, 1997).

Suresh and Vasudevan (1994) reported that *Phyllanthus emblica* enhanced Natural Killer (NK) cell activity and Antibody Dependent Cell Mediated

Cytotoxicity (ADCC) in mice bearing Dalton's lymphoma ascites tumor. The results revealed that the antitumor activity of *P. emblica* was mediated primarily through the ability of the drug to augment natural cell mediated cytotoxicity.

Immune-21, a polyherbal ayurvedic product containing *Emblica officinalis* when given at a rate of 50 mg/kg body weight orally in mice, increased both morphometric and functional changes of macrophages. It significantly potentiated humoral immunity in rabbits, showed significant protection against UV rays, cyclophosphamide and cyclosporin A induced immunosuppression. Production of plaque forming cells from spleen was stimulated (De *et al.*, 1998; Chatterjee, 2001; Nemmani *et al.*, 2002).

Rekha *et al.* (1998) found that oral administration of Brahma Rasayana (10 and 50 mg/ dose/animal) which contained *Emblica officinalis* as one of the components significantly increased the total leukocyte count and percentage of poly morpho nuclear cells. The bone marrow cellularity as well as α esterase positive cells were also increased.

Sairam *et al.* (2003) revealed the cytoprotective and immunomodulating properties of *Emblica officinalis* against chromium induced oxidative damage. Amla resulted in enhanced cell survival, decreased free radical production and higher antioxidant levels. It restored both phagocytosis and gamma interferon production by macrophages upon oxidative damage.

2.6 OTHER PHARMACOLOGICAL ACTIVITIES OF THE PLANT UNDER STUDY

The lipid lowering and antiatherosclerotic effects of *Emblica officinalis* (Amla) fresh juice were studied in cholesterol fed rabbits and found that it lowered serum cholesterol, triglyceride, phospholipids and low density lipoprotein (LDL) levels. The aortic plaques were also found regressed (Thakur *et al.*, 1988; Mathur *et al.*, 1996).

Grover and Kaur (1989) observed that water, acetone and chloroform extracts of *Emblica officinalis* fruit had antimutagenic effect on sodium azide and 4-nitro-O-phenylene diamine induced mutagenesis in *Salmonella typhimurium*.

Septilin, a patent preparation containing *Emblica officinalis* was tested for antiinflammatory and wound healing effects in rats by Udupa *et al.* (1989). It significantly enhanced gain in tensile strength in incision wounds, wound contraction and epithelialization in excision wounds. It also suppressed acute inflammation significantly without affecting chronic inflammation.

The cytotoxic effects produced by lead nitrate and aluminium sulphate administration in mice was significantly reduced by aqueous extract of *Phyllanthus emblica* fruits when administered orally (Dhir *et al.*, 1990). *Emblica officinalis* modified acute cytotoxicity of cadmium and reduced mortality and associated tissue damages (Khandelwal *et al.*, 2002).

Aqueous extract of dry fruits of *Phyllanthus emblica* when fed to mice for seven consecutive days prior to treatment with different doses of nickel or cesium chloride, a significant reduction in the percentage of aberrant cells and the frequency of micronuclei formation were noticed. Thus it acted as an effective protective agent against clastogenicity (Dhir *et al.*, 1991; Ghosh *et al.*, 1992).

Kulkarni and Verma (1992) observed that BR-16A (MENTAT), a polyherbal psychotropic preparation containing *Emblica officinalis* was effective in improving learning ability and behavioural disturbances in mentally retarded children and exerted beneficial effect in the cases of cerebral deficit. The compound improved short term memory in naive mice.

Water fraction of methanol extract of *Emblica officinalis* leaves was found to have antiinflammatory activity (Asmawi *et al.*, 1993). It inhibited migration of human polymorphonuclear cells in relatively low concentrations. It did not inhibit Leukotriene B_4 or Platelet activating factor synthesis in human polymorphonuclear cells or Thromboxane B_2 synthesis in human platelets.

Pachori *et al.* (1993) observed that oral administration of 'Abana', a polyherbal preparation containing *Emblica officinalis* decreased low density lipoprotein, very low density lipoprotein-cholesterol, serum total cholesterol, serum triglycerides and serum phospholipids and the percent change was more with Abana as compared to Metoprolol.

Oral administration of 50 percent alcoholic extract of *Phyllanthus emblica* and quercetin isolated from it at a dose rate of 100 mg and 15 mg respectively produced significant hepatoprotection against country made liquor and paracetamol (Gulati *et al.*, 1995).

Polyherbal formulations like Cauvery 100 and Rhinax which contain *Emblica officinalis* as one of its component showed protection against indomethacin induced ulcers in rabbits. The protective action was mainly due to the modulation of defensive factors by improvement in gastric cytoprotection and partly by acid inhibition and free radical scavenging properties (Manonmani *et al.*, 1995; Dhuley, 1999).

Thorat *et al.* (1995) evaluated the protective effect of *Emblica officinalis* in experimentally induced acute necrotising pancreatitis in dogs and found that it inhibited the increase in serum amylase caused pancreatitis.

The whole, aqueous, standardized extract of *Emblica officinalis* was found to offer protection against a variety of biological, physical and chemical stressors. *Emblica officinalis* strengthened the defence mechanism against free radical damage induced during stress (Dahanukar *et al.*, 1997; Rege *et al.*, 1999).

Aqueous extract of *Emblica officinalis* was found to be cytotoxic to L929 cells in culture in a dose dependent manner. *Emblica officinalis* and chyavanaprash reduced ascites and solid tumors in mice induced by DLA cells. The antitumor activity of *Emblica officinalis* extract was partially due to its interaction with cell cycle (Jose *et al.*, 1997; 2001).

Emblica officinalis extracts significantly inhibited the genotoxicity and clastogenicity of Benzopyrene as well as Cyclophosphamide. The plant extract administration resulted in an increase in antioxidant and detoxification enzymes namely glutathione peroxidase, glutathione reductase and glutathione –S-transferase (Nandi *et al.*, 1997; Sharma *et al.*, 2000; Haque *et al.*, 2001).

The dry powder and aqueous extract of *Emblica officinalis* (Amalki) showed prokinetic effect on gastrointestinal motility in mice. The incinerated powder at lower doses showed prokinetic effect whereas at higher doses, it decreased gastrointestinal motility (Dahanukar *et al.*, 1998).

The aqueous extract of *Emblica officinalis* was found to be detrimental to test dermatophytes in in-vitro studies indicating antifungal activity (Dutta *et al.*, 1998).

Bhattacharya *et al.* (1999) showed that active tannoids of *Emblica officinalis* possessed antioxidant activity as it induced an increase in both frontal, cortical and striatal superoxide dismutase, catalase and glutathione peroxidase activity when administered at the doses of 5 and 10 mg/kg intraperitoneally.

Emblica officinalis was found to inhibit hepatocarcinogenesis induced by N-nitrosodiethylamine (NDEA) in a dose dependent manner (Jose *et al.*, 1999; Rajeshkumar and Kuttan , 2001).

Nadig and Rao (1999) observed that Hepatogard, a herbal preparation containing *Emblica officinalis* reversed the dexamethasone induced decrease in breaking strength in both incised wound and granulation tissue, showing its wound healing property.

Phyllanthus emblica fruits at the dose of 50-100mg/kg body weight when orally administered to rats for 10 consecutive days enhanced secretion of gastric mucus and hexosamine in the indomethacin induced ulceration in rats and thus showed antiulcer property (Bandopadhyay *et al.*, 2000; Al-Rehaily *et al.*, 2002; Sairam *et al.*, 2002).

The effect of tannoid principles of *Emblica officinalis* in Tardive Dyskinesia induced by Haloperidol was studied by Bhattacharya *et al.* (2000). Chewing movements, buccal tremors and tongue protrusion were taken as Tardive Dyskinesia parameters, which were inhibited in a dose dependent manner by the tannoid principles of *Emblica officinalis*.

Emblicanin A and B enriched fraction of fresh juice of Emblica fruits, administered prophylactically, inhibited hepatic dysfunction (Bhattacharya *et al.*, 2000). *Emblica officinalis* and chyavanaprash inhibited the hepatotoxicity produced by acute and chronic administration of Carbontetrachloride (Jose and Kuttan , 2000).

Augusti *et al.* (2001) reported that *Emblica officinalis* showed a hypolipidaemic effect in hyperlipidaemic rats which were fed with butter fat and beef fat to induce hyperlipidaemia.

A standardised extract of *Phyllanthus emblica* was found to have long lasting and broad spectrum antioxidant activity. Emblica helped to protect the skin from damaging effects of free radicals, non radicals and transition metals induced oxidative stress. The antioxidant activity of the Amla extract was found to be both dose and concentration dependent (Khopde *et al.*, 2001; Chaudhari, 2002).

Manjunatha *et al.* (2001) observed that chyavanaprash, an ancient Indian dietary supplement derived from amla, reduced post prandial glycaemia in the oral glucose tolerance test and also reduced blood cholesterol level to a significantly greater extent than Vitamin C.

Rajeshkumar and Kuttan (2001) reported that polyphenol fraction of *Emblica officinalis* was found to scavenge superoxide and hydroxyl radicals and inhibit lipid peroxidation in vitro. *Emblica officinalis* lyophilized fresh juice also exhibited the same properties, but more quantity was needed than *Emblica officinalis* poly phenol fraction to produce same level of effect.

Rajeshkumar and Kuttan (2001) demonstrated that *Emblica officinalis* extract and its polyphenolic fraction treatment resulted in the induction of apoptosis in human and mouse carcinoma cell lines.

Flavonoids from *Emblica officinalis* reduced lipid levels in serum and tissues of rats in induced hyperlipidaemia. Hepatic HMG CoA reductase activity was significantly inhibited (Anila and Vijayalakshmi., 2002).

Kaur *et al.* (2002) observed that the chloroform and acetone extracts of a combination drug 'Triphala' (composite mixture of *Terminalia bellarica, Terminalia chebula* and *Emblica officinalis*) inhibited mutagenicity induced by both direct and S_9 dependent mutagen.

Alam and Gomes (2003) explored the anti-snake venom activity of *Emblica officinalis* and they found that its methanolic extracts significantly antagonised the *Vipera russellii* and *Naja kaouthia* venom induced lethal activity both in in vitro and in vivo studies.

Materials and Methods

3. MATERIALS AND METHODS

3.1 PLANT MATERIAL/DRUG

Fresh Emblica fruits purchased from local market were washed thoroughly, deseeded and cut into small pieces and dried under shade and then pulverized using grinder (Fig.1).

3.1.1 Preparation of Extract of Plant Material

Alcoholic extract of *Emblica officinalis* powder was prepared using ethyl alcohol in a soxhlet apparatus. The extract was evaporated to dryness with the help of a vacuum evaporator and kept in refrigerator in an airtight container. On an average, 100g of dried Emblica fruit pulp powder gave 32g of dry extract.

The aqueous extract of *Emblica officinalis* was prepared by boiling dried Emblica powder with distilled water in 1:10 ratio for 15 minutes and then filtered through a muslin cloth. The filtered solution was then evaporated to dryness using vacuum evaporator and kept in the refrigerator in an airtight container. Hundred grams of dried Emblica fruit pulp powder gave about 50g of dry extract.

3.1.2 Screening of *Emblica Officinalis* Extracts for Active Principles

The alcoholic and aqueous extracts of *Emblica officinalis* fruit pulp were tested for the presence of various active chemical constituents namely steroids, alkaloids, tannins, phenolic compounds, flavonoids, glycosides, diterpenes, triterpenes and saponins as per the procedure quoted by Harborne (1991).

3.1.2.1 *Test for Detection of Steroids*

Salkowski test

About 5mg of the extract was dissolved in 3ml of chloroform and then shaken with about 3ml concentrated sulphuric acid. If a red colour develops, indicates the presence of steroids.

Fig 1. *Emblica officinalis* fruit

Leiberman Burchardt test

About 5mg of the extract was dissolved in 3ml of chloroform. Then five drops of acetic anhydride and 1 ml of concentrated sulphuric acid were added to it through the sides. A reddish ring if obtain at the junction of two layers, indicates the presence of steroids.

3.1.2.2 *Tests for Detection of Alkaloids*

About 0.5g of the extract was mixed with 5ml ammonia and then extracted with equal volume of chloroform solution. To this, 5ml dilute hydrochloric acid was added. The acid layer obtained was used for chemical tests for the alkaloids

Mayer's test

To 1ml of the acid layer, few drops of Mayer's reagent (Potassium mercuric iodide) was added. If a creamy white precipitate is formed, indicates the presence of alkaloids.

Wagner's test

Few drops of Wagner's reagent (Solution of Iodine in Potassium iodide) was added to 1ml of the acid layer. If there is presence of reddish brown coloured precipitate, the presence of alkaloids is indicated.

Hager's test

To 1ml of the acid layer, few drops of Hager's reagent (Saturated solution of picric acid) was mixed. An yellow precipitate is formed, if the alkaloids are present.

Dragendroff's test

Few drops of Dragendroff's reagent (Solution of Potassium and Bismuth iodide) was mixed with 1ml of the acid layer. Presence of alkaloids is indicated, if a reddish brown precipitate is seen.

3.1.2.3 *Test for Detection of Tannins*

Ferric chloride test

Two milligram of the extract was mixed with 3ml of one percent ferric chloride solution. If blue, green or brownish green colour is obtained, it indicates the presence of tannins.

Gelatin test:-

About 0.5g of the extract was mixed with few drops of one percent solution of gelatin containing 10 percent sodium chloride. If white precipitate is formed, indicates the presence of tannins.

3.1.2.4 *Test for Detection of Flavonoids*

Ferric chloride test

To 2ml of alcoholic solution of the extract (0.5g extract in 10ml methanol), few drops of neutral ferric chloride solution was mixed. If development of green colour occur, indicates the presence of flavonoids.

Lead acetate test

To 2ml of alcoholic solution of the extract (0.5g extract in 10ml methanol), few drops of neutral 10 percent lead acetate was mixed. If yellow precipitate appears, the presence of flavonoids is indicated.

3.1.2.5 *Test for Presence of Glycosides*

Sodium hydroxide reagent

Dissolved a small amount of the extract (about 5mg) in 1 ml water and added 5-6 drops of sodium hydroxide solution. An yellow colour if obtain, indicates the presence of glycosides.

Benedict's test

To about 1ml of the extract (0.5g extract in 1ml water), 5ml of Benedict's reagent was added. The mixture was boiled for two minutes. Development of brown to red colour if occur, indicates the presence of glycosides.

3.1.2.6 ***Test for Presence of Phenolic Compounds***

About 5mg of the extract was dissolved in 1ml of water and five drops of 10 percent ferric chloride solution was added to it. Development of dark brown colour occur, if the phenolic compounds are present.

3.1.2.7 ***Test for Detection of Diterpenes***

About 5mg of the extract was mixed with 3ml of copper acetate solution. If there is development of green colour, the presence of diterpenes is indicated.

3.1.2.8 ***Test for the Presence of Triterpenes***

Salkowski test

About 3mg of the extract was dissolved in 3ml chloroform and then it was shaken with concentrated sulphuric acid. If lower layer turn to yellow on standing, indicates the presence of triterpenes.

Lieberman Burchardt test

Few drops of acetic acid and 1ml concentrated sulphuric acid was added to 3ml chloroform solution of the extract (about 3mg extract in 3ml chloroform). Deep red ring at the junction of two layers if appear, indicates the presence of triterpenes.

3.1.2.9 ***Test for Presence of Saponins***

Foam test

A small amount of the extract (about 5mg) was shaken with 3ml of water. If the foam produced persists for 10 minutes, the presence of saponins is confirmed.

3.2 EXPERIMENTAL ANIMALS

One hundred and forty four male Swiss Albino mice weighing between 15g to 30g and two to three months of age procured from Small Animal Breeding Station, Mannuthy were used for the study. They were maintained on identical feeding and managemental practices in the laboratory for one week before the commencement of the experiment. Water was given adlibitum.

3.3 ANTIGEN

Sheep blood was collected from the animals maintained in the University Sheep and Goat farm Mannuthy, in equal volume of Alsever's solution following sterile procedures. This was used for antigen preparation and stored at 4°C until use.

3.3.1 Preparation of Sheep Red Blood Cell (SRBC) Antigen for Immunization

Materials

Alsever's solution

Sodium chloride	4.2g
Tri sodium citrate	8.0g
Citric acid	0.55g
Glucose	20.5g
Distilled water	1000ml

Each ingredient in the above order was added to 500ml of distilled water in a graduated cylinder and stirred until the chemicals dissolved completely. The volume was then made upto 1 litre with distilled water and finally steamed for 10 minutes.

Normal Saline

Sodium Chloride	0.85g
Distilled water	100ml

Phosphate Buffered Saline(pH 7.2, 0.15 M)

Sodium Chloride	8.5g
Disodium hydrogen phosphate	1.34g
Sodium dihydrogen phosphate	0.34g
Distilled water	1000ml

Method

The sheep blood collected was centrifuged and washed three times in large volumes of sterile normal saline and concentration was adjusted to $1x10^8$ cells/ml in phosphate buffered saline (PBS) for immunization.

3.4 EVALUATION OF IMMUNOMODULATORY STATUS

Immunomodulatory status was assessed by physiological, haematological, biochemical and immunological parameters. The physiological parameters like body weight and relative organ weight, haematological parameters like the total leukocyte count and differential leukocyte count, biochemical parameters like total protein and globulin, immunological parameters like haemagglutination test to assess the humoral immune response and tests like delayed type of hypersensitivity, macrophage migration index and qualitative nitroblue tetrazolium dye reduction test to assess the cellular immune response were recorded.

Experimental Design

Seventy two mice were used for testing Haemagglutination (HA) antibody titre and Macrophage Migration Index (MMI) and NitroBlue Tetrazolium (NBT) dye reduction test. They were divided randomly into two groups of 36 each. One group was tested with alcoholic extract of the test substance and the other with the aqueous extract. The first group was then divided into three subgroups of 12 each and denoted as A_1, A_2, and A3. A_1 served as control and received vehicle alone (five percent gum acacia in distilled water) while A_2 and A_3 were fed with alcoholic extract of the test substance in five percent gum acacia at the dose rate of 100 mg/kg body

weight and 200 mg/kg body weight respectively for 19 days. The second group containing 36 mice were also divided into three subgroups of 12 each and denoted as B_1, B_2 and B_3 for testing the aqueous extract in the same manner.

