IMMUNOMODULATORY EFFECT OF FRACTIONS OF ETHANOLIC EXTRACT FROM *Emblica officinalis* (AMLA) FRUIT PULP IN MICE

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Thesis submitted in partial fulfilment of the requirement for the degree of

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DECLARATION

I hereby declare that this thesis, entitled **"IMMUNOMODULATORY EFFECT OF FRACTIONS OF ETHANOLIC EXTRACT FROM *Emblica officinalis* (AMLA) FRUIT PULP IN MICE"** is a bonafide record of research work done by me during the course of research and that the 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.

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CERTIFICATE

Certified that this thesis, entitled **"IMMUNOMODULATORY EFFECT OF FRACTIONS OF ETHANOLIC EXTRACT FROM *Emblica officinalis* (AMLA) FRUIT PULP IN MICE"** is a record of research work done independently by **Dr. Senthil Kumar. P.K.,** under my guidance and supervision and it has not previously formed the basis for the award of any degree, diploma, fellowship or associateship to him.

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We, the undersigned members of the Advisory Committee of **Dr. Senthil Kumar. P.K.,** a candidate for the degree of **Master of Veterinary Science in Pharmacology and Toxicology**, agree that this thesis entitled **"IMMUNOMODULATORY EFFECT OF FRACTIONS OF ETHANOLIC EXTRACT FROM *Emblica officinalis* (AMLA) FRUIT PULP IN MICE"** may be submitted by **Dr. Senthil Kumar. P.K.,** in partial fulfilment of the requirement for the degree.

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No man will make a great leader who wants to do it all himself

Or

To get all the credit for doing it

-Andrew Carnegie

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Senthil Kumar. P.K.

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Introduction

1. INTRODUCTION

We live in a potentially hostile world filled with a bewildering array of infectious agents of diverse shape, size, composition and subversive character which would happily use us a rich sanctuaries for propagating their "selfish genes" had we not also developed a series of defense mechanisms at least their equal in effectiveness and ingenuity (Roitt, 1997).

The tissues of living, healthy animals are resistant to microbial invasion because of the presence within the body of a vast array of defense mechanisms. Indeed, the survival of an animal depends on the successful defense of the body against microbial invasion. Because of the essential nature of the defense of the body, it is critical that the body can effectively exclude all invaders that may cause disease or reduce an animal's ability to survive. The defenses of the body form a complex system of overlapping and interlinked mechanisms that together will be able to destroy or control almost all invaders. A failure in these defenses either because the immune system is destroyed (as in AIDS) or because the invading organisms can overcome or evade the defenses (as in Rabies) will inevitably result in death. The immune system is not simply a useful system to have around. It is essential to life itself (Tizard, 2004).

The immunological mechanisms operate through two types of immunity, viz., natural and aquired. In natural immunity the pivotal role is played by monocytes, macrophages, polymorphonuclear phagocytes and natural killer cells while in aquired immunity the pivotal role is played by two classes of lymphocytes, viz., T-cells that are mainly responsible for cell-mediated immune responses and B-cells that helps in the production of highly specific proteins called antibodies which are mainly responsible for humoral immunity.

Complement and cytokines are the two molecules that have a tremendous influence over the regulation of immune system. Complement system is a complex series of proteins, which is an essential part of the body's defenses, but it must be

carefully regulated, since uncontrolled activation may lead to massive cell or tissue destruction. Cells of the immune system (Macrophages and B-cells) secrete a bewildering variety of proteins called cytokines, whose role has come to light in recent years. It is truly the language of communication between cells of the immune system.

The function and efficacy of immune system may be influenced by many exogenous and endogenous factors like food, pharmaceuticals, physical, psychological stress, hormones etc., and the compounds that enhance or inhibit the immunological responsiveness of an organism by interfering with its regulatory mechanisms are called immunomodulators. Immunostimulants are those compounds that stimulate the immune response and the immunosuppressants are those compounds that suppress the immune response.

The rationale for the development of immunostimulants is to increase the resistance against infection (Anilkumar and Rajan, 1986). On the other hand, immunosuppressants are used in condition like organ transplant, tissue grafting and autoimmune diseases.

Nature has been source of medicinal treatments for thousands of years and plant-based systems continue to play an essential role in the primary health care of 80 per cent of world's population (Ramnath *et al.*, 2002). In recent times, focus on plant research has increased all over the world and large body of evidence showed the immense potential of medicinal plants used in various traditional systems (Dahanukar *et al.*, 2000).

'Rasayanas' are a group of non-toxic herbal drug preparations that are used to improve the general health by stimulating the body's immunity (Kumar *et al.*, 1999). 'Panchagavya' is a term used in ayurveda to describe the bovine products viz., milk, curd, ghee, urine and dung that are used along with other herbs for treatment of several diseases (Fulzele *et al.*, 2002). Emphasis is also laid on the

integration of traditional medicine with the modern health practices (Ziauddin *et al.*, 1996).

A number of traditional medicinal plants have been identified as promising source of immunomodulators that will have a great deal of interest in immune system and a few of them are *Azadirachta indica, Andrographis paniculata, Picrorrhiza kurroa, Ocimum sanctum, Curcuma longa* etc. (Sen *et al.*, 1992; Puri *et al.*, 1993; Sharma *et al.*, 1994; Sadekar *et al.*, 1998; Antony *et al.*, 1999). Compared to synthetic drugs, herbal drugs are less expensive and easily available.

Fruits of *Emblica officinalis* commonly known as 'Amla' or 'Indian gooseberry', a member of genus Emblica (family Euphorbiacea) is extensively used in Indian ayurvedic and siddha system of traditional medicine for the treatment of wide spectrum of diseases (Harikumar *et al.*, 2004; Periyanayagam *et al.*, 2004; Sultana *et al.*, 2005).

Several phytochemical studies on *Emblica officinalis* revealed the presence of polyphenols, tannoids, glycosides, ascorbic acid, flavonoids, diterpenes, triterpenes, saponins (Jose *et al.*, 1997; Bhattacharya *et al.*, 1999; Zhang *et al.*, 2000; Khopde *et al.*, 2001; Anila and Vijayalakhsmi, 2002; Rani, 2003).

Aqueous and alcoholic extract of *Emblica officinalis* had shown immunostimulant activity (Rani, 2003). Though the study on immunomodulatory activity of the crude ethanolic extract was carried out with positive outcome, no studies on immunomodulatory activity of individual active principles of *Emblica officinalis* had been carried out and also it is not necessary that all the active principles present in the *Emblica officinalis* should produce immunostimulant activity. Therefore the present study was conducted to evaluate the role of acetone soluble and acetone insoluble fraction of ethanolic extract and to assess their relative advantage over crude ethanolic extract by studying the role of acetone soluble fraction and acetone insoluble fraction of ethanolic extract on immune system separately.

Review of Literature

2. REVIEW OF LITERATURE

Immuonomodulators constitute a broad class of chemicals, microbes, microbial components and plant products that can modify specific and non-specific host immune reactions. The classic observations of Freund (1956) that dead mycobacteria could further stimulate the adjuvant effect of water- in-oil emulsions led to the discovery of other immunomodulators. Medicinal plants were used to cure human and animal illness since time immemorial.

2.1 PLANT IN GENERAL

Xia *et al.* (1997) quoted that there are 17 countries in the world that use 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 anti-inflammatory activities. It is regarded as a traditional immunomodulator and a natural adaptogen.

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

Emblica officinalis is widely used in Indian medicine for the treatment of various diseases (Sultana *et al.*,2004).

2.2 PHYTOCHEMICAL STUDIES ON *Emblica officinalis*

Jose *et al.* (1997) observed that *Emblica officnalis* contained several poly phenolic compounds including tannins. These poly phenols and their glycosides were responsible for the antioxidant and anti carcinogenic activity of *Emblica officinalis.*

Bhattacharya *et al.* (1999) showed that active tannoids of *Emblica officinalis* possessed antioxidant activity as it induced an increase in frontal, cortical and striatal concentrations of superoxide dismutase, catalase and

glutathione peroxidase activity when administered at the doses of 5 and 10 mg per kg intra peritoneally in mice.

Zhang *et al.* (2000) isolated three ester glycosides from the roots of *Phyllanthus emblica* along with 15 tannins and related compounds.

Ascorbic acid and poly phenols 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 17 g concentrated extract and vacuum evaporation of ethyl acetate phase yielded 3.2 g of poly phenol fraction.

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, phyllanemblic acids B and C and phyllanemblicin D together with two new phenolic glycosides were isolated from the roots of *Phyllanthus emblica* (Zhang *et al.*, 2002).

Anila and Vijayalakshmi (2002) found that the flavonoids isolated from *Emblica officinalis* effectively reduced lipid levels in serum and tissues of rats having hyperlipidaemia.

Indian gooseberry juice that has the highest vitamin C content (478.56 mg / 100 ml) was blended with other fruits juice for the preparation of ready to serve beverages as it boosted their nutritional quality in terms of vitamin C content (Jain and Khurdiya, 2004).

2.3 HERBAL IMMUNOMODULATORS

2.3.1 Herbal Immunostimulants

Miyamoto *et al.* (1988) observed that on intra peritoneal injection of agrimoniin, tannin contained in *Agrimonia pilosa ledeb* caused the augmentation of NK cell activity as the earliest reaction, reaching a peak at 2 days after the injection. The induction of antibody-dependent cell lytic activity was a later reaction, which reached a peak at 6 days after the injection.

Total saponin extracted from *Gynostemma pentaphylla* showed increase in the weight of immune organs, content of anti-SRBC hemolysin and percentage of NK cell activity (Zhang *et al.*, 1990).

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

Acharya *et al.* (1993) reported that an interferon stimulator derived from *Glycyrrhiza glabra* offered protection to sub acute hepatic failure and many other diseases including cancer.

The 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 (Puri *et al.*, 1993).

Effect of Rasayanas on cellular immune responses were studied by Kumar *et al.* (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.

Sharma *et al.* (1994) found that fifty per cent ethanolic extract of *Picrorrhiza kurroa* leaves stimulated the cell-mediated and humoral components of the immune system as well as phagocytosis in mice.

Subramoniam *et al.* (1996) observed that *Janakia arayalpathra* root suspension stimulated immune system in mice at a dose rate of 500 mg per 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 immunosuppression (Ziauddin *et al.,* 1996; Agarwal *et al.,* 1999).

Sadekar *et al.* (1998) found that *Ocimum sanctum* dry leaf powder had potentiating effect on humoral immune response in poultry that was immunosuppressed due to IBD infection.

Fiserova *et al.* (1997) observed that the ergot alkaloids, products of *Claviceps purpurea* enhanced the rat NK cell-mediated cytotoxic activity *in vitro*, particularly against target cells of astrocyte origin (C-6 glioma) and also argue for a possible immunomodulatory role of ergot alkaloids cell-mediated immunity in tumour regression processes.

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).

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.

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 alpha esterase positive cells were also enhanced (Antony *et al.,* 1999).

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 had high immunostimulatory activity as evidenced by the increased tumor necrosis factor alpha release from mouse peritoneal macrophage. It also showed higher up regulation of immune function gene expression.

Administration of alcoholic extract of *Mangifera indica* stem bark produced an 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 lymphocytic proliferation, triggered by the potent T-cell mitogen which depends on the type and dilutions of extracts and concentrations of concanavalin 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 *a* esterase positive bone marrow cells, when administered at a nontoxic dose of 1.25 microgram per kg body weight consecutively for 5 days in normal mice (Ramnath *et al.,* 2002; Tripathi and Maiti, 2003).

2.3.2 Herbal Immunosuppressants

Mori *et al.* (1994) observed that bark of *Phellodendron amruense* suppressed the induction phase of SRBC-induced delayed type hypersensitivity in mice and tuberculin induced delayed type hypersensitivity in guinea pigs.

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 reduction in haemolytic capacity of the human complement was also noticed.

Albezzia lebbeck extract administration in mice at a dose of 25 mg per 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).

Lakshmana *et al.* (2001) observed that E-721, 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.

Lee *et al.,* (2001) observed that oral administration of *Solanum melongena* aqueous extract significantly inhibited passive cutaneous anaphylactic reaction and histamine release. It had significant inhibitory effect on Ig E induced tumor necrosis factor secretion from rat peritoneal mast cells.

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 anti-anaphylactic action (Shin *et al.,* 2001).

Kim *et al.* (2003) showed that buckwheat grain extract possessed anti-allergic action by the inhibition of histamine release and cytokine gene expression in the mast cells.

The extract of *Acoras calamus* rhizome inhibited proliferation of mitogen and antigen stimulated human peripheral mononuclear cells. It also inhibited production of nitric oxide, interleukin (IL-2) and tumour necrosis factor alpha (Mehrotra *et al.,* 2003).

2.4 PROPRIETARY IMMUNOSTIMULANTS

Levamisole restored the humoral immune responses in both X-irradiated and antibiotics induced immunosuppression in turkeys (Panigrahy *et al.*, 1979).

Buddle *et al.* (1988) suggested that glucan, a beta –1, 3-polyglucose compound could enhance chemotactic response of the neutrophils.

Biostim, obtained from the K201 strain of *Klebsiella pneumoniae* appeared to be capable of enhancing both the bactericidal activity of circulating phagocytes and non-specific resistance to intra dermal injections of *Erysipelothrix rhusiopathiae* (Laval *et al.,* 1988).

Shibata *et al.* (1988) found that FR-900483; a substance produced by a fungus *Nectria lucida* reversed the suppression of antibody synthesis to sheep erythrocytes treated with mitomycin C in mice.

Bryostatin, a macrocyclic lactone from marine organism, Bryozoan *Bugula neritina* when synergized with recombinant IL-4, produced stimulation of resting cells to proliferate and to differentiate into cytotoxic T-lymphocytes (Trenn *et al.*, 1988).

Wang *et al.* (1988) reported that N- [4-(4-Flurophenyl) sulfonylphenyl] acetamide, a synthetic compound have a wide range of immunomodulating activities in mice.

Eader *et al.* (1994) used the mouse macrophage cell line, ANA-1, and demonstrated the direct induction of interferon beta (IFN beta), interleukin-6 (IL-6), tumor necrosis factor-alpha (TNF alpha) and interferon response factor-1 (IRF-1) mRNA expression following treatment with flavone-8-acetic acid.

Macrolide antibiotics such as midecamycin acetate, josamycin and clarithromycin suppressed interleukin-2 (IL-2) production induced by mitogen stimulated T-cells and not the expression of IL-2 receptor (CD25) in a dose-dependent manner in *in vitro* conditions (Morikawa *et al.*, 1994).

Fosfomycin and clarithromycin suppressed the synthesis of tumor necrosis factor alpha, interleukin 1 alpha (IL-1 alpha), IL-1 beta, IL-1 receptor antagonist, and granulocyte-macrophage colony-stimulating factor in a concentration dependent manner in both *in vivo* and *in vitro* conditions (Morikawa *et al.*, 1996).

In vitro exposure to clindamycin, piritrexim and pyrimethamine caused an inhibition of human T lymphocyte proliferation in response to mitogen and human NK cell activity was reduced only at the highest concentration (Viora *et al.*, 1996).

Anis *et al.* (1998) showed that the trovafloxacin levels achievable in human's suppressed *in vitro* synthesis of each of the cytokines analyzed viz., interleukin-1α (IL-1α), IL-1β, IL-6, IL-10, granulocyte-macrophage colony stimulating factor and tumor necrosis factor alpha.

Ansari *et al.* (1998) found that administration of ascorbic acid 500 mg thrice daily orally for 21days increased serum immunoglobulin levels in both humans and rabbits but have no effect on cell-mediated immunity.

Lipopolysaccharide and radio detoxified lipopolysaccharide of *Salmonella typhimurium* stimulated murine peritoneal exudate cells and macrophages and there by caused an increase in lysosomal enzymes namely aryl sulfatase, beta glucuronidase and cytokines namely tumour necrosis factor alpha and interleukin-1 respectively (Naidu and Chander, 1999).

2.5 IMMUNOMODULATORY STUDIES OF *Emblica officinalis*

Septilin, a poly herbal formulation containing *Emblica officinalis* when administered orally at a dose rate of 500 mg per kg enhanced both cellular and humoral 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.

Immue-21, a Polyherbal ayurvedic product containing *Emblica officinalis,* when given at a rate of 50 mg per 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) that contained *Emblica officinalis* as one of the components significantly increased the total leukocyte count and percentage of polymorphonuclear cells. The bone marrow cellularity as well as alpha esterase positive cells were also increased.

Oral administration of *Haridradi ghrita,* a polyherbal formulation that has *Emblica officinalis* significantly increased neutrophil adhesion and delayed type hypersensitivity where as humoral response to sheep RBCs was unaffected (Fulzele *et al.*, 2002).

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 *Emblica officinalis*

Both normal and hypercholesterolaemic men of age 35-55 years showed a decrease in cholesterol levels, when fed with *Emblica officinalis* supplement for 28 days (Jacob *et al.*, 1988).

Septilin, a patent preparation containing *Emblica officinalis* was tested for anti-inflammatory 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.

Dhir *et al.* (1990) showed that the cytotoxic effects produced by lead nitrate and aluminium sulphate administration in mice were significantly reduced by oral administration of aqueous extract of *Phyllanthus emblica* fruits.

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 caesium chloride caused a significant reduction in the percentage of aberrant cells and the frequency of micronuclei formation. Thus it acted as an effective protective agent against clastogenicity (Dhir *et al.,* 1991; Ghosh *et al.,* 1992).

Methanolic extract of *Emblica officinalis* leaves was found to have anti-inflammatory 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.

Gulati *et al.* (1995) showed that the oral administration of 50 per cent 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.

Polyherbal formulations like Cauvery 100 and Rhinax, which contain *Emblica officinalis* as one of its components 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).

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

Emblica officinalis was found to inhibit hepatocarcinogenesis induced by N- nitrosodiethylamine in a dose dependent manner (Jose *et al.*, 1997).

Phyllanthus emblica fruits at the dose of 50-100 mg per 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; AI-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, which were taken as tardive dyskinesia parameters, were inhibited in a dose dependent manner.

Emblicanin A and B enriched fraction of fresh juice of Emblica fruits, administered prophylactically, inhibited hepatic dysfunction (Bhattacharya *et al.*, 2001). *Emblica officinalis* and chyavanaprash inhibited the hepatotoxicity produced by acute and chronic administration of carbon tetrachloride (Jose and Kuttan, 2000).

The poly phenol fraction of *Emblica officinalis* was found to scavenge superoxide and hydroxyl radicals and inhibit lipid per oxidation *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 (Rajesh Kumar and Kuttan, 2001).

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 S9 dependent mutagen (Kaur *et al.*, 2002).

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 in both *in vitro* and *in vivo* studies.

Emblica officinalis Gaertn decreased a number of cough efforts; frequency of cough and the intensity of cough attacks in inspirium and expirium in a dose-dependent manner in cats (Nosalova *et al.*, 2003).

The ethanolic extract from the fruits of *Emblica officinalis* Gaertn reduced T_3 and T_4 concentrations by 64 per cent and 70 per cent respectively as compared to a standard anti thyroid drug, propyl thiouracil that decreased the levels of the thyroid hormones by 59 and 40 per cent respectively in mice (Panda and Kar, 2003).

A panchagavya ayurvedic formulation containing *Emblica officinalis* produced a significant prolongation of pentobarbital-induced sleeping time, reduced spontaneous locomotor activity, significantly antagonized the amphetamine induced hyper-locomotor activity and protected mice against tonic convulsions induced by maximal electroshock (Achliya *et al.*, 2004).

Hyponidd, herbomineral formulation composed of the extracts *Emblica officinalis*, resulted in significant lowered levels of blood glucose and significant increased levels of hepatic glycogen and total hemoglobin on oral administration of hyponidd for 45 days in rats (Babu and Stanely, 2004).

Administration of *Emblica officinalis* significantly increased activities of the antioxidant enzymes catalase, superoxide dismutase, glutathione peroxidase, glutathione-S-transferase, total leukocyte count, bone marrow viability and hemoglobin which are all reduced by the effects of the radiation (Harikumar *et al.*, 2004).