Seventy two mice were used to assess Delayed type of Hypersensitivity (DTH) of which 36 were used for testing the alcoholic extract and the remaining 36 for the aqueous extract. Each group was then divided into three subgroups as C_1, C_2, and C_3 and D_1, D_2, and D_3 of 12 each. C_1 was taken as control which received vehicle only. C_2 and C_3 were given alcoholic extract of the drug at the dose rate of 100 mg/kg body weight and 200 mg/kg body weight respectively for 19 days. For the subgroups D_1, D_2 and D_3 the same procedure was repeated with the aqueous extract.

Mice from all groups (A_1, A_2, A_3, B_1, B_2, B_3, C_1, C_2, C_3, D_1, D_2, D_3) were injected with SRBC antigen ($1x10^8$cells/ml/100g body weight ie, 0.2ml per mice) intraperitoneally on fifth day of drug or vehicle administration.

The day before the commencement of study was taken as zero day and the reading of parameters recorded on that day served as experimental control.

3.4.1 Physiological Parameters

The weight of the individual mouse was recorded before and after the experiment. The weight of the organs like spleen, liver and kidney were also recorded at the time of sacrifice.

3.4.2 Haematological Parameters

Blood samples were collected from retro orbital plexus of mice from groups A_1, A_2, A_3, B_1, B_2 and B_3 with the help of heparinised capillary tube using disodium salt of Ethylene diamine tetra acetic acid as anticoagulant on zero, 5, 12, and 19 day of administration of the extract/vehicle. Total and differential leukocyte count was taken as per the method described by Schalm (1975).

3.4.3 Biochemical Parameters

Blood collected from retro orbital plexus of mice from groups A_1, A_2, A_3, B_1, B_2 and B_3 was taken in a test tube without anticoagulant for serum separation.

The total protein content in the serum was estimated by Biuret method (Gomall *et al.*, 1949). The Albumin content in the serum was estimated by Bromo Cresol Green dye method described by Doumas (1971). Serum globulin value was determined by deducting the value of serum albumin from total serum protein.

3.4.4 Immunolgical Parameters for Evaluation of Humoral Immune Response.

3.4.4.1 *Haemagglutination test*

Materials

SRBC antigen for Haemagglutination

Sheep blood collected freshly in Alsever's solution from University Sheep and Goat farm was washed thrice in normal saline and the concentration was adjusted to get one percent in PBS.

Alsever's solution

Normal saline (NS)

Phosphate buffered saline (PBS)

Method

The blood was collected from mice of groups A_1, A_2, A_3, B_1, B_2 and B_3 on day zero, 12 and 19 of drug/vehicle administration. Haemagglutination test was performed using techniques of Ray and Mediratta (1991).

Two fold dilutions of sera were performed on 0.15M Phosphate Buffered Saline (pH 7.2) and 50µl of each dilution was aliquoted into 96 well microtitire plates. A 25 µl quantity of fresh one percent SRBC suspension in PBS was dispensed into each well and mixed thoroughly. The plates were incubated at 37°C for one hour

and then inspected for haemagglutination. The reciprocal of highest dilution of the test serum giving 50 percent agglutination had been expressed as HA titre

3.4.5 Immunological Parameters for Evaluation of Cell Mediated Immune Response

3.4.5.1 *Delayed Type of Hypersensitivity (DTH)*

Materials

SRBC antigen for DTH test

Sheep blood collected in Alsever's solution from University Sheep and Goat farm was washed thrice in sterile normal saline and the concentration was adjusted to $1x10^8$ cells/0.025ml in Phosphate Buffered saline

Alsever's Solution

Normal saline

Phosphate Buffered Saline

Method

The DTH test was done according to the method of Saraf *et al.* (1989).The antigen specific cellular immune response in experimental animals upon treatment was measured using this parameter. Six mice from groups C_1, C_2, C_3, D_1, D_2, D_3 which were primed with SRBC antigen intraperitoneally on day five were challenged on day 12 with 0.025ml of the so prepared antigen containing $1x10^8$ SRBC subcutaneously on left hind foot pad. The right hind paw received 0.025 ml of saline alone. The test was repeated on 19th day in the remaining 6mice of each group. The measurement of footpad swelling was done 24hrs after challenge on hind paw using Vernier callipers at three different dimensions. The difference in paw thickness was taken as a measure of DTH.

3.4.5.2 *Macrophage Migration Index (MMI)*

Materials

Hanks Balanced Salt Solution (HBSS)*

Readymade HBSS powder was reconstituted as per manufacturer's instruction in sterile double distilled water and filtered using micropore filter (0.2µm) and stored at 4°C until use. pH of the prepared solution was adjusted to 7.2 using sterile 4.4 percent Sodium bicarbonate solution just before use.

Rosewell Park Memorial Institute-1640 (RPMI-1640)*

Readymade RPMI-1640 powder was reconstituted as per manufacturer's instruction , in sterile double distilled water and then filter sterilized using Millipore filter (0.2µm) and stored at 4°C until use. pH of the medium was adjusted to 7.2 using sterile 4.4 percent Sodium bicarbonate solution just before use.

Heparin**

Foetal Calf Serum

Foetal calf blood was collected from University Livestock farm, Mannuthy following sterile procedures. The serum was separated and heat inactivated to destroy the complement. Inactivation was done by incubating serum at 56°C for 30 minutes in a waterbath and it was stored at -20°C until use.

L-Glutamine***

The glutamine powder was dissolved in double distilled water and diluted to a concentration of 200mM solution. Then filtered using millipore filter (0.2µm) into 10ml aliquots and stored at 4°C until use. The so prepared glutamine stock solution was again diluted to 1:100 with double distilled water to get a concentration of 2mM

*Himedia, Bombay.

** Biological E, Hyderabad.

***SRL, Bombay.

Fixative

The fixative was prepared by mixing acetic acid, methanol and distilled water in the proportion 1:7:2.

Peritoneal Exudate Cells (PEC)

PEC were collected using the procedure followed by Benencia *et al* .(1996). The animals were sacrificed under ether anaesthesia by decapitation and exsanguination. The abdomen was swabbed with rectified spirit to dampen the furcoat. The mouse was then stripped off its skin by pulling the fur and avoiding touching the anterior abdominal wall.

The peritoneal cavity was then distended by injecting approximately 5-6ml of HBSS containing 5 IU Heparin per ml. The peritoneal fluid was removed by inserting a needle into the left flank and applying lateral traction. Peritoneal cavity was gently shaken to have better yield of cells. The aspirated fluid was gently transferred into a sterile tube by removing the needle from syringe.

Antibiotic Solution

Antibiotics like streptomycin and benzyl penicillin available in the powdered form was dissolved in 40ml double distilled water, filter sterilized using millipore filter (0.2μm) and stored at -20°C until use.

Method

Macrophage Migration Index was taken according to the procedure followed by Saxena *et al.* (1991) with some modifications. Peritoneal exudates cells were collected from six mice of groups A_1, A_2, A_3, B_1, B_2 and B_3 on day 12 using the method followed by Benencia *et al.* (1996) and MMI was taken. The experiment was repeated on 19^{th} day in the other six mice of each group in the same pattern.

The PEC collected were centrifuged at 250g at 4°C for 10 minutes. The cells were again washed twice with fresh cold RPMI-1640 medium and tested for viability by Trypan blue exclusion technique in which one part of Trypan blue solution (0.1

percent w/v in saline) was mixed with one part of cell suspension and loaded into a haemocytometer and the stained and unstained cells were counted separately. Viable cells excluded the dye while nonviable took up the dye and thus appeared blue in colour. Batches with 90 percent or more viability were used for the test. The cells were finally suspended in 0.5ml RPMI-1640 media containing antibiotics like benzyl penicillin and streptomycin (100 IU/ml and 100 µg/ml respectively), 2mM glutamine and 10 percent heat inactivated foetal calf serum. Then the cells were counted in Neubauer's counting chamber using WBC diluting fluid. The final cell suspension was adjusted to a concentration of 60-80x10^6 cells/ml media.

PEC thus prepared from treated and untreated animals were packed in a microhaematocrit capillary of uniform diameter, were placed in migration chambers, filled with complete RPMI-1640 medium containing five percent foetal calf serum, antibiotics (streptomycin and benzyl penicillin) and glutamine; then incubated for 18-24hrs. At the end of incubation period, the cells were fixed to the surface by flooding the plates with methanol acetic acid fixative for 15 minutes. The migration area of macrophage was measured by taking the average diameter of the opaque zone around the capillary.

The migration area of PEC from the treated group (A_1) divided by that of untreated control animals (A_2) was expressed as macrophage migration index (MMI).

3.4.5.3 *Qualitative Nitro Blue Tetrazolium (NBT) Reduction Assay*

Materials

HBSS

RPMI-1640

Peritoneal Exudate Cells (PEC)

Nitro Blue Tetrazolium (NBT)*

* Himedia, Bombay.

10 mg of NBT chloride powder was accurately measured and dissolved completely in 1ml sterile distilled water.

Dimethyl Sulphoxide (DMSO)

Sorenson's Buffer (0.5M, pH 10.5)

Stock solution (1)

Potassium dihydrogen phosphate	3.402g
Distilled water	50ml

The ingredient was completely dissolved in 50ml distilled water and kept in an airtight container

Stock solution (2)

Disodium hydrogen phosphate .$2H_2O$	3.954g
Distilled water	50ml

The ingredient was dissolved completely by stirring in 50ml distilled water and kept in an airtight container.

Working solution

4.5 ml of Disodium hydrogen phosphate was added to 0.5ml of Potassium dihydrogen phosphate and the volume was made up to 100ml with distilled water and the pH was adjusted to 10.5 using 1N Sodium hydroxide solution.

Method

Nitro Blue Tetrazolium Reduction Test was done as per the procedure quoted by Sairam *et al.* (1997). The PEC was collected from A_1, A_2, A_3, B_1, B_2 and B_3 on day 12 as explained earlier and was subjected to NBT reduction test. The experiment was repeated on 19^{th} day in the other six mice of each group in the same pattern.

The PEC collected were washed thrice with fresh cold HBSS and the final concentration of the cells was adjusted to 10-20x10^6 cells/ml HBSS. Then 100µl of

cells were plated into 96 well tissue culture plates and incubated for two hour at 37°C in a CO_2 incubator for macrophages to adsorb to the bottom of the plates. The cells were then washed three times with cold HBSS to remove non adherent cells followed by the addition of 200µl of RPMI-1640 medium to the wells

After plating the macrophages in the wells, 25µl of nitroblue tetrazolium chloride (NBT 10mg/ml) was added to 200µl of RPMI-1640 medium taken already in the wells and the cells were incubated in a CO_2 incubator at 37°C for one hour. Then the cells were washed three times with HBSS and the NBT reduced was solubilized in 200µl DMSO and 25µl of Sorenson's buffer, pH 10.5. The blue colour obtained was measured at 650nm using ELISA reader.

3.5 STATISTICAL ANALYSIS OF DATA

The data obtained were statistically analysed using one way Analysis of Variance for comparison between groups and student *t* test for within the groups, as described by Snedocor and Cochran (1985). The results are expressed as mean $\pm$ standard deviation.

Results

4. RESULTS

4.1 SCREENING OF *Emblica officinalis* FOR ACTIVE PRINCIPLES

4.1.1 Steroids

As per Salkowski test, red colour was not obtained with the extract and Liebermán Burchardt test did not give a reddish ring at the junction. Thus it could be concluded that no detectable level of steroids were present in the aqueous and alcoholic extract of dried *Emblica officinalis* fruit pulp.

4.1.2 Alkaloids

A creamy white precipitate as per Mayer's test and a reddish brown coloured precipitate as per Wagner's test was not obtained. Dragendroff's test did not yield reddish brown precipitate. Thus the tests revealed the absence of detectable level of alkaloids in the dried fruit pulp extracts of Emblica.

4.1.3 Tannins

Intense blue colour was obtained in Ferric chloride test and a white precipitate was obtained in gelatin test. These results indicated the presence of tannins in the extracts.

4.1.4 Flavonoids

A green colour in the Ferric chloride test and a yellow precipitate in lead acetate test indicated the presence of flavonoids in the extracts.

4.1.5 Glycosides

As per Benedict's test, brown colour was developed indicating the presence of glycosides. An yellow colour was obtained by mixing the extract with sodium hydroxide reagent which also indicated the presence of glycosides.

4.1.6 Phenolic Compounds

The extract mixed with 10 percent ferric chloride produced dark brown colour indicating the presence of phenolic compounds.

4.1.7 Diterpenes

Diterpenes were detected in the extract as indicated by the green colour when it was mixed with copper acetate solution.

4.1.8 Triterpenes

As per Salkowski test, lower layer turned to yellow on standing and by Lieberman Burchardt test, a deep ring appeared at the junction of two layers. These results indicated the presence of triterpenes in the *Emblica officinalis* extract.

4.1.9 Saponins

In the foam test, foam persisted for 10 minutes in case of aqueous and alcoholic extracts indicating the presence of saponins.

The results obtained in the above phytochemical study are summarised in the Table 1.

4.2 EVALUATION OF IMMUNOMODULATORY STATUS

4.2.1 Physiological Parameters

4.2.1.1 *Body Weight*

The individual and mean bodyweights of the experimental mice (groups A_1, A_2, A_3, B_1, B_2, B_3) recorded on zero, 12 and 19th day of drug/ vehicle treatment are presented in Table 2 and 3 and graphically in Fig. 2 and 3. The zero day values represented the body weight before the commencement of the experiment.

The body weights of mice in all drug treated groups (A_2, A_3, B_2, B_3) showed a dose dependent increase during the experimental period. A significant ($P<0.05$) increase could be noticed on the 19th day of drug administration for A_3 and B_3, from their control groups. These groups (A_3, B_3) also showed significantly ($P<0.05$)

Table 1. Screening of *Emblica officinalis* fruit pulp extracts for active principles

Sl.no	Active Principles	Alcoholic Extract	Aqueous Extract
1.	Steroids	Not detected	Not detected
2.	Alkaloids	Not detected	Not detected
3.	Tannins	Present	Present
4.	Flavonoids	Present	Present
5.	Glycosides	Present	Present
6.	Phenolic compounds	Present	Present
7.	Diterpenes	Present	Present
8.	Triterpenes	Present	Present
9.	Saponins	Present	Present

Table 2. Effect of alcoholic extract of *Emblica officinalis* on body weight, g

Sl.No	Zero day			12th day			Zero day			19th day		
	A_1	A_2	A_3	A_1	A_2	A_3	A_1	A_2	A_3	A_1	A_2	A_3
1	20	20	20	20	20	20	20	20	20	20	20	30
2	20	20	25	20	25	25	20	15	25	20	25	30
3	20	20	15	20	25	25	20	25	20	25	25	25
4	25	20	20	25	20	25	25	20	20	25	25	25
5	25	25	20	20	20	20	20	20	20	20	30	25
6	20	20	25	20	20	25	20	20	25	20	20	20
Mean ± SD	21.67 ±2.58	20.83 ±2.04	20.83 ±3.76	20.83 ±2.04	21.67 ±2.58	23.33 ±2.58	20.83 ±2.04	20.00 ±3.16	20.83 ±2.04	21.67 ±2.58^{B}	24.17 ±3.76^{B}	25.83 ±3.76^{A}

(Means bearing the same superscript do not differ significantly at P<0.05)

Table 3. Effect of aqueous extract of *Emblica officinalis* on body weight, g

Sl.No	Zero day			12th day			Zero day			19th day		
	B_1	B_2	B_3	B_1	B_2	B_3	B_1	B_2	B_3	B_1	B_2	B_3
1	20	20	25	20	20	20	15	20	25	20	25	30
2	20	20	25	20	25	25	20	25	20	25	25	30
3	25	20	20	20	25	25	20	20	25	25	30	25
4	20	25	15	25	20	25	20	15	20	20	25	35
5	20	20	20	20	20	25	20	20	20	20	30	30
6	15	20	20	20	20	30	20	20	20	20	20	25
Mean ± SD	20.00 ±3.16	20.83 ±2.04	20.83 ±3.76	20.83 $\pm2.04^{B}$	21.67 $\pm2.58^{B}$	25.00 $\pm3.16^{A}$	19.17 ±2.04	20.00 ±3.16	21.67 ±2.58	21.67 $\pm2.58^{B}$	25.83 $\pm3.76^{AB}$	29.17 $\pm3.76^{A}$

(Means bearing the same superscript do not differ significantly at $P<0.05$)

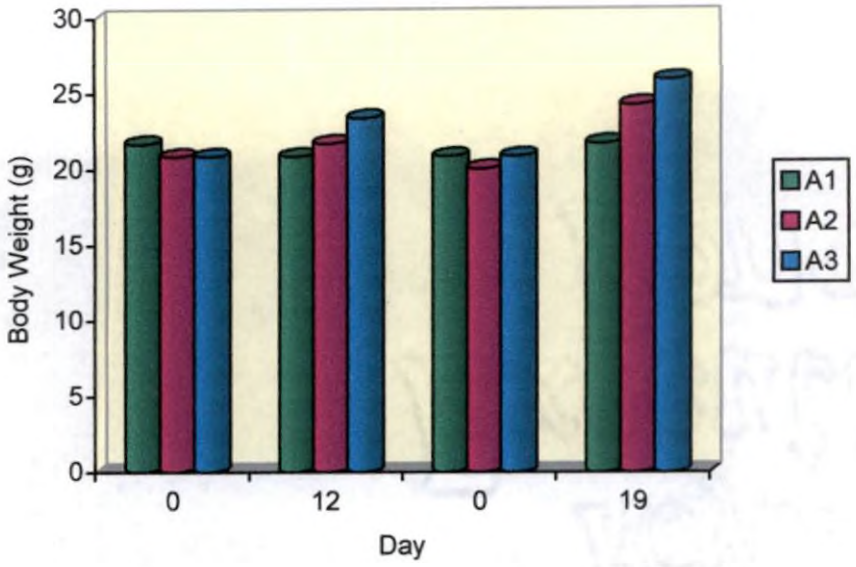


Fig. 2. Effect of alcoholic extract of *E. officinalis* on body weight

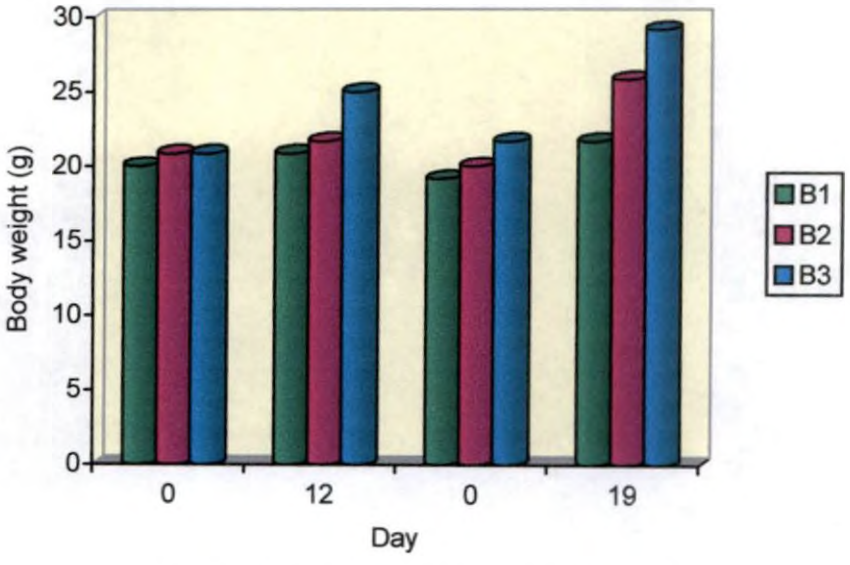


Fig. 3. Effect of aqueous extract of *E. officinalis* on body weight

higher body weight compared to the zero day. The mean body weights recorded on the 19th day for groups A_1, A_2, A_3, B_1, B_2, B_3 were 21.67±2.58, 24.17±3.76, 25.83±3.76, 21.67±2.58, 25.83±3.76 and 29.17±3.76 g respectively.