Bangladeshi medicinal plants (*Emblica officinalis, Aegle marmelos, Vernonia anthelmintica, Oroxylum indicum, Argemone mexicana*) displayed

anti-proliferative activity on MCF-7 and MDA-MB-231 breast cancer cell lines (Lambertini *et al.*, 2004).

Oral administration of *Emblica officinalis* fruit extract in various concentrations (100, 250, 500 mg per kg b.wt) for seven consecutive days prior to a single intra peritoneal injection of 7,12-dimethylbenz(a)anthracene decreased the frequency of bone marrow micronuclei induced in swiss albino mice and also there was a significant increase in the liver antioxidants, such as glutathione (GSH), glutathione peroxidase (GPx), glutathione reductase (GR) and detoxifying enzyme glutathione-S-transferase (Banu *et al.*,2004).

Perianayagam *et al.* (2004) observed that the single oral dose of ethanol and aqueous extracts of *Emblica officinalis* (500 mg / kg intra peritoneally) showed significant reduction in brewer's yeast induced hyperthermia in rats and also elicited pronounced inhibitory effect on acetic acid-induced writhing response in mice in the analgesic test.

Prophylactic treatment with *Emblica officinalis* for seven consecutive days before thioacetamide administration inhibited serum glutamic oxaloacetic transaminase, serum glutamic pyruvic transaminase, ϒ-glutamyl transpeptidase, glutathione-*S*-transferase, glutathione reductase, glucose 6-phosphate dehydrogenase and glutathione peroxidase activity and thereby reversed the oxidative stress in rats (Sultana *et al.*,2004).

Sultana *et al.* (2005) observed that pretreatment with *Emblica officinalis* at doses of 100 and 200 mg per kg body weight, prior to CCl4 intoxication showed significant reduction in the levels of SGOT, SGPT, LDH and glutathione-S-transferase synthesis. There was also increase in reduced glutathione, glutathione peroxidase and glutathione reductase in wistar rats.

2.7 IMMUNOSUPPRESSION MODELS

The mechanism of immunosuppression induced by glucocorticoids has been well characterized to induce monocytes to secrete a protein factor that inhibits the activity of neutrophils (Stevenson, 1977).

Glucocorticoids are known to induce the synthesis of protein inhibitors of phospholipase A_2 in many cell types (lipomodulin, Macrocortin) (Hirata *et al.*, 1980; Blackwell *et al.*, 1983).

Exogenous cortisol administration to chickens was associated with a decreased antibody response to sheep red blood cells and was used as a model system to evaluate the ability of ascorbic acid to overcome the steroid induced immunosuppression (Pardue and Thaxton, 1984).

The phospholipase A_2 inhibitors limit the release of arachidonic acid from membrane phospholipids and therefore inhibit the formation of prostoglandins, thromboxanes, leukotrienes and hydroxyl eicasotetraenoic acids. The activity inhibits a step in signal transduction that is known to be important to external stimuli (Valone, 1984).

Glucocorticoids have been shown to inhibit antibody response to specific antigens and lymphocyte blastogenic activity in response to mitogens (Blecha and Minocha, 1983; Pruett *et al.*, 1987).

Dexamethasone-induced immunosuppresssion has been used as a model for evaluating several potential immunomodulators in cattle (Kaeberle and Roth, 1984; Roth and Kaeberle, 1985; Chiang *et al.*, 1990).

Animals injected daily with dexamethasone (0.75 mg / kg intra peritoneally) for 2 weeks produced robust immunosuppression, resulting in loss of the spleenic cellularity, remarkable decrease in serum IFN-gamma production, In contrast, dexamethasone at a low dose (0.02 mg / kg) induced no alternation in the *in vivo* host immune activity (Tsutsui and Kamiyama, 1998).

2.8 IMMUNOMODULATORY ACTIVITY OF INDIVIDUAL ACTIVE PRINCIPLES

In *in vitro* conditions, addition of saponin preparations to mouse spleen cell cultures resulted in significant cell proliferation of B-cells in the presence of the crude saponin and proliferation of T-cellsinthe presence of Quil-A (Rao *et al.*, 1987).

Kenny *et al.* (1990) found that the flavonoids produced a wide range of immunomodulatory effects. Flavone inhibited the random migration of murine peritoneal exudate neutrophils. In contrast, six hydroxylated analogues of flavones namely fisetin, kaempferol, chrysin, flavonol, morin and quercetin significantly enhanced directed and the random migration of murine peritoneal exudate neutrophils.

Eight glycosides PF-1 to PF-8 isolated from the leaves and stems of *Formosana hayata (Liliaceae)* caused proliferative responses of mouse lymphocytes to concanavalin A and augmentation of mouse granulocyte / macrophage colony forming cells in mouse fibroblast cell L929 conditioned medium (Chiang *et al.*, 1992).

Sotomayor *et al.* (1992) observed that the 'Ukrain', a semisynthetic drug with immunomodulatory properties, derived from *Chelidonium majus L.* alkaloids and thiophosphoric acid showed a ukrain alone, in the absence of a secondary signal, induced the activation of tumouricidal function of peritoneal exudate macrophages from tumour bearing but not from normal mice and also indicated that ukrain's *in vivo* effects against the development of mammary tumours may be due, at least in part, to its ability to restore macrophage cytolytic function in *in vivo* conditions.

Thirty-four structurally different flavonoids including derivatives of chalcone, flavanone, flavan-3-ol, flavone, flavonol, and their glycosides were found to produce suppression of mitogen-induced macrophage proliferation in *in vitro* conditions (Namgoong *et al.*, 1994).

The indole alkaloids ibogaine and harmaline showed a significant increase in T-cell regulatory and effector function, B-cell function, macrophage function, and natural killer-cell function in *in vitro* conditions. But immunosuppression was noted at various concentrations in different assays, but was generally only associated with high concentrations (House *et al., 1995*).

Addition of ergot alkaloid glycosides to the mixtures of effector and target cells potentiated the peripheral blood mononuclear cells cytotoxicity against both natural killer sensitive and resistant target cells (Vladimir *et al.,* 1996).

Plohmann *et al.* (1997) observed that the triterpenoid saponins of *Solidago virgaurea* and *Helianthus annuus. L* stimulated activity of bone marrow cells, enhanced number of plaque forming cells, serum albumin and globumin concentration. It also stimulated cytotoxic macrophages and further resulted in the TNF alpha release from macrophages in mice.

Intra muscular and intra nasal immunizations with a DNA vaccine encoding env of HIV-1 along with QS-21 saponin adjuvant consistently enhanced antigen specific serum immunoglobulin G2a (IgG2a) production, delayed type of hypersensitivity reaction, cytolytic activity of splenocytes, intestinal secretory Ig A production, cytolytic activity of the mesenteric lymph node cells, interleukin-2 (IL-2) and gamma interferon in murines (Sasaki *et al.*, 1998).

Vaccine that is orally administered along with lipophilic immune stimulating complexes containing the saponin adjuvant 'Quil A' activated many cellular components of the innate immune system, including T-cells, neutrophils, macrophages and dendritic cells. In parallel, there was increased production of nitric oxide (NO), reactive oxygen intermediates (ROI), IL-1, IL-2, IL- 6, IL- 12 and gamma interferon in mice (Allan *et al.,* 1999).

The alkaloidal fraction of *Boerhaavia diffusa* induced a significant dose-related increase in antibody titre during pre and post immunization treatment in *in vitro* conditions (Mungantiwar *et al.,* 1999).

Cardiac glycosides have been found to have a significant immunomodulartory effect, inducing proliferation of IL-I, IL-6 and TNF-alpha in peripheral blood mononuclear cells from healthy individuals and suppressing production of IL-6 and TNF-alpha in peripheral blood mononuclear cells stimulated by endotoxin (Worthley and Holt, 1999).

The study showed that poly phenols extracted from *Epigallocatechin gallate* inhibited the growth of *L. pneumophila* in macrophages at a concentration as low as 0.5 μg per ml without any direct antibacterial effect on the organisms. The poly phenols selectively up regulated the production of interleukin-12 (IL-12) and tumor necrosis factor alpha (TNF-α). There was also up regulation of the levels of macrophage gamma interferon (IFN-γ) mRNA by EGCg (Kazuto *et al.*, 2001).

Kiderlen *et al.* (2001) reported that the series of 28 poly phenols produced significant macrophage activation for release of nitric oxide (NO), tumour necrosis factor (TNF) and interferon (IFN)-like properties in murines.

A series of 27 hydrolysable tannins and related compounds caused an increase in macrophage functions, including release of nitric oxide (NO), tumour necrosis factor-alpha (TNF-alpha) and interferon (IFN)-gamma in murines (Kolodziej *et al.*, 2001).

Dendroside A and dendronobilosides A and B are the new sesquiterpene glycosides, which have been isolated from the stems of *Dendrobium nobile* were found to stimulate the proliferation of murine T and B lymphocytes *in vitro* (Zhao *et al.*, 2001).

Attanasio *et al.* (2002) observed that treatment with estrogen or progesterone resulted in decreased lymphocyte numbers and in down modulation of activation markers CD25 and CD69. In addition, hormone replacement resulted in a decreasing trend for peripheral blood mononuclear cells IFN-gamma production in rhesus macaques.

Saponins enhanced production of cytokines such as interleukins and interferons and also enhanced antibody production (George *et al.,* 2002).

Cuphiin D1 (CD1), macro cyclic hydrolysable tannin isolated from *Cuphea hyssopifolia,* stimulated peripheral blood mononuclear cells, IL-1 beta, IL-2 and TNF-alpha in a dose-dependent manner. The results showed that CD1 could stimulate peripheral blood mononuclear cells release of IL-1 beta, IL-2 and TNF-alpha and then activate T-cells in *in vitro* conditions (Wang *et al.*, 2002).

The flavonoid fraction of *Tephrosia purpurea* significantly inhibited sheep red blood cells induced delayed type of hypersensitivity reactions, caused dose-related decrease in sheep erythrocyte specific haemagglutination antibody titre and failed to show a significant change in the macrophage phagocytic activity in mice (Damre *et al.*, 2003).