4.2.1.2 *Organ Weight*

The weight of the internal organs like spleen, kidney and liver were taken on 12th and 19th day of drug/vehicle treatment and expressed as relative organ weights (Tables 4, 5, 6; Fig. 4, 5).

The weight of spleen of the groups A_3 and B_3 was found to be significantly ($P<0.05$) higher than the control groups A_1 and B_1 on 19th day of the experiment. Group A_3 showed a significant increase in spleen weight even on 12th day.

The percentage increase in organ weights after drug treatment are presented in Table 7 and 8. Weight of spleen increased to a maximum of 68.65 percent in alcoholic extract treated group and 71.34 percent in aqueous extract treated group compared to controls.

The mean value for the relative weight of spleen of groups A_1, A_2, A_3, B_1, B_2 and B_3 on 19th day of the experiment were 0.303±0.03, 0.389±0.09, 0.511±0.13, 0.356±0.04, 0.413±0.08 and 0.610±0.15 g/100g body weight respectively. Appreciable variation was not seen in the weight of kidney and liver in the drug treated groups from their controls

4.2.2 Haematological Parameters

4.2.2.1 *Total Leukocyte Count*

Total leukocyte count of all the mice in groups A_1, A_2, A_3, B_1, B_2 and B_3 were recorded on zero, five, 12 and 19th day of the experiment. The individual and mean values are presented in Table 9 and 10. The mean values are graphically represented in Fig. 6 and 7.

The highest WBC count observed was in treatment groups A_3 and B_3 on 19th day of the extract administration which were 12.77±0.78 and 13.90±2.05 $x10^3$/cu.

Table 4. Effect of *Emblica officinalis* extracts on weight of spleen, g/100g body weight

Alcoholic Extract							Aqueous Extract					
Sl.No	12th day			19th day			12th day			19th day		
	A_1	A_2	A_3	A_1	A_2	A_3	B_1	B_2	B_3	B_1	B_2	B_3
1	0.310	0.334	0.430	0.299	0.520	0.457	0.382	0.365	0.438	0.335	0.468	0.817
2	0.296	0.299	0.364	0.256	0.284	0.392	0.297	0.387	0.434	0.387	0.434	0.520
3	0.328	0.481	0.340	0.314	0.315	0.667	0.335	0.384	0.384	0.384	0.364	0.763
4	0.271	0.286	0.375	0.298	0.326	0.539	0.269	0.281	0.264	0.281	0.289	0.483
5	0.311	0.323	0.450	0.350	0.425	0.656	0.350	0.373	0.528	0.373	0.524	0.593
6	0.293	0.357	0.415	0.298	0.465	0.356	0.314	0.396	0.401	0.376	0.401	0.484
Mean ± SD	0.302 $\pm 0.02^{B}$	0.347 $\pm 0.07^{B}$	0.396 $\pm 0.04^{A}$	0.303 $\pm 0.03^{B}$	0.389 $\pm 0.09^{B}$	0.511 $\pm 0.13^{A}$	0.324 ± 0.04	0.365 ± 0.04	0.408 ± 0.09	0.356 $\pm 0.04^{B}$	0.413 $\pm 0.08^{B}$	0.610 $\pm 0.15^{A}$

(Means bearing the same superscript do not differ significantly at $P<0.05$)

Table 5. Effect of *Emblica Officinalis* extracts on weight of kidney, g/100g body weight

Alcoholic Extract							Aqueous Extract					
Sl.No	12^{th} day			19^{th} day			12^{th} day			19^{th} day		
	A_1	A_2	A_3	A_1	A_2	A_3	B_1	B_2	B_3	B_1	B_2	B_3
1	1.900	2.145	1.400	1.505	2.468	1.820	1.483	2.468	1.810	1.600	1.710	1.810
2	1.124	1.681	1.165	1.295	1.141	1.750	1.648	1.141	1.740	1.541	1.650	1.740
3	1.361	1.164	1.560	1.596	1.701	2.320	2.016	1.701	2.310	2.364	2.421	2.310
4	1.242	1.013	1.584	1.640	1.344	1.960	1.136	1.344	1.960	1.820	1.468	1.936
5	1.068	1.268	2.060	1.930	1.268	1.420	1.640	1.268	1.420	1.612	1.371	1.420
6	2.142	1.661	1.505	1.640	1.974	1.034	1.814	1.974	1.031	1.470	1.924	1.570
Mean $\pm$ SD	1.47 $\pm$ 0.44	1.49 $\pm$ 0.42	1.55 $\pm$ 0.29	1.60 $\pm$ 0.21	1.65 $\pm$ 0.51	1.72 $\pm$ 0.44	1.62 $\pm$ 0.30	1.65 $\pm$ 0.51	1.71 $\pm$ 0.44	1.74 $\pm$ 0.33	1.76 $\pm$ 0.38	1.80 $\pm$ 0.31

Table 6. Effect of *Emblica officinalis* extracts on weight of liver, g/100g body weight

Sl.No	Alcoholic Extract						Aqueous Extract					
	12^{th} day			19^{th} day			12^{th} day			19^{th} day		
	A_1	A_2	A_3	A_1	A_2	A_3	B_1	B_2	B_3	B_1	B_2	B_3
1	7.84	7.48	7.24	7.34	9.12	8.68	5.29	5.24	7.68	9.46	7.91	9.63
2	5.64	7.56	8.14	5.89	7.56	5.46	7.91	8.53	9.46	8.14	5.38	8.78
3	6.96	7.26	5.48	6.48	6.68	9.03	8.49	8.64	6.64	6.48	9.69	6.36
4	6.24	5.87	6.58	8.64	8.26	7.72	6.68	7.62	8.14	5.38	8.24	7.06
5	8.12	8.44	9.04	8.73	8.18	7.46	8.56	9.04	6.26	8.24	8.46	7.52
6	7.48	6.6	7.33	7.21	5.83	8.38	7.07	6.35	7.58	8.68	7.48	8.28
Mean ± SD	7.05 ± 0.96	7.20 ± 0.88	7.30 ± 1.23	7.38 ± 1.14	7.61 ± 1.19	7.79 ± 1.28	7.33 ± 1.25	7.57 ± 1.49	7.63 ± 1.14	7.73 ± 1.51	7.57 ± 1.49	7.63 ± 1.14

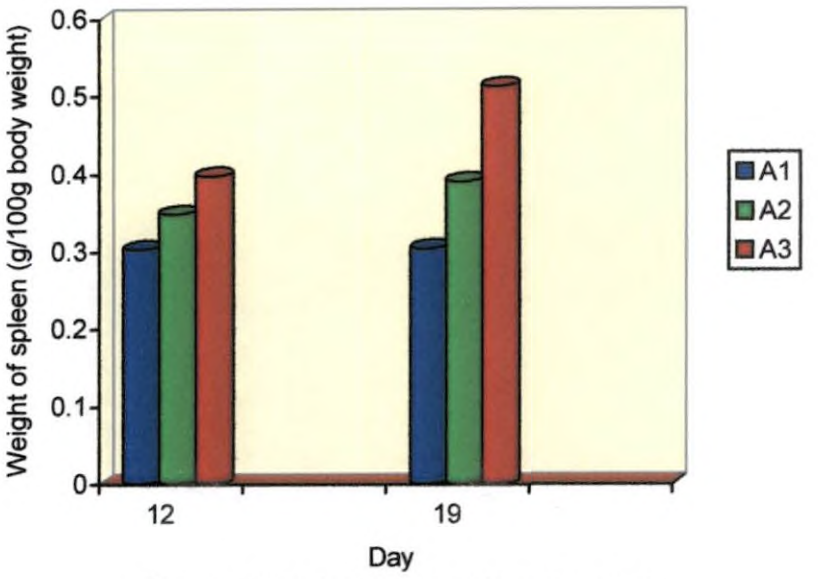


Fig. 4. Effect of alcoholic extract of *E. officinalis* on relative weight of spleen

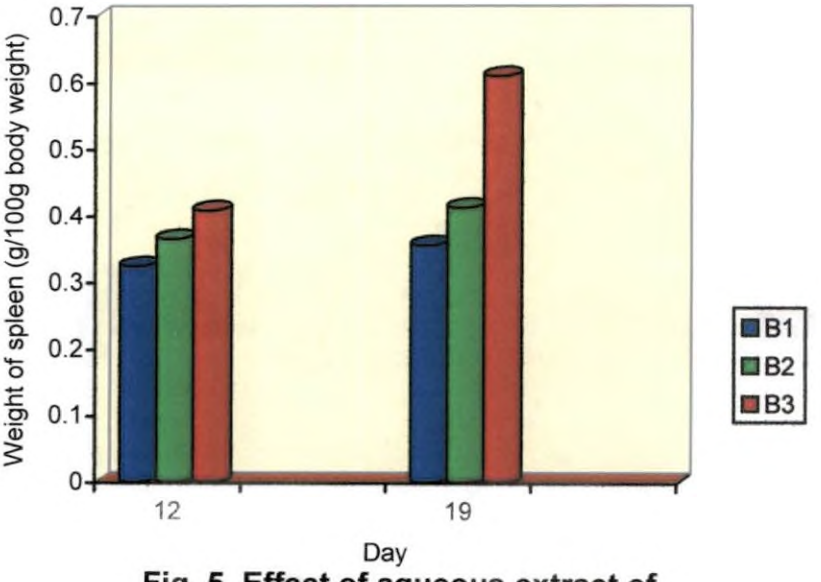


Fig. 5. Effect of aqueous extract of *E. officinalis* on relative weight of spleen

Table 7. Percentage increase in organ weights (compared to control) after alcoholic extract treatment

Dose rate (group)	Spleen		Kidney		Liver	
	12^{th} day	19^{th} day	12^{th} day	19^{th} day	12^{th} day	19^{th} day
100mg/kg (A_2)	14.9	23.38	1.09	3.0	2.17	3.02
200mg/kg (A_3)	31.13	68.65	4.96	7.25	3.62	5.5

Table 8. Percentage increase in organ weights (compared to control) after aqueous extract treatment

Dose rate (group)	Spleen		Kidney		Liver	
	12^{th} day	19^{th} day	12^{th} day	19^{th} day	12^{th} day	19^{th} day
100mg/kg (B_2)	12.65	16.01	1.6	1.26	3.2	1.6
200mg/kg (B_3)	25.92	71.34	5.48	3.63	4.01	2.69

Table 9. Effect of alcoholic extract of *Emblica officinalis* on total leukocyte count, x10^3/cu. mm

Sl.No	Zero day			Fifth day			12^{th} day			19^{th} day		
	A_1	A_2	A_3	A_1	A_2	A_3	A_1	A_2	A_3	A_1	A_2	A_3
1	6.3	8.0	7.4	6.5	7.0	7.4	7.1	9.2	10.5	7.6	10.1	13.0
2	5.8	5.3	6.1	6.0	6.6	6.5	7.3	7.6	9.8	6.7	9.1	13.4
3	7.5	7.6	6.3	7.2	7.5	6.3	6.0	10.0	9.6	6.6	10.9	11.9
4	6.3	8.0	7.4	6.5	7.0	7.4	7.1	9.2	10.5	7.6	10.1	13.0
5	5.8	5.3	6.1	6.0	6.6	6.5	7.3	7.6	9.8	8.0	9.1	13.4
6	7.5	7.6	6.3	7.2	7.5	6.3	6.0	10.0	9.6	6.6	10.9	11.9
Mean ± SD	6.53 ± 0.87	6.97 ± 1.46	6.60 ± 0.70	6.57 ± 0.60	7.03 ± 0.45	6.73 ± 0.59	6.80 ± 0.70^{C}	8.93 ± 1.22^{B}	9.97 ± 0.47^{A}	7.18 ± 0.72^{C}	10.03 ± 0.90^{B}	12.77 ± 0.78^{A}

(Means bearing the same superscript do not differ significantly at $P<0.05$)

Table 9. Effect of alcoholic extract of *Emblica officinalis* on total leukocyte count, x10^3/cu. mm

Sl.No	Zero day			Fifth day			12^{th} day			19^{th} day		
	A_1	A_2	A_3	A_1	A_2	A_3	A_1	A_2	A_3	A_1	A_2	A_3
1	6.3	8.0	7.4	6.5	7.0	7.4	7.1	9.2	10.5	7.6	10.1	13.0
2	5.8	5.3	6.1	6.0	6.6	6.5	7.3	7.6	9.8	6.7	9.1	13.4
3	7.5	7.6	6.3	7.2	7.5	6.3	6.0	10.0	9.6	6.6	10.9	11.9
4	6.3	8.0	7.4	6.5	7.0	7.4	7.1	9.2	10.5	7.6	10.1	13.0
5	5.8	5.3	6.1	6.0	6.6	6.5	7.3	7.6	9.8	8.0	9.1	13.4
6	7.5	7.6	6.3	7.2	7.5	6.3	6.0	10.0	9.6	6.6	10.9	11.9
Mean ± SD	6.53 ± 0.87	6.97 ± 1.46	6.60 ± 0.70	6.57 ± 0.60	7.03 ± 0.45	6.73 ± 0.59	6.80 ± 0.70^{C}	8.93 ± 1.22^{B}	9.97 ± 0.47^{A}	7.18 ± 0.72^{C}	10.03 ± 0.90^{B}	12.77 ± 0.78^{A}

(Means bearing the same superscript do not differ significantly at $P<0.05$)

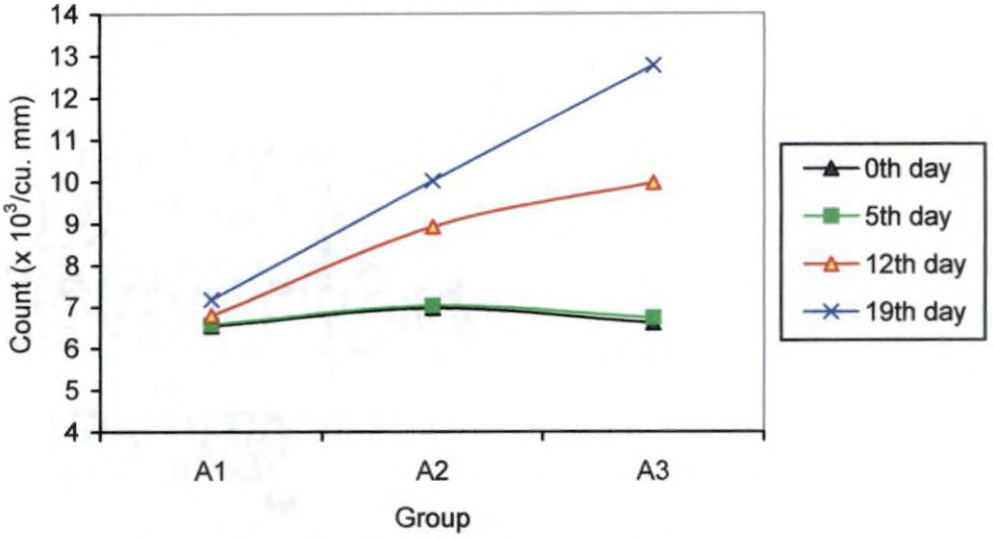


Fig. 6. Effect of alcoholic extract of *E.officinalis* on total leukocyte count

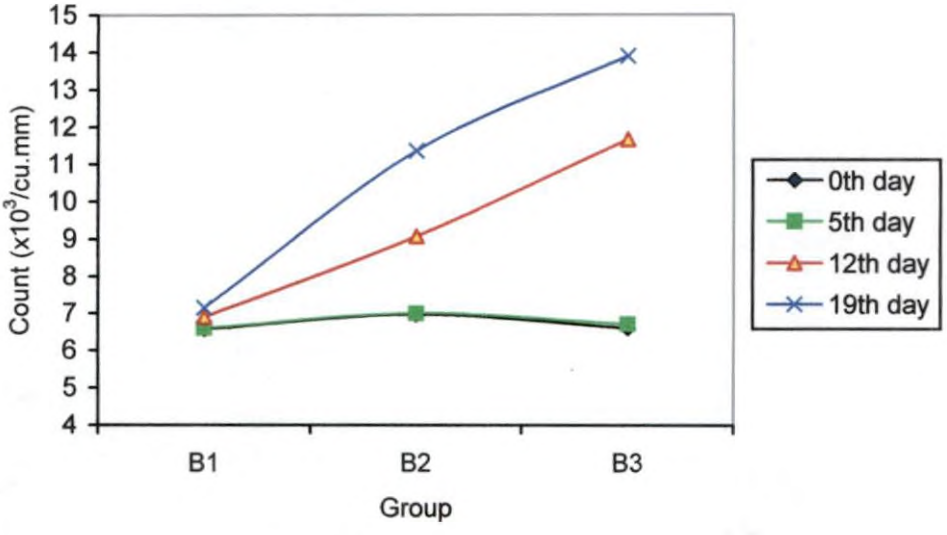


Fig. 7. Effect of aqueous extract of *E. officinalis* on total leukocyte count

mm respectively. The aqueous and alcoholic extracts at both dose rates produced significantly ($P<0.05$) higher leukocyte counts from the control groups on 12th and 19th day. The mean values of groups A_1, A_2, A_3, B_1, B_2 and B_3 on 12th day were 6.80±0.70, 8.93±1.22, 9.97±0.47, 6.90± 0.78, 9.07±1.17 and 11.67± 0.72 $x10^3$/ cu mm respectively and their corresponding values on 19th day were 7.18±0.72, 10.03±0.90, 12.77±0.78, 7.15±0.72, 11.37±1.16 and 13.9±2.05 $x10^3$/cu. mm respectively.