Chiang *et al.* (2003) found that the flavonoids, monoterpenoids, triterpenoids, iridoid glycosides and phenolic compounds of *Plantago species* enhanced the activity of human lymphocyte proliferation and secretion of IFN-γ in mice.

Submerged mycelium of *Lentinus edodes* grown in laboratory fermenters had 23–24 per cent proteins, 8–9 per cent lipids, up to 1.8 per cent phenolic substances. The submerged mycelium stimulated the development of humoral immune response elicited by sheep red blood cells (Lobanok *et al.*,2003).

Mikhaeil *et al.* (2003) observed that the frankincense essential oil contain monoterpenes, sesquiterpenes and diterpenes. Biologically, the oil exhibited a strong immunostimulant activity (lymphocyte transformation) by a significant macrophage proliferation in *in vitro* conditions.

Raphael and Kuttan (2003) showed that naturally occurring triterpenoid compounds such as glycyrrhizic acid, ursolic acid, lanolin acid, and nomilin were found to enhance the total white blood cells, bone marrow cellularity,

alpha-esterase positive cells, specific antibody titre and the number of plaque forming cells in the spleen of mice.

The methanolic extract of *Andrographis paniculata* was fractionated into dichloromethane and this resulted in three diterpene compounds, [1] andrographolide, [2] 14-deoxyandrographolide and [3] 14-deoxy-11, 12-didehydroandrographolide that augments the proliferation of human peripheral blood lymphocytes as well as all the three molecules showed enhanced interleukin-2 (IL-2) induction (Kumar *et al.*, 2004).

Flavonoids isolated from the acetone extract of the leaves of *Syzygium samarangense* inhibited interleukin-2 (IL-2) and interferon-gamma (IFN-gamma) production and caused reduction in the proliferation of human peripheral blood mononuclear cells in *in vitro* conditions (Kuo *et al.*, 2004).

Poly phenolic compounds isolated from propolis produced the antitumor activity that was related to the immunomodulatory properties of the compounds, their cytotoxicity to tumor cells and their capacity to induce apoptosis and necrosis (Orsolic *et al.*, 2004).

Three flavone glucosides, pleiosides A-C, isolated from the leaves of *Pleioblastus amarus* significantly stimulate the proliferation of murine B-lymphocytes *in vitro* (Wang *et al.*, 2004).

Materials and Methods

3. MATERIALS AND METHODS

3.1 PLANT MATERIAL

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

3.1.1 Preparation of acetone soluble and acetone insoluble fractions of ethanolic extract of plant material

Alcoholic extract of *Emblica officinalis* powder was prepared using ethyl alcohol in a soxlet apparatus. The extract was evaporated to dryness with the help of a vacuum evaporator and kept in a refrigerator in an airtight container. On an average, 100 g of dried Emblica fruit pulp powder gave 40 g of dry extract. The acetone soluble fraction of alcoholic extract was prepared by extracting 100 g of the dried alcoholic extract with 500 ml of acetone (repeated extraction with aliquots of 300 ml in the beginning and 100 ml subsequently). The insoluble residue was separated by filtration. Acetone was removed by evaporation at room temperature and the acetone soluble residue was stored under refrigeration. The acetone insoluble fraction also was recovered, dried and stored under refrigeration.

3.1.2 Screening of acetone soluble and acetone insoluble fractions of ethanolic extract for active principles

The acetone soluble and acetone insoluble fraction of ethanolic extract of *Emblica officinalis* were tested for the presence of various active principles namely steroids, alkaloids, tannins, phenolic compounds, flavonoids, glycosides, diterpenes, triterpenes and saponins as per the procedure quoted by Harborne (1991).

Fig 1. ***Emblica officinalis*** **fruit**

3.1.2.1 *Tests for Detection of Steroids*

Salkowski test

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

Liberman Burchardt test

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

3.1.2.2 *Tests for Detection of Alkaloids*

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

Mayer's test

To 1 ml of acid extract, few drops of Mayer's reagent (1.358 g of mercuric chloride dissolved in 60 ml of water and poured into a solution of 5 g of Potassium iodide in 10 ml of water and then make up the volume to 100 ml with distilled water). Development of a creamy white precipitate indicates the presence of alkaloids.

Wagner's test

Few drops of Wagner's reagent (2 g of iodine and 6 g of potassium iodide dissolved in 100 ml of water) were added to 1 ml of the acid extract. Development of reddish brown precipitate indicates the presence of alkaloids.

Hager's test

To 1 ml of the acid extract, few drops of Hager's reagent (1 g of picric acid dissolved in 100 ml of water) were mixed. Development of yellow precipitate indicates the presence of alkaloids.

Dragendroff's test

Few drops of Dragendroff's reagent (Stock solution (1) 0.6 grams of bismuth sub nitrate was dissolved in 2 ml of concentrated hydrochloric acid and 10 ml of water was added. Stock solution (2) six grams of potassium iodide was dissolved in 10 ml of water. Then both the stock solutions (1) and (2) were mixed together and then it was mixed with 7 ml of concentrated hydrochloric acid and 15 ml of water. Sufficient amount of distilled water was added to the mixture to make up the volume to 400 ml) was mixed with 1 ml of acid extract. Development of a reddish brown precipitate indicates the presence of alkaloids.

3.1.2.3 *Test for Detection of Phenolic compounds*

About 5 mg of the extract was dissolved in 1 ml of water and five drops of ten per cent ferric chloride was added to it. Development of dark blue colour indicates the presence of phenolic compounds.

3.1.2.4 *Tests for Detection of Tannins*

Ferric chloride test

Two milligram of the extract was mixed with 3 ml of one per cent ferric chloride solution. Development of a blue, green or brownish colour indicates the presence of tannins.

Gelatin test

About 0.5 g of the extract was mixed with few drops of one per cent solution of gelatin containing ten per cent sodium chloride. Development of a white precipitate indicates the presence of tannins.

3.1.2.5 *Tests for Detection of Flavonoids*

Ferric chloride test

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

Lead acetate test

To 2 ml of alcoholic solution of the extract (0.5 g extract in 10 ml methanol), few drops of neutral ten per cent lead acetate was mixed. Development of a yellow precipitate indicates the presence of flavonoids.

3.1.2.6 *Tests for Detection of Glycosides*

Sodium hydroxide test

Dissolved a small amount of the extract (about 5 mg) in 1 ml water and added 5-6 drops of sodium hydroxide solution (10 %). Development of a yellow colour indicates the presence of glycosides.

Benedict's test

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

3.1.2.7 *Test for Detection of Diterpenes*

About 5 mg of the extract was dissolved in 3 ml of copper acetate solution (5 %). Development of green colour indicates the presence of diterpenes.

3.1.2.8 *Tests for Detection of Triterpenes*

Salkowski test

About 5 mg of the extract was dissolved in 3 ml of chloroform and then it was shaken with 3 ml of concentrated sulphuric acid. Development of yellow colour in lower layer on standing indicates the presence of triterpenes.

Lieberman Burchardt test

Few drops of acetic acid and 1 ml concentrated sulphuric acid were added to 3 ml of chloroform solution of the extract (about 3 mg extract in 3 ml chloroform). Development of deep red ring at the junction of two layers indicates the presence of Triterpenes.

3.1.2.9 *Test for the Detection of Saponins*

Foam test

A small amount of the extract (about 5 mg) was shaken with 3 ml of water. Development of the foam that persists for ten minutes indicates the presence of saponins.

3.2 EXPERIMENTAL ANIMALS

One hundred and forty four male swiss albino mice of 2-3 months age, weighing 15 g to 30 g 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

Blood was collected from the sheep 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 $4^{O}C$ until use.

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

Materials

Alsever's solution

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

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

Normal Saline

Sodium Chloride	0.85 g
Distilled water	100 ml

Phosphate Buffered Saline

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

Method

The sheep blood collected was centrifuged and washed three times in large volumes of sterile normal saline and concentration was adjusted to 1×10^8 cells per ml in Phosphate Buffered Saline (PBS) for immunization.

3.4 EVALUATION OF IMMUNOMODULATORY STATUS

Immunomodulatory status was assessed by physiological, haematological, biochemical, enzymatic and immunological parameters. The physiological parameters like body weight and relative organ weight, haematological parameters like total leukocyte count and differential leukocyte count, biochemical parameters like total serum protein, serum globulin and albumin-globulin ratio, enzymatic parameters like quantification of superoxide dismutase and catalase, immunological parameters like haemagglutination test, Jerene's plaque forming assay to assess the humoral immune response and tests like delayed type of hypersensitivity, macrophage migration index and nitro blue 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, Jerene's plaque forming assay, enzymes like superoxide dismutase and catalase, bone marrow cellularity, macrophage migration index (MMI) and nitro blue tetrazolium (NBT) dye reduction test. They were divided randomly into six groups of 12 each and denoted as A_1, A_2 A_3, A_4, A_5 and A_6.

A_1 - Administered with vehicle alone (five per cent gum acacia in distilled water).

A_2 - Administered with dexamethasone alone at the dose rate of 0.75 mg per kg intra peritoneally for seven days.

A_3 - Administered with acetone soluble fraction of ethanolic extract of the test substance in five per cent gum acacia orally at the dose rate of 200 mg per kg body weight respectively for 19 days.

A_4 - Administered with dexamethasone at the dose rate of 0.75 mg per kg intra peritoneally for seven days and then fed with acetone soluble fraction of ethanolic extract of the test substance in five per cent gum acacia orally at the dose rate of 200 mg per kg body weight respectively for 19 days.

A_5 - Administered with acetone insoluble fraction of ethanolic extract of the test substance in five per cent gum acacia orally at the dose rate of 200 mg per kg body weight respectively for 19 days.

A_6 - Administered with dexamethasone at the dose rate of 0.75 mg per kg intra peritoneally for seven days and then fed with acetone insoluble fraction of ethanolic extract of the test substance in five per cent gum acacia orally at the dose rate of 200 mg per kg body weight respectively for 19 days.

Seventy two mice were used to assess delayed type of hypersensitivity. They were divided randomly into six groups of 12 each and it is denoted as B_1, B_2 B_3, B_4, B_5 and B_6.