There was significant ($P<0.05$) increase in the total leukocyte count value of drug treated groups A_2,A_3, B_2 and B_3 on 12th and 19th day from their zero day value.

4.2.2.2 *Differential Leukocyte Count*

The differential leukocyte count of groups A_1, A_2, A_3, B_1, B_2 and B_3 was taken on zero, five, 12 and 19th day of the experiment. The results obtained are presented in Table 11 and 12.

4.2.2.2.1 Lymphocytes

The percentage distribution of lymphocyte in the drug/vehicle treatment group are presented in Table 11 and 12 and Fig. 8 and 9.

A significant ($P<0.05$) increase was noticed in extract treated groups A_3, B_2 and B_3 from the control groups (A_1, B_1) on 12th and 19th day of experiment, but the values on zero and fifth day were almost the same. The mean percentage distribution of lymphocytes in groups A_1, A_2, A_3, B_1, B_2 and B_3 on 12th day were 75.67±3.93, 77.17±4.26, 82.00±3.22, 74.33±3.61, 81.67±3.08, 83.83± 2.64 % respectively and the corresponding values on 19th day were 76.0±3.22, 81.33±5.20, 85.67±2.80, 75.33±4.32, 83.83±2.32, 89.33±1.63 %respectively.

A significant ($P<0.05$) increase in percentage lymphocyte distribution was noticed in drug treated groups (A_2, A_3, B_2, B_3) on 12th and 19th day compared to the zero day.

Table 11. Effect of alcoholic extract of *Emblica officinalis* on differential leukocyte count, %

	Zero day				Fifth day				12th day				19th day			
	L	N	E	M	L	N	E	M	L	N	E	M	L	N	E	M
A_1	78	20			76	23	1		70	29		1	77	22		1
	78	19	2	1	79	21			78	22			79	21		
	75	24		1	70	29		1	79	20			72	27	1	
	71	28		1	77	22		1	80	20	1		77	22		1
	72	28			79	21			73	25		2	79	21		
	75	25			70	29	1		74	25	1		72	27		1
Mean ± SD	74.83 ± 2.93	24.00 ± 3.85			75.17 ± 4.17	24.17 ± 3.82			75.67 ± 3.93^{B}	23.5 ± 3.51^{B}			76.00 ±3.22^{B}	23.33 ± 2.88^{A}		
A_2	74	26			77	22	1		73	27			87	13		
	77	23			79	21			77	23			81	19		
	74	25	1		74	26			82	18			76	23		1
	71	28		1	72	27	1		72	27	1		88	12		
	79	20		1	76	23	1		77	23			80	20		
	70	29	1		75	25			82	18			76	23		1
Mean ± SD	74.17 ± 3.43	25.17 ± 3.31			75.50 ± 2.43	24.00 ± 2.37			77.17 ± 4.26^{B}	22.67 ± 4.03^{A}			81.33 ±5.20^{B}	18.33 ± 4.80^{A}		
A_3	70	29		1	82	18			85	15			88	12		
	76	23	1		73	26		1	78	21		1	85	15		
	74	26			77	23			83	17			86	14		
	73	26		1	78	22			85	15			89	11		
	77	23			74	24	1	1	78	21	1		85	15		
	78	22			75	24	1		83	17			81	19		
Mean ± SD	74.67 ± 2.94	24.83 ± 2.64			76.5 ± 3.27	22.83 ± 2.71			82.00 ± 3.22^{A}	17.67 ± 2.73^{A}			85.67 ±2.80^{A}	14.33 ± 2.80^{B}		

(Means bearing the same superscript do not differ significantly at P<0.05)

Table 12. Effect of aqueous extract of *Emblica officinalis* on differential leukocyte count, %

	Zero day				Fifth day				12th day				19th day			
	L	N	E	M	L	N	E	M	L	N	E	M	L	N	E	M
B_1	72	27		1	77	23			75	24		1	78	22		
	80	20			72	26	1	1	78	22			70	28	1	1
	68	29		1	70	29	1		70	29	1		76	24		
	69	28	2	1	71	28		1	75	24	1		70	29	1	
	78	22			76	24			78	22			80	19		1
	68	30	1	1	70	29	1		70	29		1	78	22		
Mean ± SD	72.5 ± 5.28	26.0 ± 4.05			72.67 ± 3.08	26.50 ± 2.59			74.33 ± 3.61^{B}	25.00 ± 3.22^{A}			75.33 ± 4.32^{C}	24.00 ± 3.84^{A}		
B_2	75	25			77	23			79	20	1		86	14		
	76	24			73	26	1		80	19		1	83	16	1	
	69	30	1		71	29			85	15			82	18		
	75	25			77	23			78	21	1		87	13		
	76	24			73	26		1	83	16		1	84	15		1
	69	30	1		71	29			85	15			81	19		
Mean ± SD	73.38 ± 3.79	26.33 ± 2.87			73.67 ± 3.06	26.00 ± 2.68			81.67 ± 3.08^{A}	17.67 ± 2.66^{B}			83.83 ± 2.32^{B}	15.83 ± 2.32^{B}		
B_3	70	28	1	1	80	20			83	17			87	13		
	77	23			70	28	1		82	17		1	91	9		
	74	25			73	27			86	14			90	9	1	
	71	28	1		80	20			87	13			91	9		
	79	20		1	70	28	1		80	19	1		89	11		
	70	29	1		73	27			85	14		1	88	11	1	
Mean ± SD	73.5 ± 3.83	25.5 ± 3.50			74.33 ± 4.59	25.00 ± 3.90			83.83 ± 2.64^{A}	15.67 ± 2.34^{B}			89.33 ± 1.63^{A}	10.33 ± 1.63^{C}		

(Means bearing the same superscript do not differ significantly at P<0.05)

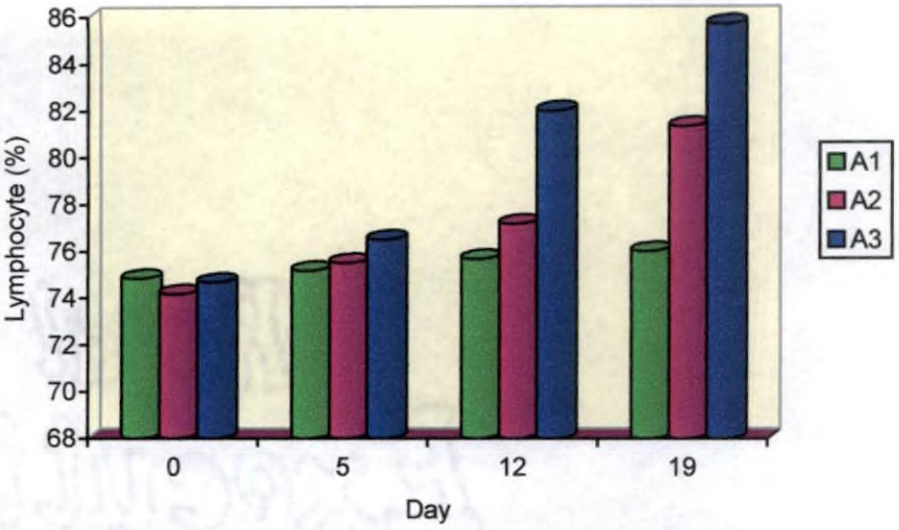


Fig. 8. Effect of alcoholic extract of *E.officinalis* on percentage distribution of lymphocytes

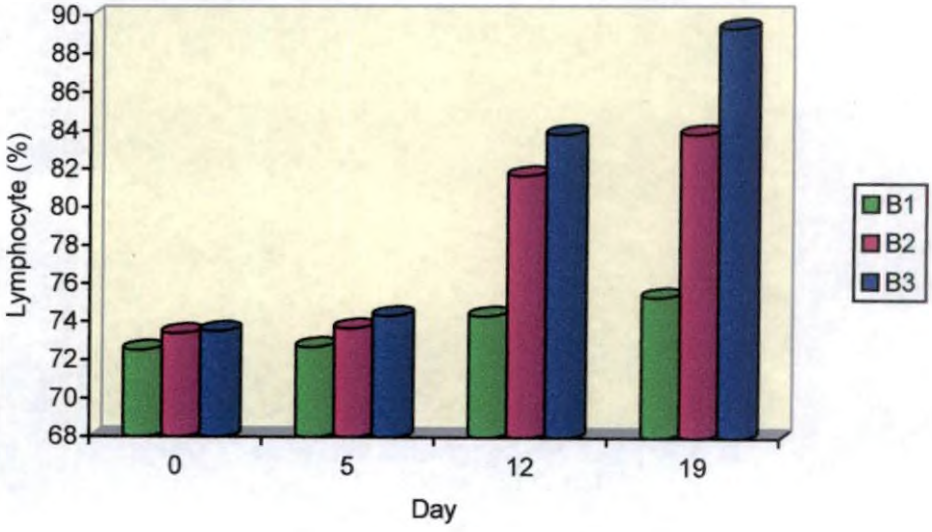


Fig. 9. Effect of aqueous extract of *E. officinalis* on percentage distribution of lymphocytes

4.2.2.2.2 Neutrophils

Mean percentage distribution of neutrophils in control and drug treated groups is presented in Table 11 and 12 .

The drug treated groups showed lower percentage distribution of neutrophils in the blood, but significant reduction from the control groups were recorded in groups A_3, B_2 and B_3 on both 12^{th} and 19^{th} day of the experiment. The mean percentage distribution of neutrophils in groups A_1, A_2, A_3, B_1, B_2 and B_3 on 12^{th} day were 23.5 ± 3.51, 22.67 ± 4.03, 17.67 ± 2.73, 25.00 ± 3.22, 17.67 ± 2.66 and 15.67 ± 2.34% respectively and their corresponding values on 19^{th} day were 23.33 ± 2.88, 18.33 ± 4.80, 14.33 ± 2.80, 24.00 ± 3.84, 15.83 ± 2.32 and 10.33 ± 1.63 % respectively.

The percentage neutrophil distribution on 12^{th} and 19^{th} day showed a significant decrease in drug treated groups A_2, A_3, B_2 and B_3 from their zero day value.

4.2.2.2.3 Eosinophils, Monocytes

The percentage distribution of eosinophils and monocytes on zero, five, 12 and 19^{th} day of the experiment did not differ significantly from the control. The data is presented in Table 11 and 12.

4.2.3 Biochemical Parameters

4.2.3.1 *Serum Total Protein*

The individual and mean values of serum protein concentration of drug treatment groups (A_2, A_3, B_2, B_3) and control groups (A_1, B_1) on zero, five, 12 and 19^{th} day of the experiment are given in table 13 and 14. The mean values are graphically presented in Fig. 10 and 11.

Significantly ($P<0.05$) higher values are recorded in the serum total protein of the extract treated groups on 19^{th} day of the experiment. The alcoholic extract could produce a significantly higher total protein value even on 12^{th} day of the experiment.

Table 13. Effect of alcoholic extract of *Emblica officinalis* on serum total protein, g/dl

Sl.No	Zero day			Fifth day			12th day			19th day		
	A_1	A_2	A_3	A_1	A_2	A_3	A_1	A_2	A_3	A_1	A_2	A_3
1	3.75	3.60	4.25	4.20	4.30	4.29	4.40	4.20	4.63	4.22	5.74	7.28
2	3.81	3.74	3.72	3.84	3.65	4.56	3.76	4.75	4.29	3.85	5.46	5.54
3	4.56	4.73	4.35	4.17	4.17	3.77	4.06	4.22	5.34	4.24	4.69	5.11
4	3.75	3.60	4.25	4.20	4.30	4.29	4.40	4.20	4.63	4.22	5.74	7.28
5	3.81	3.74	3.72	3.84	3.65	4.56	3.76	4.75	4.29	3.85	5.46	5.54
6	4.56	4.73	4.35	4.17	4.17	4.77	4.06	4.22	5.34	4.24	4.69	5.11
Mean ± SD	4.04 ± 0.45	4.02 ± 0.62	4.11 ± 0.34	4.07 ± 0.20	4.04 ± 0.34	4.21 ± 0.40	4.07 ± 0.32^{B}	4.39 ± 0.31^{AB}	4.75 ± 0.22^{A}	4.10 ± 0.22^{B}	5.30 ± 0.54^{A}	5.98 ± 1.15^{A}

(Means bearing the same superscript do not differ significantly at $P<0.05$)

Table 14. Effect of aqueous extract of *Emblica officinalis* on serum total protein, g/dl

Sl.No	Zero day			Fifth day			12^{th} day			19^{th} day		
	B_1	B_2	B_3	B_1	B_2	B_3	B_1	B_2	B_3	B_1	B_2	B_3
1	3.78	4.69	4.60	3.57	4.20	4.61	3.63	4.00	5.18	3.66	5.09	6.93
2	3.76	3.55	3.68	4.13	4.30	3.95	4.17	5.29	5.68	4.08	5.06	5.84
3	4.83	4.42	4.41	4.69	4.28	4.42	4.69	3.75	4.15	4.95	6.73	6.33
4	3.78	4.69	4.60	3.57	4.20	4.61	3.63	4.00	5.18	3.66	5.09	6.93
5	3.76	3.55	3.68	4.13	4.30	3.95	4.17	5.29	5.68	4.08	5.06	5.84
6	4.83	4.42	4.41	4.69	4.28	4.42	4.69	3.75	4.15	4.95	6.73	6.33
Mean ± SD	4.12 ± 0.61	4.22 ± 0.60	4.23 ± 0.49	4.13 ± 0.56	4.26 ± 0.05	4.33 ± 0.34	4.16 ± 0.53	4.35 ±0.83	5.07 ± 0.87	4.23 ± 0.67^{B}	5.63 ± 0.96^{A}	6.37 ± 0.55^{A}

(Means bearing the same superscript do not differ significantly at $P<0.05$)

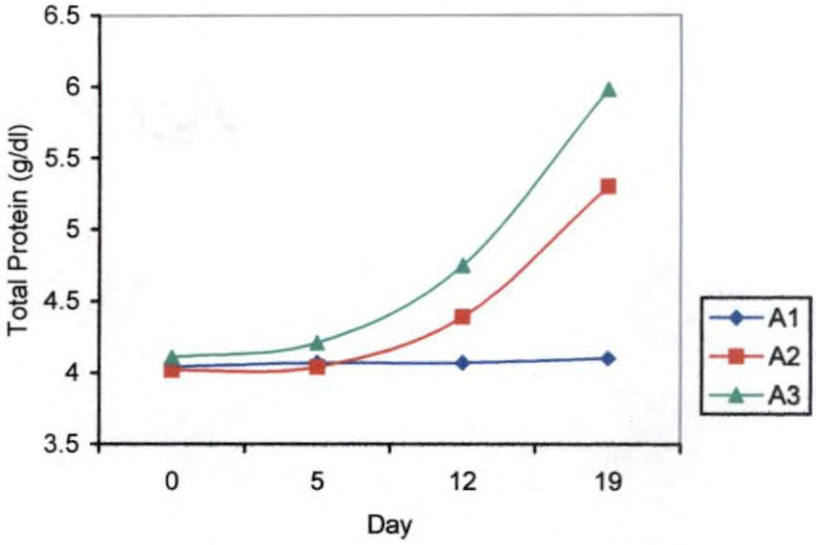


Fig 10. Effect of alcoholic extract of *E. officinalis* on serum total protein

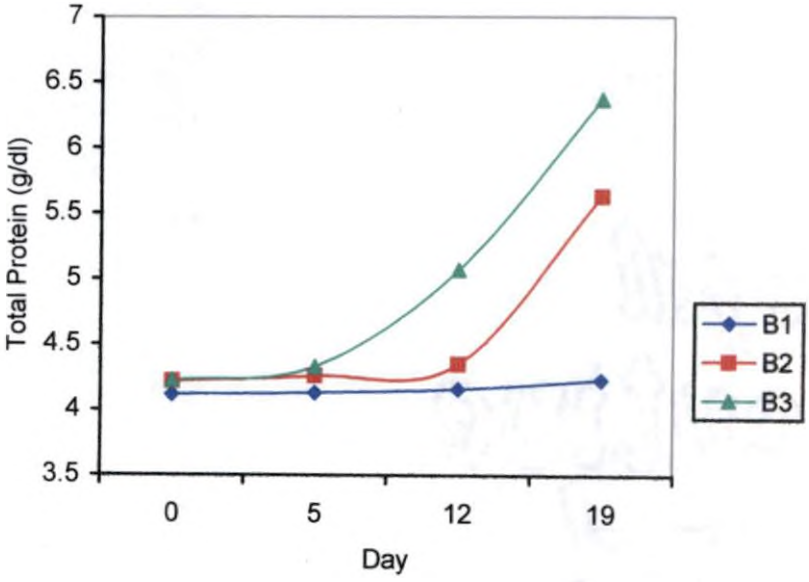


Fig 11. Effect of aqueous extract of *E. officinalis* on serum total protein

The mean values of the treatment groups vary from 4.02±0.62 to 6.37±0.55 g/dl. The maximum total protein value was seen for group B_3 on 19th day of extract administration.