B_1 - Administered with vehicle alone (five per cent gum acacia in distilled water).

B_2 - Administered with dexamethasone alone at the dose rate of 0.75 mg per kg intra peritoneally for seven days.

B_3 - Administered with acetone soluble fraction of Ethanolic extract of the test substance in five per cent gum acacia orally at the dose rate of 200 mg per kg body weight respectively for 19 days.

B_4 - Administered with dexamethasone at the dose rate of 0.75 mg per kg intra peritoneally for seven days and then fed with acetone soluble fraction of ethanolic extract of the test substance in five per cent gum acacia orally at the dose rate of 200 mg per kg body weight respectively for 19 days.

B_5 - Administered with acetone insoluble fraction of ethanolic extract of the test substance in five per cent gum acacia orally at the dose rate of 200 mg per kg body weight respectively for 19 days.

B_6 - Administered with dexamethasone at the dose rate of 0.75 mg per kg intra peritoneally for seven days and then fed with acetone insoluble fraction of ethanolic extract of the test substance in five per cent gum acacia orally at the dose rate of 200 mg per kg body weight respectively for 19 days.

All the mice of groups (A_1, A_2, A_3, A_4, A_5, A_6, B_1, B_2, B_3, B_4, B_5 and B_6) were injected with SRBC antigen 0.2 ml ($1x10^8$ cells / ml / kg body weight) intra peritoneally on day five 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 all mice from groups A_1-A_6 with the help of heparinised capillary tube using disodium salt of ethylene diamine tetra acetic acid as anticoagulant on day 0 and day 5, from six mice of groups A_1-A_6 on day 12 and from remaining six mice of groups A_1-A_6 on day 19 of extract / vehicle administration. Total and differential leukocyte count was taken as per the method described by Schalm (1975).

3.4.3 Biochemical Parameters

Blood samples were also collected from these animals in a test tube without anticoagulant for serum separation.

The total protein content in the serum was estimated by biuret method (Gornall *et al.*, 1949). The Albumin content in the serum was estimated by bromo cresol green dye method described by Doumas *et al.* (1971). Serum globulin value was determined by deducting the value of serum albumin from serum protein.

3.4.4 Enzymatic Parameters

Six mice from each groups A_1-A_6 were sacrificed by cervical strangulation on day twelve, liver was removed and used for quantification of enzymes.

3.4.4.1 ***Quantification of Superoxide Dismutase*** (Mimami and Yoshikawa, 1979)

Reagents

Tris cacodylic acid buffer (50 mM, pH 8.2)

Tris cacodylic acid 50 mM

Diethylene triamine penta acetic acid (0.03%)

Nitroblue tetrazolium 0.1M

Triton X 100 (0.001%)

All reagents were mixed in equal quantities and the pH was adjusted to 8.2 using 0.1 N sodium hydroxide solution.

0.9 per cent sodium chloride

0.2 mM pyrogallol

Procedure

1. Freshly excised liver was homogenized in tissue homogeniser with 10 volumes of 0.9 per cent sodium chloride followed by centrifugation at 400 rpm for 10 minutes at 4°C to harvest the supernatant.
2. The assay mixture consists of 1.4 ml of 50 mM tris cacodylic acid buffer, 1.4 ml of 0.2 mM pyrogallol and 0.2 ml enzyme preparation.

3. Blank contained distilled water instead of enzyme preparation.

4. The absorbance due to auto oxidation of pyrogallol was read at 420 nm using spectrophotometer.

5. One unit of superoxide dismutase activity was the amount of enzyme that inhibited pyrogallol auto oxidation by 50 per cent under experimental conditions.

6. The values were expressed in units per milligram of protein after quantifying the protein content of supernatant by method of Lowry *et al*, (1951).

3.4.4.2 ***Quantification of Catalase*** (Cohen *et al.*, 1970)

Reagents

Phosphate buffer-hydrogen peroxide solution (10 mM)

Phosphate buffer (0.05 M, pH 7.0)

0.2 M sodium dihydrogen phosphate solution	39 ml
0.2 M disodium hydrogen phosphate solution	61 ml
Distilled water	300 ml

Immediately before use 0.02 ml of hydrogen peroxide was added to 100 ml buffer

Procedure

1. Three ml of Phosphate buffer hydrogen peroxide solution was taken in test tubes.

2. Samples prepared in sodium chloride (as described in case of superoxide dismutase) were added and the absorbance was read at 240 nm at the 40^{th} second of addition of the sample against blank (distilled water).

3. The time required for the initial absorbance to decrease by 0.05 units was calculated using the formula- log E1 / E2 X 2300 / 6.93 X 1 / Ct.

E1- Initial absorbance

E2- Absorbance after decrease by 0.05 units

Ct- Time taken for the decrease in absorbance by 0.05 units (seconds).

3.4.5 Immunological Parameters for Evaluation of Humoral Immune Response.

3.4.5.1 *Haemagglutination Test*

Materials

SRBC antigen for Haemagglutination.

Sheep blood collected from the animals maintained in the university Sheep and Goat farm was washed thrice in normal saline and the concentration was adjusted to get $1x10^8$ cells per ml in phosphate buffered saline.

Alsever's solution

Normal Saline

Phosphate Buffered Saline.

Method

Part of the serum samples that have been collected for carrying out biological parameters are used in performing haemagglutination test. Haemagglutination test was performed using techniques of Ray *et al.* (1991).

Two fold dilutions of sera were prepared in 0.15 M phosphate buffered saline (pH 7.2) and 50 µl of each dilution was aliquoted into 96 well microtitre plates. A 25 µl quantity of fresh one per cent SRBC suspension in PBS (1 ml of SRBC in 99 ml of 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 per cent agglutination had been expressed as HA titre.

3.4.5.2 *Determination of Antibody forming Cells (Spleenic Plaque forming Cells)*

Antibody produced by the lymphoid cells from animals immunized with SRBC cause lysis of red cells in its vicinity (plaques) in the presence of complement (Jerne and Nordin, 1963).

The animals sacrificed already for quantifying the enzymes are used in this method. Spleen was removed on day 12 and made into single cell suspension in RPMI-1640 medium. A volume of 2.0 ml of agarose (0.6 %) was taken in tubes kept at 45°C and 0.1 ml of fresh twenty per cent SRBC suspension in PBS (20 ml of SRBC in 80 ml of PBS) and 1 ml spleen cells of treated mice (1 X 10^6 cells / ml in RPMI-1640 medium) were added and mixed well. The contents of the tubes were poured into grease free slide, spread into an area of 1" X 2", and allowed to solidify. Fresh rabbit serum, (1:10 diluted with PBS, pH 7.2) was used as the complement source. The slides were kept in an incubation rack and the space between the slides and rack was filled with complement. The slides were incubated for one hour at 37°C. The plaques so formed, found as empty spaces due to haemolysis were counted in a colony counter and expressed as the number of plaques per million lymphocytes (spleenocytes). The experiment was repeated on day nineteen in the other six mice of each group in the same pattern.

3.4.6 Immunological Parameters for Evaluation of Cell Mediated Immune Response

3.4.6.1 *Determination of Bone Marrow Cellularity*

Bone marrow cellularity was determined by the method of Mehra and Vaidya (1985). The animals sacrificed already for quantifying the enzymes are used for this method. Both the hind legs were dissected out and femurs were taken. After removing the condyles of femurs, the bone marrow was flushed with 5 ml of ten per cent foetal calf serum in both the femurs separately and the number of cells present were counted haemocytometrically and expressed as million cells per femur. The experiment was repeated on day nineteen in the other six mice of each group in the same pattern.

3.4.6.2 *Delayed Type of Hypersensitivity (DTH)*

Materials

SRBC antigen for DTH test

Sheep blood collected in alsever's solution was washed thrice in normal saline and the concentration was adjusted to 1 x 10^8 cells per 0.025 ml 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 delayed type of hypersensitivity. Six mice from groups B_1, B_2 B_3, B_4, B_5 and B_6 which were primed with SRBC antigen intra peritoneally on day five were challenged on day 12 with 0.025 ml of SRBC antigen sub cutaneously on right hind foot pad. The left hind footpad received 0.025 ml of saline alone. The footpad swelling was measured at three different dimensions using verniar calipers after 24 hrs of challenge. The difference in footpad thickness was taken as a measure of DTH. The test was repeated on 19^{th} day in the remaining 6 mice of each group.

3.4.6.3 *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 (0.2 µm) and stored at $4^{O}C$ until use. The pH of the prepared solution was

* Himedia, Bombay

adjusted to 7.2 using sterile 4.4 per cent Sodium bicarbonate solution just before use.

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

Readymade RPMI-1640 was reconstituted as per manufacturer's instruction in sterile double distilled water and filtered using micropore filter (0.2 μm) and stored at $4^{O}C$ until use. The pH of the prepared solution was adjusted to 7.2 using sterile 4.4 per cent 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 incubated at 56 °C for 30 minutes in a water bath to inactivate the complement and it was stored at –20 ^{O}C until use.

L-Glutamine*

The glutamine powder was dissolved in double distilled water and diluted to a concentration of 200 mM 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 2 mM

Fixative

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

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Peritoneal Exudate Cells (PEC)

Peritoneal exudate cells were collected following the procedure explained by Benencia *et al.* (1996). The animals sacrificed for quantifying the enzymes are used in this method. The abdomen was swabbed with rectified spirit to dampen the furcoat. The mouse was stripped off its skin by pulling the fur and avoiding touching the anterior abdominal wall.

The peritoneal cavity was then distended by injecting approximately 5-6 ml of HBSS containing 5 IU Heparin per ml. The peritoneal fluid was aspirated 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 40 ml double distilled water and filtered using micropore filter (0.2 μm) and stored at $4^{O}C$ until use.

Methods

Macrophage Migration Index was taken according to the procedure followed by Saxena *et al.* (1991). Peritoneal exudates were collected from six mice of groups A_1-A_6 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 remaining 6 mice of each group in the same pattern.