The serum total protein value obtained for extract treated groups at both dose rates showed a significant ($P<0.05$) increase on 19th day from their zero day value. The 19th day total protein value of groups A_1, A_2, A_3, B_1, B_2 and B_3 were 4.10±0.22, 5.30±0.54, 5.98±1.15, 4.23±0.67, 5.63±0.96 and 6.37±0.55 g/dl respectively.

4.2.3.2 *Globulin*

The individual and mean globulin value in serum of all the groups (A_1, A_2, A_3, B_1, B_2, B_3) on zero, five, 12 and 19th day of the experiment are shown in Table 15, 16 and graphically in Fig. 12 and 13.

The mean values of groups A_2, A_3, B_2 and B_3 on 19th day were significantly ($P<0.05$) higher than their control groups A_1 and B_1. The mean globulin value noticed on 19th day were 1.28±0.07, 1.63±0.23, 1.88±0.42, 1.29±0.10, 1.74±0.27 and 1.97±0.21 g/dl for groups A_1, A_2, A_3 B_1, B_2 and B_3 respectively. The highest value was observed after 19 days of aqueous extract administration at the rate of 200mg/kg body weight. However, A_3 and B_3 could produce a significant increase in globulin on 12th day also.

The extracts administered at the rate of 200mg/kg level had produced a significantly higher value on 12th and 19th day from their zero day value. The mean globulin value for the groups A_1, A_2, A_3, B_1, B_2 and B_3 on 12th day were 1.26±0.17, 1.37±0.05, 1.49±0.24, 1.27±0.10, 1.33±0.23 and 1.56±0.22 g/dl respectively.

4.2.4 Immunological Parameters

4.2.4.1 *Haemagglutination*

Haemagglutination titre of sera of the experimental mice were taken on zero, 12th and 19th day of the experiment (Fig. 14). The titre values obtained are presented in Table17 and 18 and Fig. 15 and 16.

Table 15. Effect of alcoholic extract of *Emblica officinalis* on serum globulin, g/dl

Sl.No	Zero day			Fifth day			12^{th} day			19^{th} day		
	A_1	A_2	A_3	A_1	A_2	A_3	A_1	A_2	A_3	A_1	A_2	A_3
1	1.25	1.20	1.41	1.20	1.26	1.34	1.43	1.41	1.51	1.24	1.84	2.36
2	1.19	1.17	1.14	1.18	1.14	1.52	1.09	1.39	1.24	1.25	1.67	1.70
3	1.34	1.39	1.28	1.39	1.38	1.08	1.27	1.32	1.72	1.36	1.38	1.59
4	1.25	1.20	1.41	1.20	1.26	1.34	1.43	1.41	1.51	1.24	1.84	2.36
5	1.19	1.17	1.14	1.18	1.14	1.52	1.09	1.39	1.24	1.25	1.67	1.70
6	1.34	1.39	1.28	1.39	1.38	1.08	1.27	1.32	1.72	1.36	1.38	1.59
Mean ± SD	1.26 ± 0.08	1.25 ± 0.12	1.28 ± 0.14	1.26 ± 0.12	1.26 ± 0.12	1.31 ± 0.22	1.26 ± 0.17^{B}	1.37 ± 0.05^{AB}	1.49 ± 0.24^{A}	1.28 ± 0.07^{B}	1.63 ± 0.23^{A}	1.88 ± 0.42^{A}

(Means bearing the same superscript do not differ significantly at $P<0.05$)

Table 16. Effect of aqueous extract of *Emblica officinalis* on serum globulin, g/dl

Sl.No	Zero day			Fifth day			12^{th} day			19^{th} day		
	B_1	B_2	B_3	B_1	B_2	B_3	B_1	B_2	B_3	B_1	B_2	B_3
1	1.26	1.43	1.41	1.19	1.40	1.43	1.20	1.30	1.64	1.22	1.53	2.20
2	1.14	1.13	1.16	1.18	1.24	1.23	1.22	1.58	1.72	1.25	1.64	1.80
3	1.38	1.30	1.29	1.38	1.27	1.30	1.38	1.12	1.31	1.40	2.04	1.92
4	1.26	1.43	1.41	1.19	1.40	1.43	1.20	1.30	1.64	1.22	1.53	2.20
5	1.14	1.13	1.16	1.18	1.24	1.23	1.22	1.58	1.72	1.25	1.64	1.80
6	1.38	1.30	1.29	1.38	1.27	1.3	1.38	1.12	1.31	1.40	2.04	1.92
Mean ± SD	1.26 ± 0.12	1.29 ± 0.15	1.29 ± 0.13	1.25 ± 0.11	1.30 ± 0.09	1.32 ± 0.10	1.27 $\pm 0.10^{B}$	1.33 $\pm 0.23^{B}$	1.56 $\pm 0.22^{A}$	1.29 $\pm 0.10^{C}$	1.74 $\pm 0.27^{B}$	1.97 $\pm 0.21^{A}$

(Means bearing the same superscript do not differ significantly at $P<0.05$)

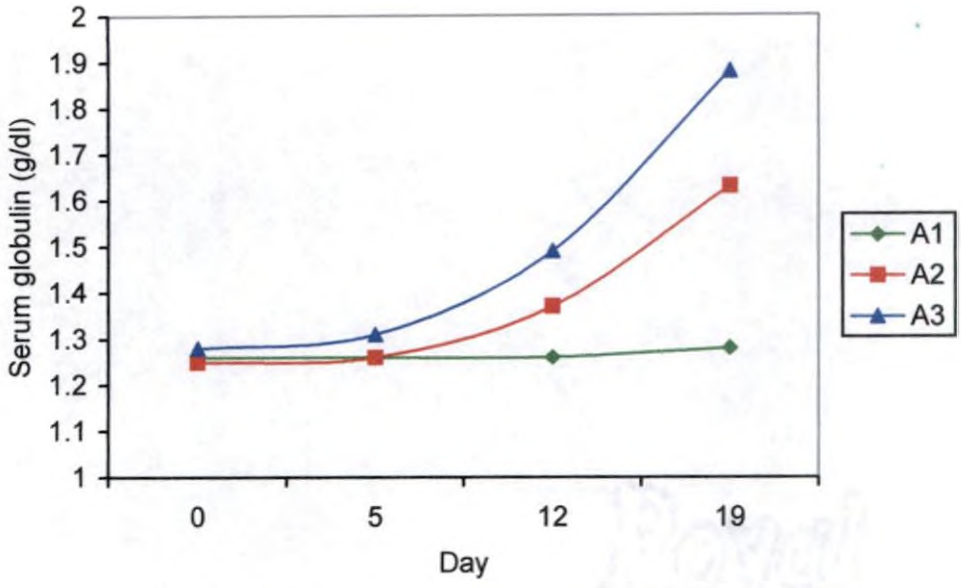


Fig.12. Effect of alcoholic extract of *E. officinalis* on serum globulin

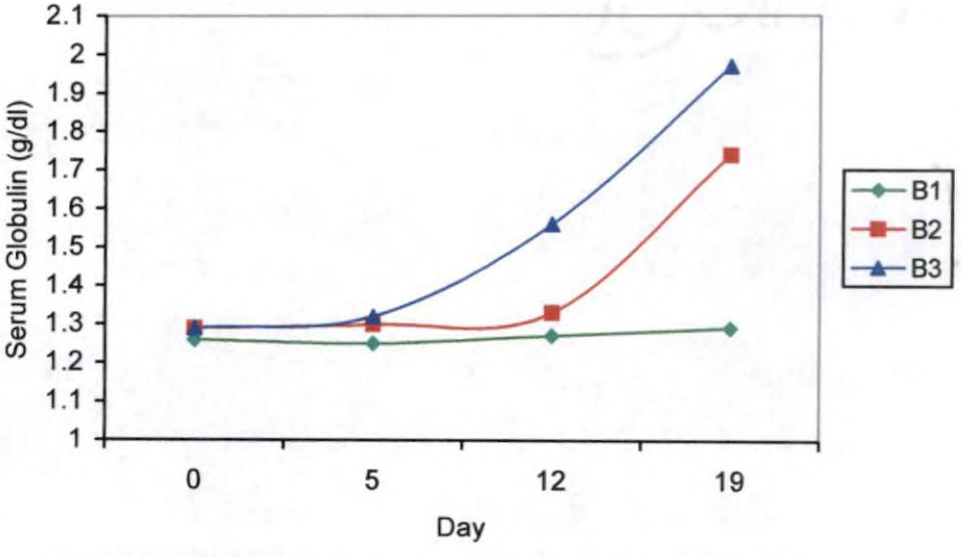


Fig.13. Effect of aqueous extract of *E. officinalis* on serum globulin

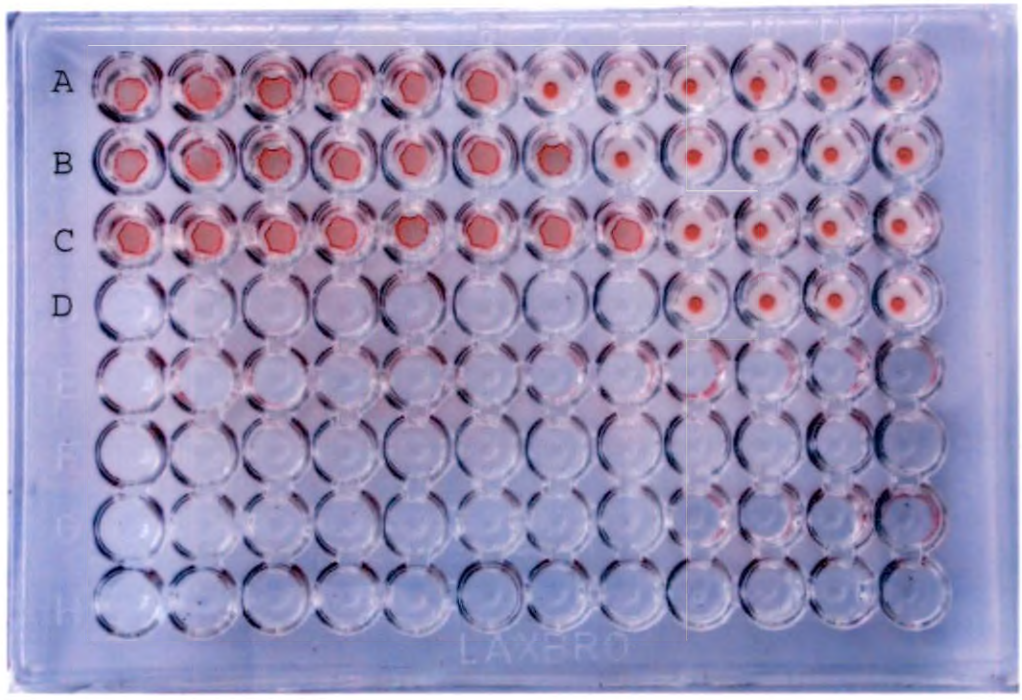


Fig. 14. Haemagglutination test: (A) Control group
(B) Treatment group (100mg extract/kg body weight)
(C) Treatment group (200mg extract/kg body weight)
(D) RBC control

Table 17. Effect of alcoholic extract of *Emblica officinalis* on haemagglutination titre

Sl. No	Zero day			12th day			19th day		
	A_1	A_2	A_3	A_1	A_2	A_3	A_1	A_2	A_3
1	2	2	2	32	32	64	32	64	128
2	4	4	4	32	64	128	32	64	128
3	4	2	4	64	64	128	64	128	256
4	2	2	2	32	32	64	32	64	128
5	4	4	4	32	64	128	32	64	128
6	4	2	4	64	64	128	64	128	256
Mean ± SD	3.33 ± 1.16	2.67 ± 1.16	3.33 ± 1.16	42.67 ± 18.48^{B}	53.33 ± 18.48^{B}	106.67 ± 36.95^{A}	42.67 ± 18.48^{B}	85.33 ± 36.95^{B}	170.67 ± 73.90^{A}

(Means bearing the same superscript do not differ significantly at $P<0.05$)

Table 18. Effect of aqueous extract of *Emblica officinalis* on haemagglutination titre

Sl. No	Zero day			12th day			19th day		
	B_1	B_2	B_3	B_1	B_2	B_3	B_1	B_2	B_3
1	2	2	2	32	64	128	32	64	256
2	4	2	4	32	64	256	64	128	256
3	4	4	4	64	128	256	64	128	512
4	2	2	2	32	64	128	32	64	256
5	4	2	4	32	64	256	64	128	256
6	4	4	4	64	128	256	64	128	512
Mean ± SD	3.33± 1.16	2.67 ± 1.16	3.33 ± 1.16	2.67 ± 18.48^{B}	85.33 ± 36.95^{B}	213.33 ± 73.90^{A}	53.33 ± 18.48^{B}	106.67 ± 36.95^{B}	341.33 ± 147.80^{A}

(Means bearing the same superscript do not differ significantly at $P<0.05$)

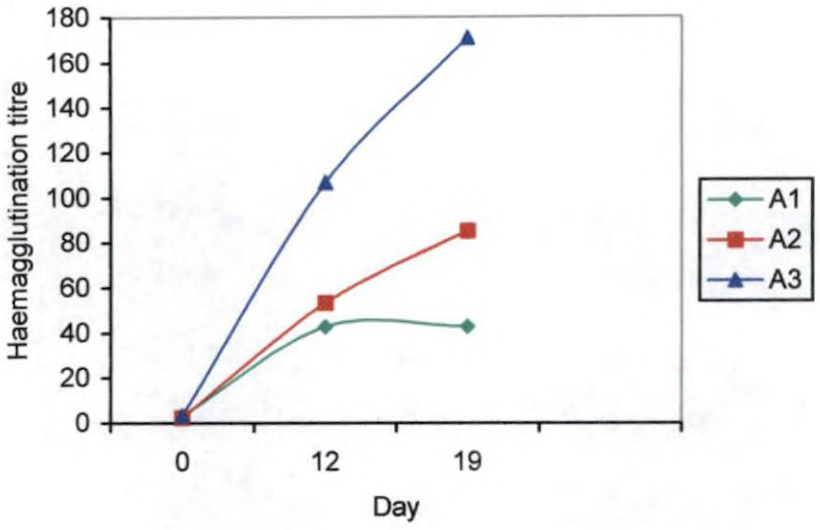


Fig. 15. Effect of alcoholic extract of *E. officinalis* on haemagglutination titre

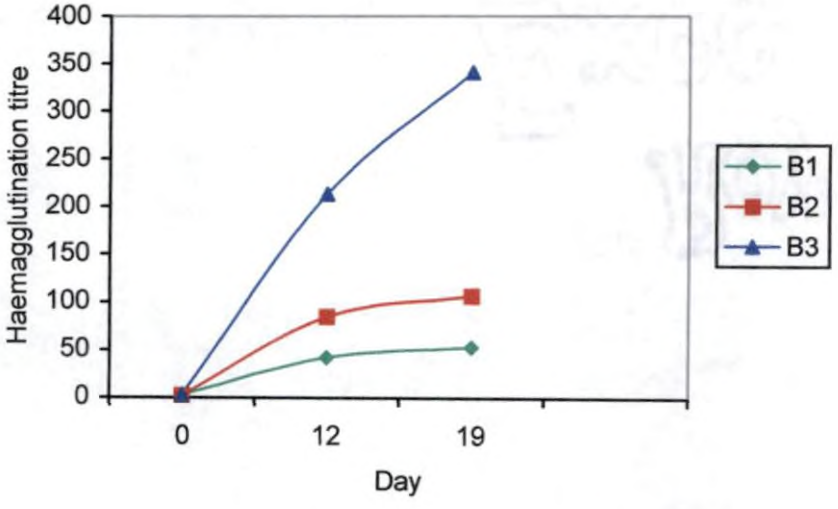


Fig. 16. Effect of aqueous extract of *E. officinalis* on haemagglutination titre

The maximum titre level observed during the experimental period was on 19th day for group B_3. The treatment groups showed a dose dependent increase in the titre values on 12th and 19th day of experiment. The mean haemagglutination titres of groups A_1, A_2, A_3, B_1, B_2 and B_3 noticed on 12th day were 42.67±18.48, 53.33±18.48, 106.67±36.95, 42.67±18.48, 85.33±36.95 and 213.33±73.90 respectively.

A significant ($P<0.05$) difference was noticed between 12th and 19th day titre values of groups A_3 and B_3.

4.2.4.2. Delayed Type of Hypersensitivity

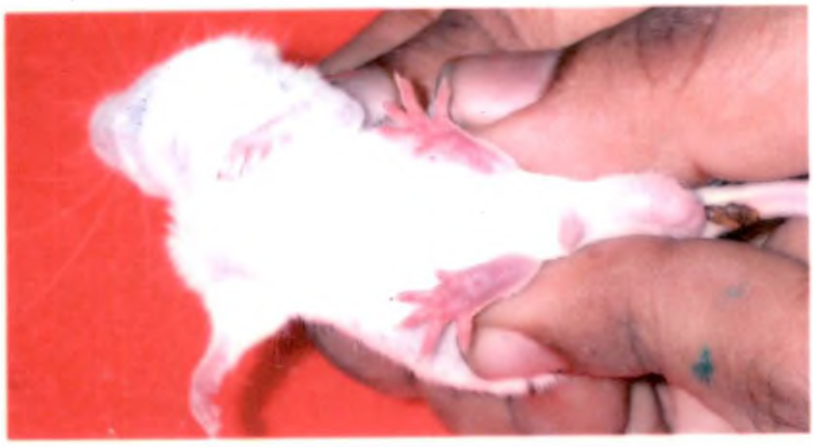
Delayed Type of Hypersensitivity (DTH) was measured as the increase in thickness of the foot pad injected with SRBC as compared to the control foot pad of the same mouse injected with saline (Plate 1). Footpad thickness was taken as the mean of six readings. The increase in footpad thickness of the treatment and control groups on 12th and 19th day are presented in Table 19 and Fig.17 and 18.