The peritoneal exudate cells collected were centrifuged at 250 g at $4^{O}C$ for 10 minutes. The cells were again washed twice with fresh cold RPMI-1640 and tested for viability by trypan blue exclusion technique in which one part of Trypan blue solution (0.1 % 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 non viable took up the dye and thus appeared blue in colour. Batches of peritoneal exudate cells with 90 per cent or more viability were used for the test. The cells were finally suspended in 0.5 ml RPMI-1640 media containing antibiotics like streptomycin and benzyl penicillin (100 µg / ml and 100 IU / ml respectively), 2 mM glutamine and ten per cent 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 60-80 X 10^6 cells per ml media.

Peritoneal exudate cells thus prepared from treated and untreated animals were packed in a microhaematocrit capillary of uniform diameter. They were placed in migration chamber, filled with complete RPMI-1640 medium containing five per cent foetal calf serum, antibiotics (streptomycin and benzyl penicillin) and glutamine and then incubated at 37^0C for 18-24 hrs. 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 divided by that of untreated group animals was expressed as Macrophage Migration Index.

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

Materials

HBSS

RPMI-1640

Peritoneal Exudate Cells (PEC)

Nitro Blue Tetrazolium (NBT)* (Dissolved 10 mg of NBT chloride powder in 1 ml of sterile distilled water)

Dimethyl Sulphoxide (DMSO)

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Sorenson's Buffer (0.5 M, pH 10.5)

Stock solution (1) - Potassium dihydrogen phosphate (3.402 g) dissolved in 50 ml of distilled water

Stock solution (2) - Disodium hydrogen phosphate (3.954 g) dissolved in 50 ml of distilled water

Working Solution

Four point five ml of Stock solution (1) was added to 0.5 ml of Stock solution (2) and the volume was made up to 100 ml with distilled water and pH was adjusted using 1 N sodium hydroxide solution.

Method

Nitro Blue Tetrazolium (NBT) Reduction Assay was done as per the procedure explained by Sairam *et al.* (1997). Part of the peritoneal exudate cells collected from A_1- A_6 on day 12 for carrying out macrophage migration index are subjected to Nitro Blue Tetrazolium (NBT) Reduction Assay. The experiment was repeated on 19^{th} day in the remaining 6 mice of each group in the same pattern.

The peritoneal exudate cells collected were washed thrice with fresh cold HBSS and the final concentration of the cells was adjusted to 10-20 X 10^6 cells per ml HBSS. Then 100 µl of cells were placed into a 96 well tissue culture plates and incubated for two hours at 37°C in a CO_2 incubator for macrophages to adsorb to the bottom of the plates. The cells were washed three times with cold HBSS to remove non-adherent cells followed by the addition of 200 µl of RPMI-1640 medium to the wells.

After plating the macrophages in the wells, 25 µl of nitroblue tetrazolium chloride (NBT 10 mg / ml) was added to 200 µl 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 200 µl DMSO and 25 µl sorenson's buffer (0.5 M, pH 10.5). The blue colour obtained was measured at 650 nm using ELISA reader.

3.5 STATISTICAL ANALYSES OF DATA

The data obtained were statistically analyzed using one way Analysis of Variance for comparison between groups and L.S.D for pair wise comparison of means if the analysis of variance was found significant.

Results

4. RESULTS

4.1 SCREENING OF ACETONE SOLUBLE AND ACETONE INSOLUBLE FRACTION OF ETHANOLIC EXTRACT OF *Emblica officinalis* FOR ACTIVE PRINCIPLES

4.1.1 Steroids

As per the salkowski test, red colour was not obtained and liberman burchardt test also did not give a reddish ring at the junction in both the fractions. Thus it could be concluded that no detectable level of steroids was present in both acetone soluble and acetone insoluble fractions of ethanolic extract of *Emblica officinalis.*

4.1.2 Alkaloids

A creamy white precipitate as per mayer's test and a reddish brown coloured precipitate as per wagner's test were not obtained and dragendroff's test did not yield reddish brown precipitate as well as hager's test also did not yield yellow precipitate in both the fractions. Thus the tests revealed the absence of detectable level of alkaloids in both the fractions.

4.1.3 Phenolic Compounds

A dark blue colour was not produced, when the acetone soluble fraction was mixed with ten percent of ferric chloride indicating the absence of phenolic compounds. In acetone insoluble fraction dark brown colour was obtained, which indicates the presence of Phenolic compounds in the acetone insoluble fraction.

4.1.4 Tannins

Intense blue colour was not obtained in ferric chloride test and a white precipitate was not obtained in gelatin test. These results indicated the absence of

tannins in the acetone soluble fraction. But in acetone insoluble fractions, dark blue colour in ferric chloride test and white precipitate in gelatin test were obtained, which revealed the presence of tannins in the acetone insoluble fraction.

4.1.5 Flavonoids

A green colour in the ferric chloride test and a yellow precipitate in lead acetate test were not obtained and it indicated the absence of flavonoids in acetone soluble fraction but in acetone insoluble fraction, dark blue colour in ferric chloride test and yellow precipitate in lead acetate test were obtained, which showed the presence of flavonoids in the acetone insoluble fraction.

4.1.6 Glycosides

In the Benedict's test, brown colour was not obtained indicating the absence of glycosides. Yellow colour was not obtained by mixing the extract with sodium hydroxide, which also indicated the absence of glycosides in acetone soluble fraction. In acetone insoluble fraction, brown colour was obtained in Benedict's test and yellow colour was obtained in sodium hydroxide test. It showed the presence of glycosides in the acetone insoluble fraction.

4.1.7 Diterpenes

Diterpene was detected in the extract as indicated by the green colour when it was mixed with copper acetate solution in acetone soluble fraction, but no green colour was obtained in acetone insoluble fraction, which indicates the presence of diterpenes in acetone soluble fraction and its absence in acetone insoluble fraction.

4.1.8 Triterpenes

As per Salkowski test, lower layer turned to yellow on standing and by Liberman Burchardt test, a deep red ring appeared at the junction of two layers in the acetone soluble fraction but no such colour was obtained in the acetone

Table 1. Screening of acetone soluble and acetone insoluble fraction of ethanolic extract of *Emblica officinalis* for active principles

Sl.no	Active Principles	Acetone Soluble Fraction	Acetone Insoluble Fraction
1	Steroids	Not Detected	Not Detected
2	Alkaloids	Not Detected	Not Detected
3	Tannins	Not Detected	Present
4	Flavonoids	Not Detected	Present
5	Glycosides	Not Detected	Present
6	Phenolic compounds	Not Detected	Present
7	Diterpenes	Present	Not Detected
8	Triterpenes	Present	Not Detected
9	Saponins	Not Detected	Present

insoluble fraction. These results indicated the presence of triterpenes in the acetone soluble fraction alone.

4.1.9 Saponins

In the foam test, foam was not persisted for 10 minutes in the acetone soluble fraction but the foam persisted in the acetone insoluble fraction, which indicates the presence of saponins in acetone insoluble fraction.

4.2 EVALUATION OF IMMUNOMODULATORY STATUS

4.2.1 Physiological Parameters

4.2.1.1 *Body Weight*

The individual and mean bodyweights of the mice of the groups A_1, A_2 A_3, A_4, A_5 and A_6 recorded on days 0,12 and 19 of drug / vehicle administration are given in Table 2. The zero day values represented the bodyweight before the commencement of the experiment.

There was no significant increase in body weights on 12^{th} day of experiment in any of the groups. The body weights of mice in drug treated group A_3 alone showed a significant ($P<0.05$) increase on 19^{th} day of the experiment compared to control group. The highest mean body weight was recorded in group A_3 on 19^{th} day of the experiment and its mean body weight is 26.67±2.58, whereas the mean body weights recorded in groups A_1, A_2, A_4, A_5 and A_6 on 19^{th} day are 22.50±2.74, 22.50±2.74, 21.67±2.58, 24.16±2.04 and 23.33±4.08 respectively.

4.2.1.2 *Organ Weight*

The weight of the individual organs like spleen, kidney and liver were taken on day 12 and 19 of drug / vehicle administration and expressed as relative organ weights (Table 3, 4, 5; Fig. 2, 3). The weight of spleen in groups A_3 and

Table 2. Effect of acetone soluble and acetone insoluble fractions of ethanolic extracts of *Emblica officinalis* on body weight of mice in grams

Sl.No	Zero day						12th day						19th day					
	A_1	A_2	A_3	A_4	A_5	A_6	A_1	A_2	A_3	A_4	A_5	A_6	A_1	A_2	A_3	A_4	A_5	A_6
1	20	20	25	20	15	20	20	20	25	20	15	20	20	25	25	20	25	30
2	20	25	20	20	25	20	20	20	20	20	25	25	20	20	25	20	25	25
3	25	20	25	25	20	20	25	20	25	25	20	20	25	25	30	25	25	20
4	20	20	20	20	20	20	20	15	20	20	20	20	25	20	25	20	20	20
5	15	25	25	25	25	25	15	25	25	25	25	25	20	20	30	25	25	25
6	20	25	20	20	20	20	20	20	25	20	20	20	25	25	25	20	25	20
7	20	20	20	15	25	20	20	20	20	20	25	20	-	-	-	-	-	-
8	20	25	15	20	25	20	20	20	15	20	25	20	-	-	-	-	-	-
9	20	20	20	20	20	20	15	20	20	20	20	20	-	-	-	-	-	-
10	25	20	25	25	20	15	25	20	25	25	20	20	-	-	-	-	-	-
11	25	25	20	25	20	25	15	25	20	25	20	25	-	-	-	-	-	-
12	20	20	25	20	15	20	25	20	25	20	20	15	-	-	-	-	-	-
Mean	20.83	22.08	21.67	21.25	20.83	20.41	20.00	20.42	22.08	21.67	21.25	20.83	22.50^{B}	22.50^{B}	26.67^{A}	21.67^{B}	24.16^{AB}	23.33^{AB}
±SD	±2.89	±2.57	±3.26	±3.11	±3.59	±2.57	±3.69	±2.57	±3.34	±2.46	±3.10	±2.89	±2.74	±2.74	±2.58	±2.58	±2.04	±4.08

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

Table 3. Effect of acetone soluble and acetone insoluble fractions of ethanolic extract of *Emblica officinalis* on weight of spleen in grams per 100 gram body weight