The mean values of the treatment groups C_2, C_3, D_2 and D_3 were significantly ($P<0.05$) higher than their control groups C_1 and D_1. The maximum DTH response was seen in aqueous extract (200mg/kg) treated group. The mean values obtained on 19th day for the groups C_1, C_2, C_3, D_1, D_2 and D_3 were 0.56±0.10, 1.42±0.13, 1.62±0.14, 0.70±0.14, 1.67±0.23 and 1.98±0.46 mm respectively.

The DTH response values obtained for the drug/vehicle treatment groups did not differ significantly between 12th and 19th day.

4.2.4.3 Macrophage Migration Index

Macrophages were isolated from peritoneal washing of drug/vehicle treatment groups (A_1, A_2, A_3, B_1, B_2, B_3) without red cell contamination. On an average 90 percent cells were found to be viable by dye exclusion method. The macrophage suspension prepared at a concentration of 60-80 $x10^6$ cells/ml did not show clumping or reduction in viability.



A

B

C

Plate 1. Delayed hypersensitivity reaction on footpad of mice:
(A) Control group (B) Treatment group (100mg extract/kg body weight)
(C) Treatment group (200mg extract/kg body weight)

Table 19. Effect of *Emblica officinalis* extracts on delayed hypersensitivity response, mm

Alcoholic Extract							Aqueous Extract					
Sl.No	12th day			19th day			12th day			19th day		
	C_1	C_2	C_3	C_1	C_2	C_3	D_1	D_2	D_3	D_1	D_2	D_3
1	0.47	1.33	1.73	0.63	1.6	1.6	0.6	1.3	1.8	0.8	1.6	1.9
2	0.5	1.03	1.6	0.53	1.43	1.76	0.5	1.4	1.6	0.8	1.9	2.1
3	0.4	1.2	1.13	0.6	1.4	1.36	0.6	1.4	2.0	0.6	1.9	2.3
4	0.3	0.36	1.5	0.7	1.46	1.66	0.6	1.6	2.4	0.5	1.7	1.7
5	0.5	1.43	1.43	0.5	1.2	1.7	0.4	1.3	1.6	0.8	1.6	1.3
6	0.4	1.2	1.5	0.42	1.43	1.66	0.7	1.6	1.6	0.7	1.3	2.6
Mean ± SD	0.43 ± 0.08^C	1.26 ± 0.14^B	1.48 ± 0.20^A	0.56 ± 0.10^C	1.42 ± 0.13^B	1.62 ±0.14^A	0.57 ± 0.10^C	1.43 ± 0.14^B	1.83 ±0.32^A	0.70 ± 0.14^B	1.67 ± 0.23^A	1.98 ± 0.46^A

(Means bearing the same superscript do not differ significantly at P<0.05)

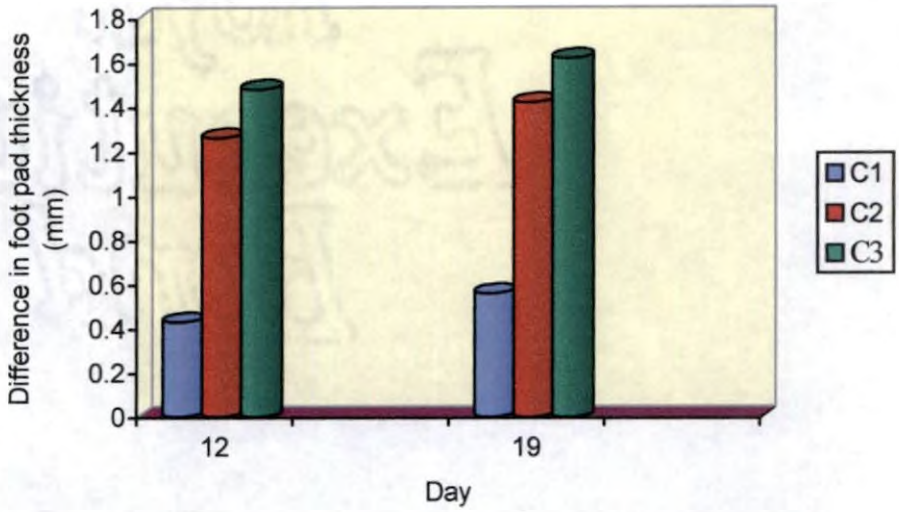


Fig. 17. Effect of alcoholic extract of *E. officinalis* on delayed hypersensitivity response

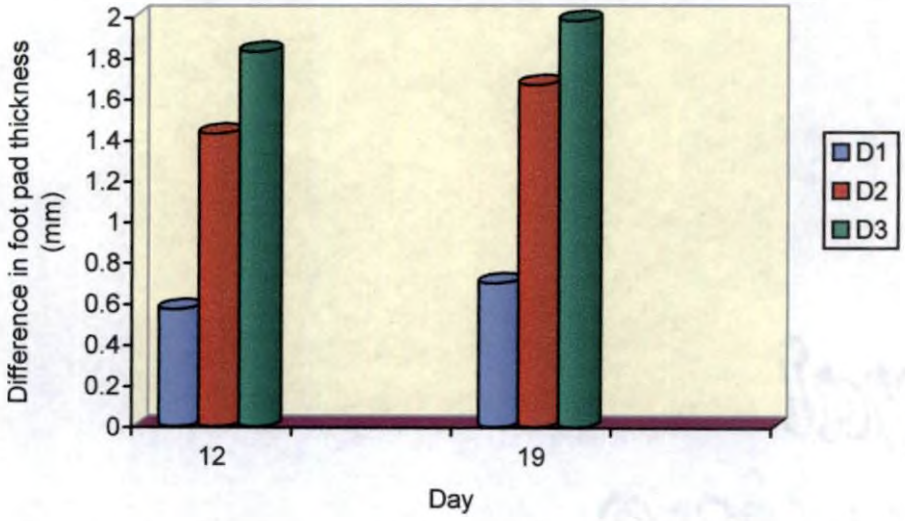


Fig. 18. Effect of aqueous extract of *E.officinalis* on delayed hypersensitivity response

Macrophage from all mice showed migration on the tissue culture plate and the zone of migration was well discernible. The zone of migration of cells appeared as circular opaque area around the capillary (Plate 2). The diameter of the area was measured at different angles viewing through a magnifying lens.

Macrophage Migration Index (MMI) of all mice was taken on 12^{th} and 19^{th} day of drug/ vehicle administration. The macrophage migration area of the drug treated groups (A_2, A_3, B_2, B_3) on 12^{th} and 19^{th} day of experiment showed significant ($P<0.05$) increase from the control groups A_1 and B_1. The individual and mean values of the migration area are shown in table 20 and graphically in Fig. 19 and 20. The Macrophage Migration Index for the drug treated groups are presented in Table 21 and Fig. 21 and 22.

The maximum MMI level was 3.71 in aqueous extract fed group and 2.36 in alcoholic extract fed group. The mean MMI values obtained for groups A_2, A_3, B_2 and B_3 on 12^{th} day were 1.36 ± 0.27, 1.65 ± 0.49, 1.76 ± 0.56 and 2.03 ± 0.74 respectively and their corresponding 19^{th} day values were 1.55 ± 0.36, 1.93 ± 0.41, 1.82 ± 0.45 and 2.48 ± 0.74 respectively. Thus generally a 1.5 to 2.5 fold increase in MMI was seen on extract administration.

4.2.4.5 Nitroblue Tetrazolium (NBT) Dye Reduction Test

Phagocytosis of particles by macrophage is usually accompanied by a burst of oxidative metabolism leading to the generation of reactive oxygen species which can be detected through an assay based on the reduction of NBT. The NBT readings of the control and treatment group is presented in Table 22 and Fig. 23 and 24.

The extract administration at both dose levels produced a significant($P<0.05$) increase in the NBT dye reduction test values from the controls on 19^{th} day, in a dose dependent manner. Thus the highest value was obtained for the group B_3 on 19^{th} day of the drug administration. Group A_3 and B_3 had a significantly higher NBT reading on 12^{th} day also. The mean values of NBT dye reduction test for the groups A_1, A_2, A_3, B_1, B_2 and B_3 on 12^{th} day were 0.310 ± 0.02, 0.331 ± 0.03, 0.417 ± 0.03,

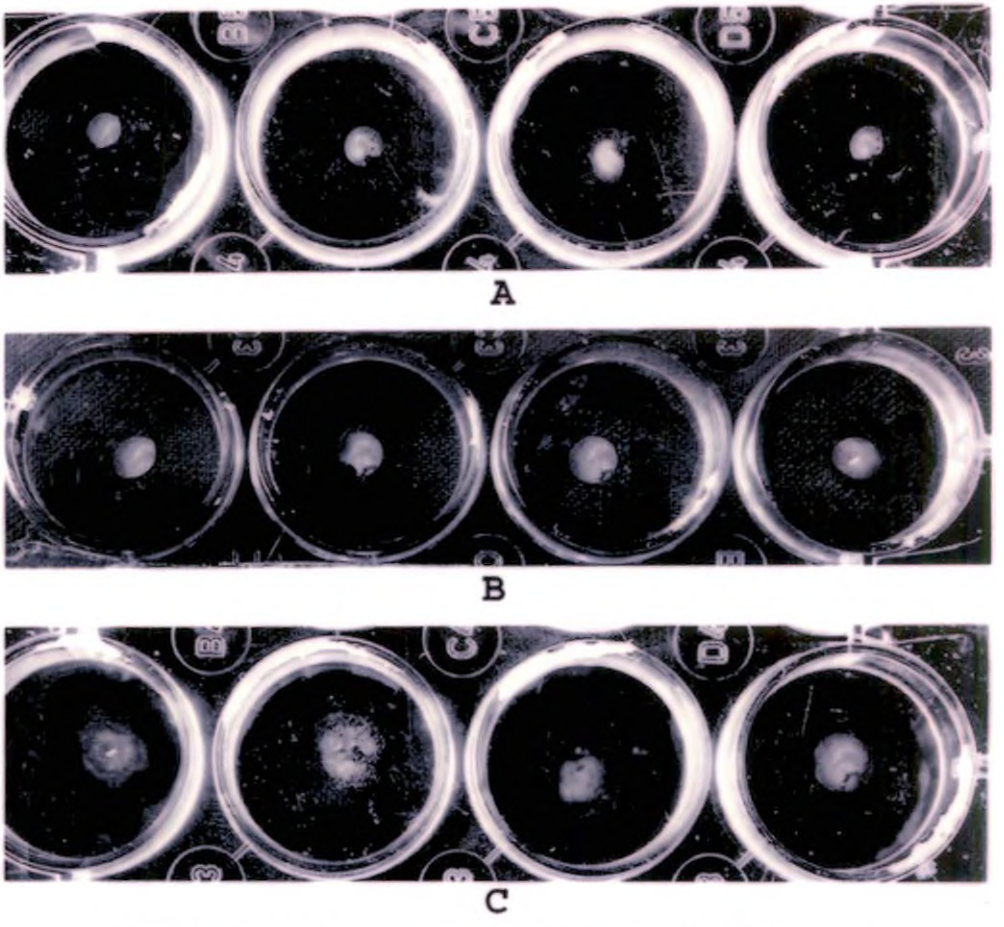


Plate 2. Macrophage migration test : (A) Control group
(B) Treatment group (100mg extract/kg body weight)
(C) Treatment group (200mg extract/kg body weight)

Table 20. Effect of *Emblica officinalis* extracts on macrophage migration area, mm^2

Alcoholic Extract							Aqueous Extract					
Sl.No	12^{th} day			19^{th} day			12^{th} day			19^{th} day		
	A_1	A_2	A_3	A_1	A_2	A_3	B_1	B_2	B_3	B_1	B_2	B_3
1	7.67	7.69	11.81	3.55	7.67	12.56	3.55	6.5	12.56	4.43	7.66	17.71
2	3.14	5.93	7.69	5.4	11.78	8.29	6.5	7.66	7.66	3.55	7.66	13.35
3	3.99	9.61	5.93	5.4	6.5	8.95	3.99	6.5	8.95	2.76	12.56	7.66
4	7.67	5.93	11.81	4.43	8.95	8.29	3.55	8.29	3.99	8.29	8.29	11.78
5	5.4	6.5	6.5	4.9	7.07	13.35	5.93	7.07	13.35	6.5	6.5	8.95
6	3.55	7.07	8.29	8.29	7.67	10.33	4.43	13.35	10.33	3.14	9.61	11.78
Mean ± SD	5.24 ± 2.03^{B}	7.12 ± 1.4^{AB}	8.67 ± 2.57^{A}	5.33 ± 1.61^{B}	8.27 ± 1.90^{A}	10.93 ± 2.21^{A}	4.66 ± 1.26^{B}	8.23 ± 2.60^{A}	9.47 ± 3.43^{A}	4.78 ± 2.18^{B}	8.71 ± 2.14^{A}	11.87 ± 3.54^{A}

(Means bearing the same superscript do not differ significantly at $P<0.05$)

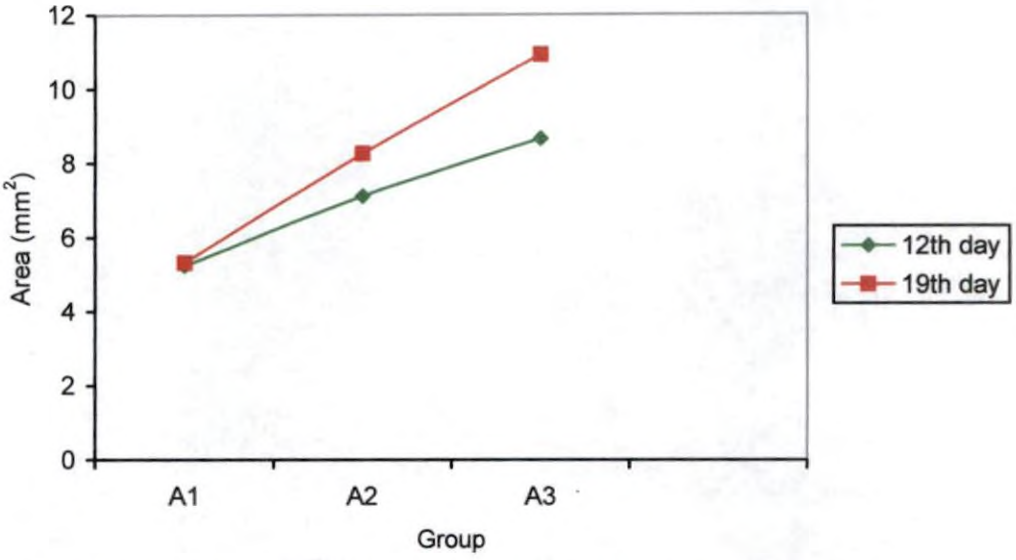


Fig. 19. Effect of alcoholic extract of *E.officinalis* on macrophage migration area

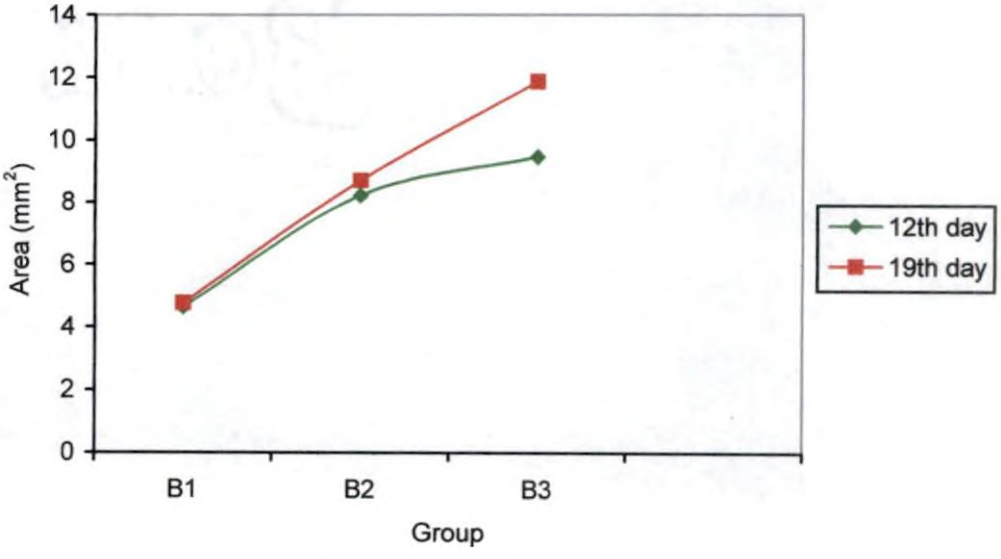


Fig. 20. Effect of aqueous extract of *E. officinalis* on macrophage migration area

Table 21. Macrophage migration index after *Emblica officinalis* extract treatment

Sl.No	Alcoholic Extract				Aqueous Extract			
	12^{th} day		19^{th} day		12^{th} day		19^{th} day	
	A_2	A_3	A_2	A_3	B_2	B_3	B_2	B_3
1	1.47	2.25	1.44	2.36	1.39	2.7	1.6	3.71
2	1.13	1.47	2.21	1.56	1.64	1.64	1.6	2.79
3	1.83	1.13	1.22	1.68	1.39	1.92	2.63	1.6
4	1.13	2.25	1.68	1.56	1.78	0.86	1.73	2.46
5	1.24	1.24	1.33	2.5	1.52	2.86	1.36	1.87
6	1.35	1.58	1.44	1.94	2.86	2.22	2.01	2.46
Mean $\pm$ SD	1.36$\pm$0.27	1.65$\pm$ 0.49	1.55$\pm$ 0.36	1.93$\pm$ 0.41	1.76$\pm$ 0.56	2.03$\pm$ 0.74	1.82$\pm$ 0.45	2.48$\pm$ 0.74