S.No	12th day						19th day					
	A_1	A_2	A_3	A_4	A_5	A_6	A_1	A_2	A_3	A_4	A_5	A_6
1	0.310	0.295	0.365	0.325	0.345	0.322	0.330	0.299	0.575	0.405	0.560	0.420
2	0.295	0.270	0.324	0.310	0.321	0.305	0.299	0.275	0.534	0.402	0.585	0.396
3	0.325	0.285	0.375	0.340	0.360	0.325	0.330	0.305	0.625	0.428	0.610	0.408
4	0.286	0.240	0.338	0.308	0.324	0.302	0.270	0.258	0.702	0.395	0.685	0.401
5	0.296	0.255	0.340	0.302	0.332	0.305	0.305	0.258	0.687	0.485	0.652	0.428
6	0.312	0.270	0.354	0.298	0.340	0.302	0.322	0.305	0.679	0.462	0.650	0.448
Mean	0.30^B	0.27^C	0.35^A	0.31^B	0.34^A	0.31^B	0.31^C	0.28^C	0.63^A	0.43^B	0.62^A	0.42^B
± SD	±0.01	±0.02	±0.02	±0.02	±0.01	±0.01	±0.02	±0.02	±0.07	±0.04	±0.04	±0.02

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

Table 4. Effect of acetone soluble and acetone insoluble fractions of ethanolic extract of *Emblica officinalis* on weight of kidney in grams per 100 gram body weight

S.No	12th day						19th day					
	A_1	A_2	A_3	A_4	A_5	A_6	A_1	A_2	A_3	A_4	A_5	A_6
1	1.895	1.765	1.994	1.880	1.985	1.875	1.898	1.810	1.990	1.895	1.990	1.880
2	1.224	1.202	1.359	1.262	1.456	1.210	1.231	1.202	1.525	1.275	1.505	1.255
3	1.365	1.295	1.465	1.390	1.405	1.375	1.365	1.302	1.685	1.402	1.650	1.397
4	1.268	1.215	1.365	1.301	1.305	1.275	1.264	1.220	1.520	1.325	1.545	1.295
5	1.814	1.685	1.962	1.795	1.865	1.771	1.802	1.695	2.202	1.925	1.995	1.810
6	1.956	1.825	2.182	1.992	2.050	2.085	1.961	1.828	2.254	1.980	2.350	2.175
Mean	1.59^A	1.50^A	1.72^A	1.60^A	1.68^A	1.58^A	1.59^A	1.51^A	1.86^A	1.63^A	1.84^A	1.62^A
± SD	±0.34	±0.29	±0.37	±0.32	±0.33	±0.35	±0.34	±0.30	±0.33	±0.33	±0.33	±0.35

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

Table 5. Effect of acetone soluble and acetone insoluble fractions of ethanolic extract of *Emblica officinalis* on weight of liver in grams per 100 gram body weight

SI.No	12^{th} day						19^{th} day					
	A_1	A_2	A_3	A_4	A_5	A_6	A_1	A_2	A_3	A_4	A_5	A_6
1	7.74	7.45	8.48	8.04	8.24	8.06	7.92	7.68	8.98	8.42	8.68	8.22
2	6.84	6.42	7.24	6.86	7.02	6.84	7.28	6.85	7.94	7.63	7.82	7.42
3	6.98	6.48	7.36	6.89	7.22	6.83	7.45	6.95	8.24	7.12	8.16	7.22
4	7.23	6.88	8.22	7.56	8.22	7.42	7.68	7.42	9.26	8.22	8.96	8.06
5	6.98	6.71	7.46	7.08	7.36	7.12	7.52	7.04	7.98	7.65	7.82	7.38
6	7.74	7.08	8.28	7.74	8.11	7.82	8.02	7.82	9.36	8.34	8.92	8.46
Mean	7.25^{BC}	6.84^{C}	7.84^{A}	7.36^{BC}	7.70^{AB}	7.34^{BC}	7.64^{CD}	7.29^{D}	8.62^{A}	7.90^{B}	8.39^{AB}	7.80^{CD}
± SD	±0.40	±0.39	±0.54	±0.49	±0.55	±0.51	±.028	±0.40	±0.65	±0.51	±0.53	±0.52

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

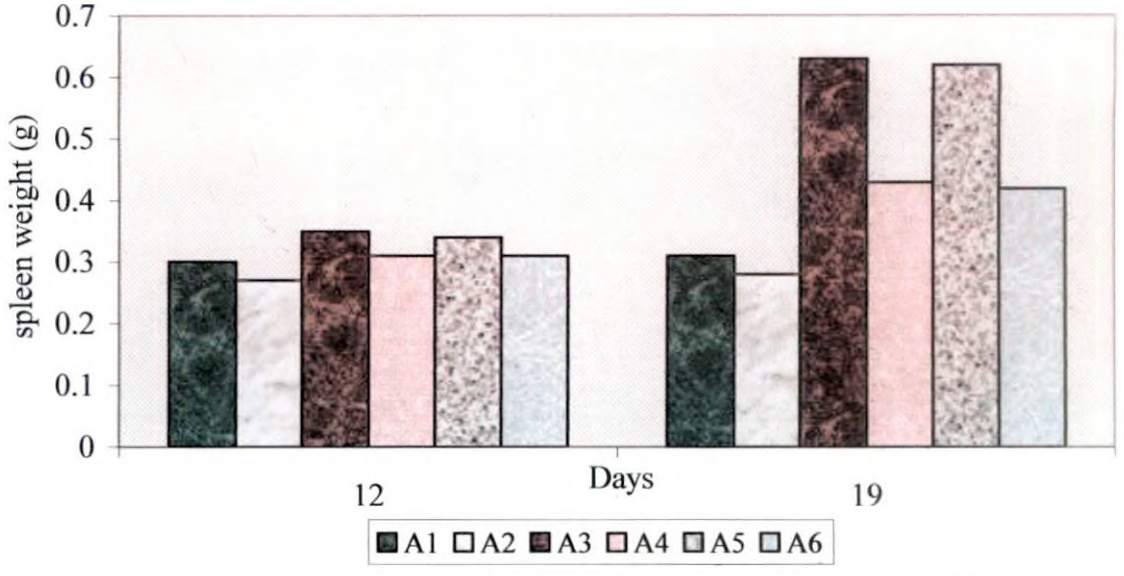


Fig. 2. Effect of acetone soluble and acetone insoluble fractions of ethanolic extract of *Emblica officinalis* on weight of spleen in grams per 100 gram body weight

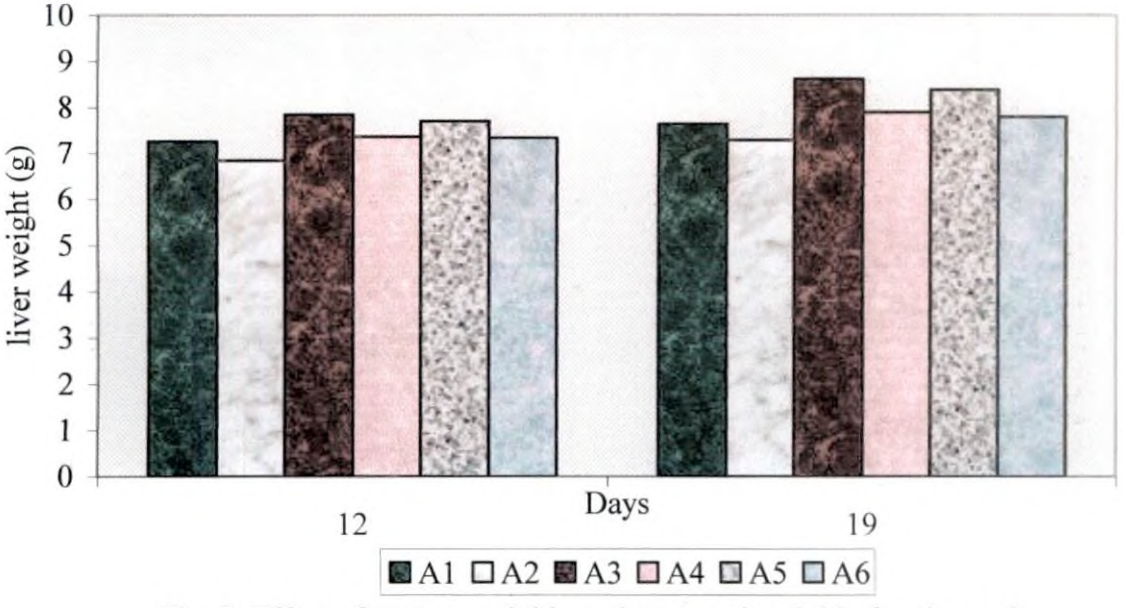


Fig. 3. Effect of acetone soluble and acetone insoluble fractions of ethanolic extract of *Emblica officinalis* on weight of liver in grams per 100 gram body weight

A_5 showed a significant ($P<0.05$) increase, where as group A_2 (immunosuppressed with dexamethasone) showed a significantly lower value and the rest of the groups showed no significant difference on 12th day of the experiment than their control groups. The weight of spleen in groups A_3, A_4, A_5 and A_6 showed a significant increase and group A_2 showed no significant difference on 19th day of the experiment than their control groups. The mean spleen weights of groups A_3, A_4, A_5 and A_6 are 0.63±0.07, 0.43±0.04, 0.62±0.040 and 0.42±0.02 respectively. The highest mean spleen weight was noticed in A_3 on 19th day.

Appreciable variation was not seen in the weight of kidney in any of the groups on both 12th and 19th day of the experiment.

The weight of liver in group A_3 showed a significant ($P<0.05$) increase, whereas all other groups showed no significant difference on 12th day of the experiment than their control groups. The weight of liver in groups A_3, A_4 and A_5 showed a significant increase but no such significant difference was found in other groups on 19th day of the experiment than their control groups. The highest mean liver weight was noticed in A_3 on 19th day of the experiment and its mean weight is 8.62±0.65.

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, A_4, A_5 and A_6 recorded on days 0, 5, 12 and 19 of drug / vehicle administration is presented in Table 6 and Fig. 4.