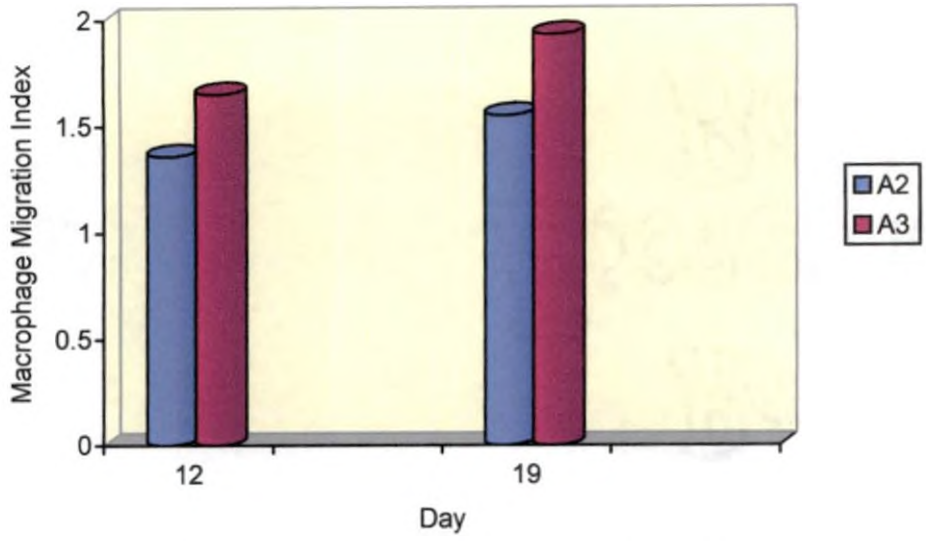


Fig. 21. Macrophage migration index after treatment with alcoholic extract of *E. officinalis*

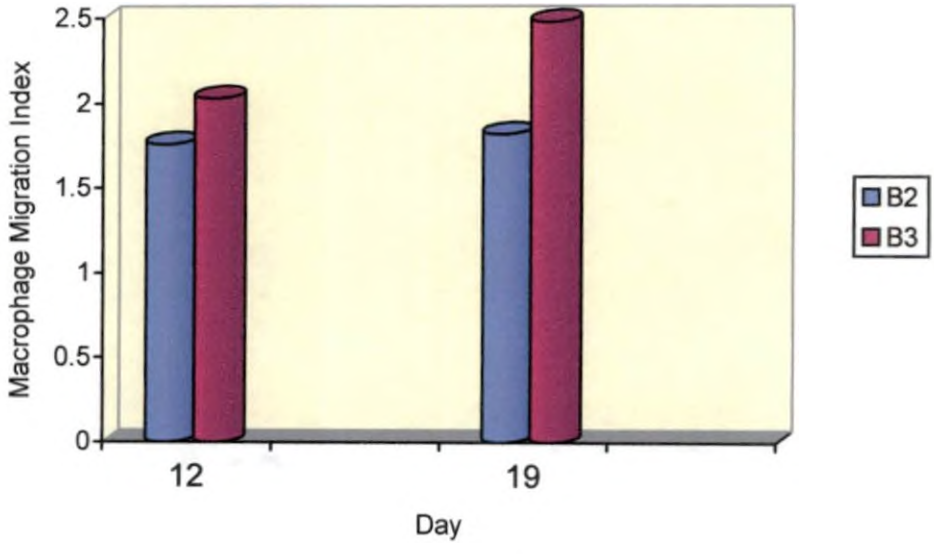


Fig. 22. Macrophage migration index after treatment with aqueous extract of *E. officinalis*

Table 22. Effect of *Emblica officinalis* extracts on nitroblue tetrazolium dye reduction test

Alcoholic Extract							Aqueous Extract					
Sl.No	12th day			19th day			12th day			19th day		
	A_1	A_2	A_3	A_1	A_2	A_3	B_1	B_2	B_3	B_1	B_2	B_3
1	0.272	0.300	0.448	0.393	0.358	0.401	0.272	0.308	0.433	0.383	0.399	0.507
2	0.323	0.322	0.400	0.345	0.386	0.458	0.323	0.353	0.465	0.394	0.445	0.576
3	0.336	0.330	0.416	0.372	0.416	0.521	0.349	0.388	0.421	0.349	0.438	0.505
4	0.305	0.364	0.398	0.289	0.401	0.484	0.305	0.360	0.411	0.305	0.413	0.550
5	0.311	0.358	0.468	0.246	0.420	0.514	0.311	0.291	0.454	0.361	0.398	0.494
6	0.313	0.311	0.374	0.323	0.376	0.496	0.313	0.310	0.471	0.313	0.428	0.599
Mean ± SD	0.310 ± 0.02^B	0.331 ± 0.03^B	0.417 ± 0.03^A	0.328 ± 0.05^C	0.393 ± 0.02^B	0.479 ± 0.04^A	0.312 ± 0.03^B	0.335 ± 0.04^B	0.443 ± 0.02^A	0.351 ± 0.04^C	0.420 ± 0.02^B	0.538 ± 0.04^A

(Means bearing the same superscript do not differ significantly at $P<0.05$)

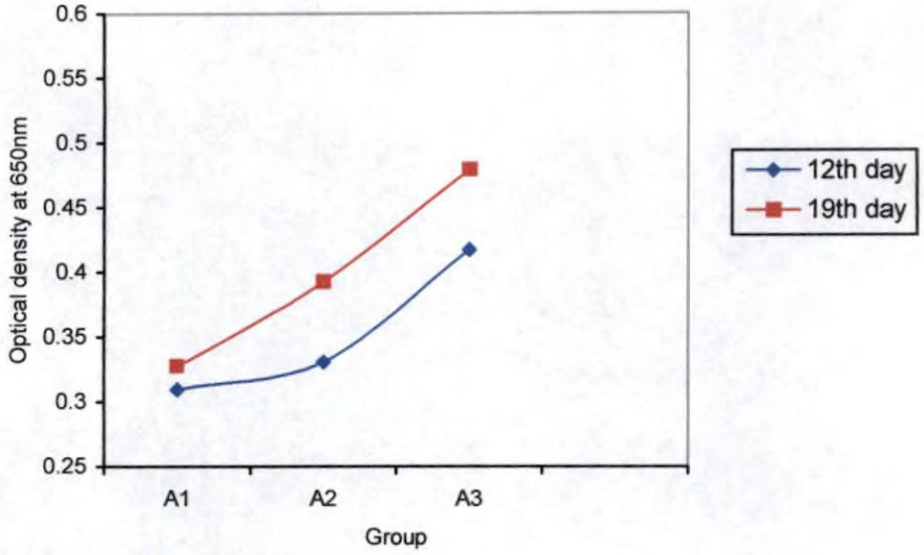


Fig. 23. Effect of alcoholic extract of *E.officinalis* on nitroblue tetrazolium dye reduction test

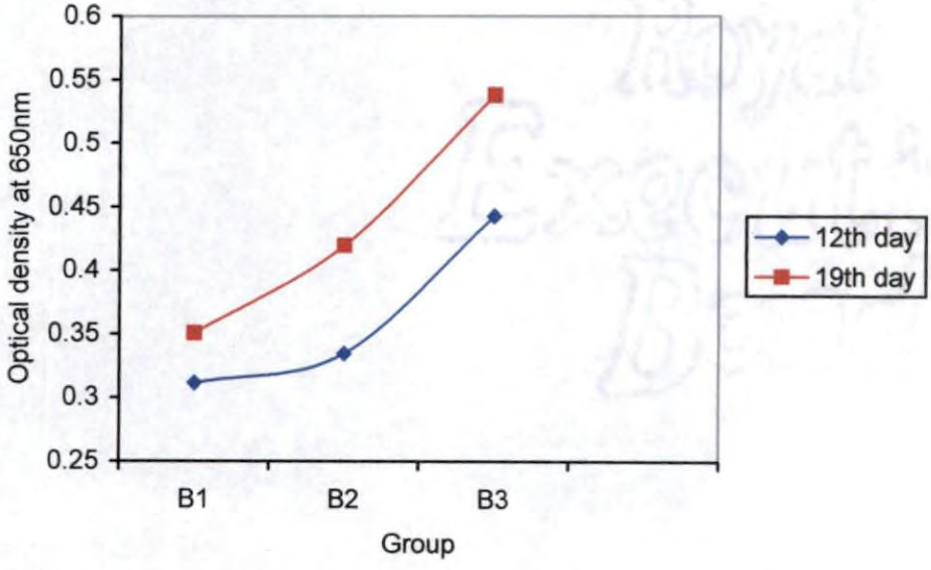


Fig. 24. Effect of aqueous extract of *E. Officinalis* on nitroblue tetrazolium dye reduction test

0.312±0.03, 0.335±0.04 and 0.443±0.02 respectively and their corresponding 19th day values were 0.328±0.05, 0.393±0.02, 0.479±0.04, 0.351±0.04, 0.420±0.02 and 0.538±0.04 respectively.

The NBT values obtained for drug treatment groups on 19th day were significantly ($P<0.05$) higher than its 12th day values.

The NBT dye reduction test values showed a maximum of 53.28 percent increase in oxidative metabolism in the aqueous extract treated group while the alcoholic extract treated group had a maximum of 46.04 percent increase from their control groups. The percentage increase in oxidative metabolism of the drug treatment groups are presented in Table 23.

Table 23. Percentage increase in oxidative metabolism after *Emblica officinalis* extract treatment

Dose rate	Alcoholic Extract		Aqueous Extract	
	12^{th} day	19^{th} day	12^{th} day	19^{th} day
100mg/kg	6.78	19.82	7.37	19.66
200mg/kg	34.51	46.04	41.98	53.28

Discussion

5. DISCUSSION

Human beings and animals are in a constant struggle against the changing environmental conditions to maintain optimum health and vigour through out their life. This imparts a potential threat to the immune function. If the immune system is competent, the individual will survive the condition. So immunomodulatory compounds especially those derived from medicinal plants have potential as therapeutic agents in this regard. Immunomodulators are those agents which can enhance or inhibit the immunological responsiveness of an organism by interfering with its regulatory mechanisms (Devasagayam and Sainis, 2002). *Emblica officinalis* is one of the principal ingredients in various Ayurvedic preparations used as preventive/ curative/ health restorative agents. Hence the study was undertaken to evaluate the immunomodulatory effect of *Emblica officinalis* fruit pulp extracts in experimental animals. The alcoholic and aqueous extracts of *Emblica officinalis* fruit pulp were also tested for presence of various active chemical constituents namely steroids, alkaloids, tannins, phenolic compounds, flavonoids, glycosides, diterpenes, triterpenes and saponins.

In the present study, one group of mice was tested with alcoholic extract of *Emblica officinalis* and the other with aqueous extract. The control group in both set received vehicle alone. The extracts were fed at two dose levels, 100 mg/kg body weight and 200 mg/kg bodyweight. The immunological activity of the extracts in experimental animal was assessed by various physiological, haematological, biochemical and immunological parameters which included body weight, organ weight, total leukocyte count, differential leukocyte count, total serum protein, serum globulin, haemagglutination titre, delayed type of hypersensitivity, macrophage migration index and nitroblue tetrazolium dye reduction test.

5.1 SCREENING OF *Emblica officinalis* FOR ACTIVE PRINCIPLES

There is a growing interest in identifying and characterising the natural compounds with immunomodulatory activity ever since they have been suggested in

modern medicine. The phytochemical study on alcoholic and aqueous extract of dried Emblica *officinalis* fruit pulp revealed that no detectable level of steroids and alkaloids are present in them. The various active principles detected in *Emblica officinalis* extracts by different qualitative tests included tannins, flavonoids, glycosides, phenols, diterpenes, triterpenes and saponins.

Tannins are polyphenolic compounds formed from several molecules of phenolic acids such as gallic acid and ellagic acid. Tannins are considered to have astringent and antiseptic activity. Flavonoids are also phenols beneficial as powerful antioxidants, stress modifiers and antiallergic agents. Antiviral activity and anticarcinogenic action was also noted. Some flavonoids may be classified as flavonoid glycoside which are demonstrated to have antiinflammatory, antiallergic, antithrombotic and vasoprotective properties. It can be seen that phenols act as a parent group in many phytochemical classification and they are commonly used as antioxidants. Terpenoids form the largest group of plant product and are the most common ingredient in volatile oils. They are categorized as monoterpenoids, diterpenoids, triterpenoids, sesquiterpenoids etc. They exert various biological activities. Saponins are glycoside compounds and its activities include antioxidant effect, selected cytotoxicity of cancer cells, immune modulation and regulation of cell proliferation (Batchelder, 1995; Lipkin, 1998).

Jose *et al.* (1997) have observed that *Emblica officinalis* fruit contains several polyphenolic compounds including tannins which was later substantiated by Bhattacharya *et al.* (1999) by their finding that Amla fruits had ascorbic acid conjugated to gallic acid and reducing sugars, forming a tannoid complex , which was more stable. It was shown that the potent vitamin C like activity of *Emblica officinalis* fruit is located in the low molecular weight hydrolysable tannins which includes emblicanin A, emblicanin B, punigluconin and pedunculagin (Ghosal *et al.*, 1996). Zhang *et al.* (2001) have tried to isolate six new ellagitannins, phyllanemblins A-F (1-6) along with 30 known tannins from *Phyllanthus emblica* fruit. They have also isolated six new phenolic constituents from its fresh juice. Khandelwal *et al.*

(2002) have reported that *Emblica officinalis* fruit contains tannins such as gallic and ellagic acid, crude cellulose, nicotinic acid, amino acids, minerals like chromium, zinc, iron, copper etc.

5.2. EVALUATION OF IMMUNOMODULATORY STATUS

5.2.1 Body weight and Organ Weight

In the present study, the body weight of mice in both aqueous and alcoholic extract treated group showed a dose dependent increase during the experimental period. (Table 2, 3 and Fig. 2, 3). At higher dose rate (200mg/kg) both extracts could produce a significantly higher body weight and spleen weight than the control groups, on 19th day of the treatment. However, the alcoholic extract could produce a significant increase in spleen weight even on 12th day. The initial body weight (zero day body weight) had increased significantly after 19 days of experiment when the extracts were fed at 200mg/kg body weight dose rate.

It can be seen that the weight of spleen reached to a maximum of 68.65% in alcoholic extract treated group and 71.34% in aqueous extract treated group compared to the controls but appreciable variation was not seen in the weight of kidney and liver in the treated group.

Gulati *et al.* (1995) and Sajitha (2002) have studied the effect of *Phyllanthus emblica* on body weight of rats having liver damage and found that there was increase in body weight. There are reports of significant increase in body weight after treatment with polyherbal preparations like Septilin and Brahma Rasayana which contain *Emblica officinalis* as a major component (Sharma and Ray, 1997; Rekha *et al.*, 1998). A significant increase in spleen/body weight ratio was also observed in Septilin treated animals. Administration of immunostimulants like curcumin, *Trigonella foenum-graecum* and *Helicanthus elastica* have resulted in a significant increase in relative weights of lymphoid organs such as spleen and thymus (Mary *et* al., 1994; Antony *et al.*, 1999; Hafeez *et al.*, 2003).

The increase in body weight after administration of *Emblica officinalis* could be due to better feed utilization, as this agent is found good in the regulation of gastric function and also possessing hepatoprotective activity (Xia *et al.*, 1997).

Secondary lymphoid organs facilitate antigen trapping (by phagocytes) and provide maximum opportunities for processed antigen to be presented to antigen sensitive cells (T and B lymphocytes) which mediate immune response. Spleen is a secondary lymphoid organ containing many phagocytes as well as T and B lymphocytes. The increase in spleen weight after Emblica treatment can be viewed as increased immunocompetancy in the treated animals (Tizard, 2000; Lydyard *et al.*, 2003).

5.2.2 Haematological Parameters

The administration of aqueous and alcoholic extracts of *Emblica officinalis* increased the total WBC count in a dose dependent manner and a maximum value was observed on 19th day of drug administration in mice fed with the aqueous extract at the rate of 200 mg/kg body weight. (Table 9, 10 and Fig 6, 7). All the extract treated groups showed a significant increase in the leukocyte count and percentage lymphocyte distribution on 12th and 19th day from their zero day value. Immunostimulatory effect was characterised by lymphocytic leukocytosis on 12th and 19th day compared to the controls at both the dose rates, along with a lower percentage distribution of neutrophils.

Rekha *et al.* (1998) have shown that a preparation made from *Emblica officinalis* (Brahma Rasayana) stimulated bone marrow cells. There was proliferation of WBC in normal and cyclophosphamide treated groups. Rasayanas could increase the total count of leukocytes and percentage of neutrophils in mice.

Emblica officinalis and Chyavanaprash which contain Emblica extracts were found to increase total WBC count in leukopenia associated with chemotherapy. They also increased bone marrow cellularity of animal after treatment (Jose and Kuttan, 1998).

Septilin, a polyherbal formulation containing *Emblica officinalis* has improved total leukocyte count and led to a preferential increase in polymorpho nuclear cell (Kumar *et al.*, 1992; Sharma and Ray, 1997).

Sairam *et al.* (2003) have observed that Amla could inhibit immunosuppressive effects of chromium on lymphocyte proliferation. Kumar *et al.* (1996) and De *et al.* (1998) have reported that Emblica and its products could inhibit myelosuppression induced by radiation and cyclophosphamide or cyclosporine treatments.

Ansari *et al.* (1997) have evaluated the immunostimulatory potential of the medicinal plant *Azadirachta indica* and found that it potentiated cell mediated immunity by increasing the total lymphocyte count similar to the results obtained in the present study. The lymphocytic leukocytosis might be due to the activation of macrophages by Emblica, leading to enhanced production of colony stimulating factors resulting in proliferation of cells in the bone marrow as reported by Chatterjee (2001).

5.2.3 Biochemical Parameters

From the present investigation, it is evident that the alcoholic and aqueous extract treated groups could produce significantly higher total serum protein and serum globulin on 19th day compared to the controls (Tables13, 14, 15, 16 and Fig. 10, 11, 12, 13). However, the alcoholic extract could produce higher serum total protein on 12^{th} day of experiment also. But the highest value for serum protein and globulin was observed on 19^{th} day for aqueous extract fed group at the rate of 200 mg/kg body weight.

Serum protein consists of hundreds of protein with a wide range of structure and function. Based on protein electrophoresis, they are divided into albumin and globulin. They have role in maintaining homeostasis, regulating inflammatory response and providing resistance to infection etc (Kaneko, 1997). Earlier in 1990, Bhasin has demonstrated a marked increase in serum IgG level following treatment

with Septilin, a polyherbal preparation containing *Emblica officinalis*. This was substantiated by Ansari *et al.* (1998) through his finding that ascorbic acid, a component of *Emblica officinalis* produced rise in IgG levels. The mechanism of action by which the extract of Emblica result in an increased Ig level is yet to be studied. This may be due to the enhanced responsiveness to B lymphocyte subset to the stimuli. Anilkumar and Rajan (1986) have studied on a synthetic immunostimulant, Levamisole and found an increase in serum globulin concentration of kids after treatment.

5.2.4 Immunological Parameters

5.2.4.1 *Haemagglutination Titre*

The present study denoted a significant increase in antibody titre in the sera of mice exposed to higher concentration of *Emblica officinalis* (200mg/kg body weight) on 19th day of experiment. However, no significant change in antibody titre was seen in mice treated with 100mg/kg body weight of *Emblica officinalis* extract. Immune-21, a poly herbal product containing *Emblica officinalis*, significantly potentiated the humoral immunity in rabbits, as evidenced by an increase in antibody titre similar to septilin which is also an Amla containing patent preparation (De *et al.*, 1998). Ansari *et al.* (1998) have reported that rabbits treated with ascorbic acid, which is considered as a component of *Emblica officinalis*, produced a highly significant rise in antibody titre, after injection of typhoid H-antigen.

The augmentation of humoral response to Sheep Red Blood Cells (SRBC) by *Emblica officinalis*, as evidenced by an increase in haemagglutination titre in mice indicated the enhanced responsiveness of macrophages and T and B lymphocyte subsets involved in the antibody synthesis. This was supported by Sharma *et al.* (1994) by their finding on *Picrorrhiza kurroa* for its immunostimulant property. In view of the pivotal role played by macrophages in coordinating the processing and presentation of antigen to immunocompetent cells, it can be suggested that the

augmentation of humoral response to SRBC by Emblica may be by facilitating this process.

5.2.4.2 *Delayed Type of Hypersensitivity (DTH)*

The effect of the plant extracts on the antigen specific cellular immune response was measured by determining the degree of DTH reaction using the footpad swelling test in experimental animal. In the present study, the DTH of all the aqueous and alcoholic extract treated mice was found to be significantly higher than their control groups, on both 12^{th} and 19^{th} day. (Table 19 and Fig 17, 18). The maximum DTH response was seen in aqueous extract treated group fed at the rate of 200mg/kg on 19^{th} day. But the DTH response obtained on 19^{th} day was almost similar to that of 12^{th} day.

Sharma and Ray (1997) have reported that Septilin containing *Emblica officinalis* as one of its components facilitated the footpad thickness response to SRBC in sensitised mice. The increase in DTH response as evidenced by increased foot pad thickness has been demonstrated in various immunostimulatory plant products like *Centella asiatica, Trigonella foenum-graecum, Azadirachta indica* etc. (Sen *et* al., 1992; Jayadev *et al.*, 1998; Hafeez *et al.*, 2003).

In the Delayed Type of Hypersensitivity reaction, the major part is played by macrophages and T lymphocyte. It has been suggested that primary contact with antigen (Ag) followed by one to two week induction phase result in activation of T-helper cells. When the same Ag is reintroduced, the reacting cells secrete cytokines such as monocyte chemotactic factor and migration inhibition factor that recruit and activate macrophages and other non-specific inflammatory cells. Macrophage thus become the principal effector cell in DTH. These events lead to a localised inflammatory response, resulting in damage to the invading agent (Singh, 1971). Thus the increased DTH response obtained in the present study revealed that Emblica may enhance the cell mediated immune response by favouring these processes.

5.2.4.3 *Macrophage Migration Index (MMI)*

The macrophage migration area of all the drug treated groups ie, mice fed with aqueous and alcoholic extract at both dose levels on 12th and 19th day of experiment showed a significant increase from the control groups (Table 20 and Fig.19, 20). The migration area of macrophages from the drug treated group divided by that of control group of animals was expressed as Macrophage Migration Index (MMI). The MMI observed was maximum at higher dose rate (200mg/kg) for both aqueous and alcoholic extract. Generally a 1.5 to 2.5 fold increase in MMI was seen on extract administration.

Peritoneal macrophage from mice and guinea pig pretreated with any immunostimulant when packed in a glass capillary and placed in a migration chamber, migrated to a larger area than macrophage from normal untreated animals. The extent of migration could be correlated with the dose of immunostimulant and the period of treatment. A close correlation was observed between macrophage migration and delayed type of hypersensitivity response in animals sensitised with SRBC. MMI appear to be a close correlate of macrophage activation and possibly also of the status of cell mediated immune response (Saxena *et al.*, 1991).

The capillary tube method of handling peritoneal exudate macrophage provides certain operational advantages over other techniques for assessing macrophage migration because packing of free macrophage may increase their resemblance to macrophage within lymphoid and connective tissue and the migration of macrophage from the cut surface of the capillary tube 'explants' may be formally equivalent to the migration of macrophage from 'explants' of solid lymphoid tissue (Morley *et al.*, 1979).

Agarwal *et al.*(1994) and Puri *et al.*(1994) have evaluated the immunostimulatory potential of *Nyctanthes arbor-tristis* and pippali rasayana

respectively using MMI as an index of immune status and concluded that these plants induced a significant increase in MMI. De *et al* (1998) and Chatterjee (2001) have suggested the mechanism of immune protection offered by Septilin and Immune-21 containing Emblica could be due to increase in number of activated macrophages which was also supported by Dahanukar (2000) and Devasagayam and Sainis (2002) in their finding that Emblica administration stimulated macrophages.

Thus the present study revealed that the aqueous and alcoholic extracts of *Emblica officinalis* is enhancing the macrophage activation. Macrophages play a major role in nonspecific and specific immune response. In innate immunity, the phagocytosis of foreign bodies by macrophage and other phagocytes provides the first line of defence against infection. In acquired immunity, macrophages and other phagocytes contribute to the regulation of both humoral and cellular immune responses. Macrophages serve as effector cells to provide immune surveillance against tumor cells also (Kapil and Sharma, 1997).

5.2.4.4 *NitroBlue Tetrazolium Dye Reduction Test (NBT test)*

NBT test is routinely being employed for assay of respiratory burst activity or oxidative metabolism in phagocytes viz, granulocytes and macrophages. The present study showed that the aqueous and alcoholic extracts of *Emblica officinalis* produced a dose dependent increase in NBT dye reduction test values. At higher dose rate, both the extracts could produce a significant increase from the control groups on 12^{th} and 19^{th} day (Table 22 and Fig 23, 24). The highest value was seen with mice fed with aqueous extract at the rate of 200mg/kg on 19^{th} day. The values obtained on 19^{th} day differed significantly from those on 12^{th} day. Thus the NBT values showed a maximum of 53.28 percent increase in oxidative metabolism in the aqueous extract treated group while the alcoholic extract treated group had a maximum of 46.04 percent increase from their control groups.

Phagocytosis of particles by macrophage is usually accompanied by a burst of oxidative metabolism which result from activation of NADPH oxidase within the

cell leading to the generation of reactive oxygen species. This can be detected by its capacity to ingest pale yellow salt of NBT and reduce it to a dark blue coloured compound formazan by association with reactive oxygen radical. The intensity of NBT reduction roughly correlates with the bactericidal activity (Park *et al.*, 1968; Tsuji and Kondoh, 1997).

The increase in NBT reduction test values indicate the enhanced functioning of macrophage system. The primary target of most of the immunomodulatory compounds is believed to be macrophages which play a key role in generation of immune response. Activated macrophage produce not only effector molecules like free radicals, nitric oxide, but also cytokines like TNFα, IL-1, 6, 12 etc. These cytokines have direct effector function or could mediate the effector response of other immune cell population (Subramoniam *et al.*, 2000).

Though no reports are currently available regarding the oxidative burst of Emblica and its products, Antony *et al.* (1999) have demonstrated the increased macrophage phagocytic activity of *curcuma longa* in mice after immunization with SRBC. Benencia *et al.* (1995) and Nores *et al.* (1997) has showed the diminution of both phagocytosis and respiratory burst activity by *Cedrela tubiflora* leaves with the help of NBT reduction test.

The result of the present study indicates a positive immunomodulatory activity of *Emblica officinalis* fruit pulp extracts. It has significant cytoprotective and immunomodulatory properties against oxidative damage also (Sairam *et al.*, 2003). It may be presumed that the immunostimulatory effect of *Emblica officinalis* is due to its various active principles like tannins, flavonoids, terpenoids, saponins etc.

Many plant products used in traditional medicine have been reported to have immunomodulatory activities. Some of these stimulated both humoral and cell mediated immunity, while others activate only cellular components of the immune system ie, phagocytic function without affecting humoral and cell mediated immunity. The present study thus establishes a strong immunostimulant activity of

dried *Emblica officinalis* fruit pulp extracts in a concentration dependent manner as evidenced by an increase in total leukocyte count, percentage lymphocyte distribution, lymphoid organ weight, globulin concentration, delayed type of hypersensitivity response, macrophage migration index and respiratory burst activity indicating its action on humoral, cell mediated and cellular mechanism of immune response.

Summary

6. SUMMARY

The present study was undertaken to evaluate the immunomodulatory potential of dried *Emblica officinalis* fruit pulp extracts in mice and also to assess the active principles present in it. One hundred and forty four male swiss albino mice weighing 15 to 30g were divided into two in which one group of mice was tested with alcoholic extract of Emblica while the other with aqueous extract. The extracts were fed at two dose levels ie, 100mg and 200mg/kg body weight for 19 days. The controls in both groups received vehicle alone (five percent gum acacia).

Different parameters like body weight, organ weight, total leukocyte count, differential leukocyte count, total serum protein, serum globulin, Haemagglutination (HA) titre, Delayed Type of Hypersensitivity (DTH), Macrophage Migration Index (MMI) and NitroBlue Tetrazolium (NBT) reduction test were studied for evaluating the immunomodulatory effect of Emblica. The alcoholic and aqueous extracts of *Emblica officinalis* fruit pulp were tested qualitatively for the presence of various active chemical constituents.

The body weight gain showed a gradual increase during the experimental period. But a significant increase in weight from the control group was noted when the extracts were fed at the rate of 200mg/kg for 19 days. Spleen weight increased to a maximum of 68.65 percent in alcoholic extract treated group and 71.34 percent in aqueous extract treated group compared to controls when fed at 200mg/kg body weight.

Haematological studies indicated that the administration of aqueous and alcoholic extract of *Emblica officinalis* could increase total WBC count in a dose dependent manner. All the extract treated groups showed a significant increase in total leukocyte count as well as percentage distribution of lymphocytes on 12^{th} and 19^{th} day from their initial zero day value. Immunostimulatory effect was characterised by lymphocytic leukocytosis on 12^{th} and 19^{th} day compared to the controls at both dose rates, along with a lower percentage distribution of neutrophils.

Serum profile revealed a significant increase in the total protein and globulin level in the alcoholic and aqueous extract treated groups compared to the controls, on 19th day of experiment. At higher dose rate (200mg/kg) both extracts could produce a significantly higher globulin concentration even on 12th day. However, the highest value for serum protein and globulin was observed on 19th day for aqueous extract fed at the rate of 200mg/kg body weight.

The augmentation of humoral immune response to SRBC by *Emblica officinalis* was evidenced by a significant increase in haemagglutination titre on 19th day of the experiment, in mice fed with aqueous and alcoholic extract of Emblica at the rate of 200mg/kg body weight.

The effect of Amla extracts on antigen specific cell mediated immune response was established by a significantly higher delayed hypersensitivity response in all the extract treated groups than their controls on 12th and 19th day. The maximum delayed hypersensitivity response was seen in aqueous extract treated group fed at the rate of 200mg/kg on 19th day.

The effect of aqueous and alcoholic extract on cellular components of the immune system like macrophage was determined by using parameters, Macrophage Migration Index (MMI) and Qualitative NitroBlue Tetrazolium (NBT) Dye reduction test. The macrophage migration area of all the drug treated groups at both dose levels showed a significant increase from the control groups on 12th and 19th day of experiment. The MMI observed was maximum at higher dose rate (200mg/kg body weight) for both aqueous and alcoholic extract. Generally, a 1.5 to 2.5 fold increase in MMI was noticed when extract was administered.

The respiratory burst activity of macrophage was increased dose dependently when Emblica extracts were administered as evidenced by the increased NBT test reading from the controls. Significantly higher values for NBT test was obtained on 12th and 19th day of experiment when extracts were fed at a higher dose rate (200mg/kg). The NBT test values indicated a maximum of 53.28 percent increase in

respiratory burst activity in aqueous extract treated group while the alcoholic extract treated group had a maximum of 46.04 percent increase from their controls.

The phytochemical study on alcoholic and aqueous extract of dried *Emblica officinalis* fruit pulp revealed that various active principles like tannins, flavonoids, glycosides, phenols, diterpenes, triterpenes and saponins are present in the extracts while there was no detectable level of steroids and alkaloids.

The results of the present study indicate that *Emblica officinalis* extracts could effectively stimulate both humoral and cell mediated immunity in addition to the activation of cellular components of the immune system like macrophage.

Diseases are in fact manifestations of the interaction between the disease causing agent and the immune system of the host. Once a disease is contracted, its outcome is largely determined by the immunological competency of the host. If the immune system is adequately competent, the individual survives the infection. Therefore, it would be a better profitable proposition to attempt stimulation of the immune system in the management of disease. Non specific immunostimulation has been extensively used as an adjunct to many infections.

A number of traditional medicinal plants have been identified as promising source of immunomodulators that will have a great deal of interest in enhancing the immune function. In the current medicinal era, the use of the immunomodulatory plants is much promising, greatly because of its less toxicity and easy availability.

Considering the above facts and the findings of the present study, it could be concluded that dried *Emblica officinalis* fruit pulp extracts could be effectively used as a positive immunomodulator in veterinary practice.

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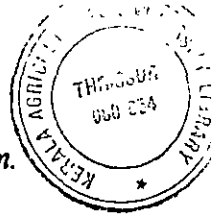

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EVALUATION OF THE IMMUNOMODULATORY EFFECT OF *Emblica officinalis* (AMLA) FRUIT PULP EXTRACTS IN MICE

SUJA RANI. S.

ABSTRACT OF THE THESIS

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Department of Pharmacology and Toxicology
COLLEGE OF VETERINARY AND ANIMAL SCIENCES
MANNUTHY. THRISSUR - 680651
KERALA, INDIA

ABSTRACT

The immunomodulatory activity of alcoholic and aqueous extracts of *Emblica officinalis* was investigated on the basis of their effects on humoral, cell mediated and cellular immune mechanism in mice. The extracts were also qualitatively tested for the presence of various active principles in it. One hundred and forty four mice taken for the study were divided into two in which one group was tested with alcoholic extract while the other with aqueous extract. The extracts were fed at two dose levels ie, 100mg and 200 mg/kg bodyweight for 19 days. The controls in both groups received vehicle alone (five percent gum acacia).

Various physiological, biochemical, haematological and immunological parameters like bodyweight, organ weight, total leukocyte count, differential leukocyte count, serum total protein, serum globulin, Haemagglutination (HA) titre, Delayed Type of Hypersensitivity (DTH), Macrophage Migration Index (MMI) and NitroBlue Tetrazolium (NBT) dye reduction test were performed for evaluating the immunomodulatory potential of the extract.

Both the extracts were found to increase the bodyweight and spleen weight significantly when fed at a higher dose rate of 200mg/kg for 19 days. The total leukocyte count was increased to a maximum of 13.90 ± 2.05 and 12.77 ± 0.78 $x10^3$/cu.mm respectively in aqueous and alcoholic extract treated groups on 19^{th} day of experiment, compared to control groups (7.18 ± 0.72 and 7.15 ± 0.72). Lymphocytic leukocytosis was seen after drug treatment.

Serum total protein and globulin levels were also increased by the administration of extracts of Emblica. The drug administration increased the globulin concentration to 1.88 ± 0.42 and 1.97 ± 0.21g% on 19^{th} day for alcoholic and aqueous extract treated groups respectively which was significantly higher than the control groups.

The increase in HA antibody titre indicated the augmentation of humoral immune response to SRBC by *Emblica officinalis.* Administration of Amla extracts

significantly increased cell mediated immune response as evidenced by increase in DTH response.

Both the extracts were found to increase the macrophage migration area to 10.93 ± 2.21 and 11.87 ± 3.54 mm^2 in aqueous and alcoholic extract treated group respectively on 19^{th} day of experiment compared to the controls (5.33 ± 1.61 and 4.78 ± 2.18). Thus a 1.5 to 2.5 fold increase in MMI could be noticed. The results of NBT test gave a maximum of 53.28 percent increase in respiratory burst activity of macrophage in aqueous extract treated group while the alcoholic extract treated group had a maximum of 46.04 percent increase from their controls.

The phytochemical study of the Emblica extracts revealed the presence of active principles like tannins, flavonoids, glycosides, phenols, diterpenes, triterpenes and saponins in it.

Thus the present study establishes the positive immunomodulatory activity of dried *Emblica officinalis* fruit pulp extracts, in a concentration dependent manner acting via humoral, cell mediated and cellular immune response.