Total leukocyte count of the mice in groups A_2, A_4 and A_6 showed a significantly lower value (immunosuppressed with dexamethasone before the start of the experiment) on day zero and day five of the experiment than their control groups. But on 12th day, significantly lower value was noticed in group

Table 6. Effect of acetone soluble and acetone insoluble fractions of ethanolic extract of *Emblica officinalis* on total leukocyte count, x 10^3/ cu.mm

Sl.No	Zero day						5th day					
	A_1	A_2	A_3	A_4	A_5	A_6	A_1	A_2	A_3	A_4	A_5	A_6
1	20	20	25	20	15	20	20	20	25	20	15	20
2	20	25	20	20	25	20	20	20	20	20	25	25
3	25	20	25	25	20	20	25	20	25	25	20	20
4	20	20	20	20	20	20	20	15	20	20	20	20
5	15	25	25	25	25	25	15	25	25	25	25	25
6	20	25	20	20	20	20	20	20	25	20	20	20
7	20	20	20	15	25	20	20	20	20	20	25	20
8	20	25	15	20	25	20	20	20	15	20	25	20
9	20	20	20	20	20	20	15	20	20	20	20	20
10	25	20	25	25	20	15	25	20	25	25	20	20
11	25	25	20	25	20	25	15	25	20	25	20	25
12	20	20	25	20	15	20	25	20	25	20	20	15
Mean	6.36^B	5.26^C	6.80^A	5.34^C	6.46^{AB}	5.17^C	6.37^B	5.44^C	7.00^A	5.54^C	6.73^A	5.40^C
± SD	±0.42	±0.27	±0.34	±0.34	±0.31	±0.34	±0.40	±0.40	±0.39	±0.27	±0.41	±0.36

Sl.No	12th day						19th day					
	A_1	A_2	A_3	A_4	A_5	A_6	A_1	A_2	A_3	A_4	A_5	A_6
1	6.4	6.1	8.1	7.2	7.5	7.0	6.4	5.9	12.2	9.8	12.0	9.7
2	5.8	5.5	7.8	7.0	7.5	7.0	5.9	6.0	11.2	9.6	11.0	9.2
3	6.5	5.4	8.2	7.0	8.0	6.5	6.6	6.2	13.1	9.5	11.4	9.5
4	6.1	5.9	8.0	6.5	7.9	6.1	6.2	6.3	12.0	8.9	10.8	8.8
5	5.8	5.5	7.2	6.6	7.4	6.2	5.8	6.5	11.4	9.1	10.2	9.0
6	7.2	6.5	6.8	6.4	7.0	6.1	7.1	6.9	11.2	8.8	11.4	8.5
7	6.9	5.8	8.0	7.1	7.9	6.9	-	-	-	-	-	-
8	6.8	5.6	8.1	7.2	7.5	6.5	-	-	-	-	-	-
9	6.5	6.2	8.2	6.9	7.9	6.6	-	-	-	-	-	-
10	6.9	5.9	8.4	6.8	8.4	6.4	-	-	-	-	-	-
11	7.1	5.8	8.1	7.4	7.9	6.5	-	-	-	-	-	-
12	6.5	5.5	7.9	7.3	8.2	6.8	-	-	-	-	-	-
Mean	6.54^C	5.81^D	7.90^A	6.95^B	7.76^A	6.55^C	6.33^A	6.30^A	11.85^C	9.28^B	11.13^C	9.11^B
± SD	±0.47	±0.33	±0.46	±0.32	±0.39	±0.32	±0.48	±0.36	±0.74	±0.41	±0.62	±0.44

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

A_2 alone, whereas significant ($P<0.05$) increase was noted in group A_4 and no significant difference was found in group A_6. On 19th day, groups A_4 and A_6 only showed a significant increase.

Groups A_3 and A_5 showed no significant difference on day zero but on 5th, 12th and 19th day they showed a significant ($P<0.05$) increase than their control groups. The highest WBC count observed was in the treatment group A_3 on day 19 of the experiment and its mean leukocyte count is 11.85±0.74.

4.2.2.2 *Differential Leukocyte Count*

Differential leukocyte count of all the mice in groups A_1, A_2 A_3, A_4, A_5 and A_6 recorded on days 0, 5, 12 and 19 of all the groups are presented in Table 7, 8, 9 and 10.

4.2.2.2.1 Lymphocytes

The percentage distribution of lymphocytes in drug / vehicle administration group is presented in Table 7 and Fig. 5.

The percentage distribution of lymphocytes of the mice in groups A_2, A_4 and A_6 showed a significantly lower value (immunosuppressed with dexamethasone before the start of the experiment) on day zero and day five of the experiment than their control groups. But on 12th and 19th day, only groups A_2 and A_6 showed such a significantly lower value. A significant increase was noticed in group A_4.

Groups A_3 and A_5 showed no significant difference than control on zero and fifth day of the experiment. However, a significant increase was noticed on 12th and 19th day. The highest mean lymphocyte value was obtained on day 19 in group A_3 and its mean value is 86.50±1.38

Table 7. Effect of acetone soluble and acetone insoluble fractions of ethanolic extract of *Emblica officinalis* on distribution of lymphocytes in percentage

S.No	Zero Day						Fifth Day					
	A_1	A_2	A_3	A_4	A_5	A_6	A_1	A_2	A_3	A_4	A_5	A_6
1	78	71	79	68	79	68	78	72	79	70	79	71
2	78	70	80	69	80	69	79	70	78	72	78	72
3	76	70	75	67	77	70	79	69	78	73	75	69
4	74	69	76	65	79	70	77	68	79	72	77	73
5	76	70	78	69	78	71	78	69	80	72	78	72
6	72	70	78	70	79	69	79	70	80	71	79	70
7	78	70	78	69	78	68	77	72	82	73	81	72
8	76	68	75	68	79	71	79	70	83	75	82	73
9	75	72	79	71	80	69	78	73	82	76	80	74
10	78	71	77	66	75	68	75	72	80	77	80	74
11	73	73	76	71	77	70	77	72	81	79	81	77
12	72	69	75	70	79	68	80	72	82	80	81	77
Mean ± SD	75.5^{AB} ±2.3	70.2^{C} ±1.3	77.1^{A} ±1.7	68.5^{D} ±1.8	78.3^{A} ±1.4	70.2^{CD} ±1.3	78.0^{AB} ±1.3	70.7^{D} ±1.6	80.3^{A} ±1.6	74.1^{C} ±3.2	79.2^{AB} ±2.0	70.7^{D} ±1.6

S.No	Twelfth Day						Nineteenth Day					
	A_1	A_2	A_3	A_4	A_5	A_6	A_1	A_2	A_3	A_4	A_5	A_6
1	78	73	84	78	83	74	79	73	88	80	87	80
2	76	73	83	79	82	75	76	74	87	82	86	81
3	75	74	82	78	81	75	75	74	86	82	83	81
4	77	70	79	78	81	76	80	75	85	84	83	80
5	72	70	84	74	83	73	74	76	88	84	84	82
6	80	71	82	75	82	74	74	74	85	85	81	82
7	75	71	82	79	82	73	-	-	-	-	-	-
8	77	72	83	78	80	75	-	-	-	-	-	-
9	76	70	84	77	83	74	-	-	-	-	-	-
10	73	74	79	78	81	75	-	-	-	-	-	-
11	75	72	84	80	82	76	-	-	-	-	-	-
12	78	72	82	75	84	72	-	-	-	-	-	-
Mean ± SD	76.0^{C} ±2.2	71.8^{E} ±1.4	82.3^{A} ±1.7	77.4^{B} ±1.8	82.0^{A} ±1.1	71.8^{B} ±1.4	76.3^{D} ±2.5	74.3^{E} ±1.0	86.5^{A} ±1.3	82.8^{B} ±1.8	84.0^{B} ±2.1	74.3^{B} ±1.0

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

Table 8. Effect of acetone soluble and acetone insoluble fractions of ethanolic extract of *Emblica officinalis* on distribution of neutrophils in percentage

S.No	Zero Day						Fifth Day					
	A_1	A_2	A_3	A_4	A_5	A_6	A_1	A_2	A_3	A_4	A_5	A_6
1	22	28	21	32	20	31	22	28	21	30	20	29
2	21	30	20	30	20	31	20	30	21	28	22	27
3	24	29	24	33	22	29	21	29	22	26	24	30
4	25	29	24	34	21	29	23	31	20	28	23	27
5	24	29	21	30	22	29	21	31	19	27	21	28
6	27	30	22	29	20	31	21	30	20	29	20	30
7	21	29	22	31	20	31	23	27	18	27	19	28
8	24	31	24	30	21	29	21	30	17	24	17	27
9	24	28	21	27	20	30	21	26	17	23	19	25
10	22	29	21	32	23	30	25	27	19	23	19	24
11	25	26	23	28	22	28	22	28	18	20	19	23
12	26	31	24	30	21	32	20	27	17	20	18	23
Mean ± SD	23.7^{CD} ±1.9	29.3^{B} ±1.3	22.2^{D} ±1.4	30.5^{A} ±2.0	21.0^{D} ±1.0	29.0^{AB} ±1.3	21.6^{C} ±1.4	28.6^{A} ±1.7	19.0^{D} ±1.7	25.4^{B} ±3.3	20.0^{CD} ±2.0	28.6^{A} ±1.7

S.No	Twelfth Day						Nineteenth Day					
	A_1	A_2	A_3	A_4	A_5	A_6	A_1	A_2	A_3	A_4	A_5	A_6
1	21	26	16	22	16	25	20	27	11	20	13	20
2	23	27	17	21	18	25	23	26	12	17	14	19
3	24	26	17	21	19	24	24	24	13	18	16	18
4	23	30	20	21	18	24	20	25	15	15	16	19
5	28	29	16	25	16	27	26	24	12	15	15	18
6	20	29	18	25	18	26	26	25	14	15	18	18
7	25	29	17	21	17	26	-	-	-	-	-	-
8	22	27	16	20	19	25	-	-	-	-	-	-
9	25	29	15	22	17	26	-	-	-	-	-	-
10	25	26	20	22	19	24	-	-	-	-	-	-
11	24	26	15	20	17	24	-	-	-	-	-	-
12	22	28	17	23	15	27	-	-	-	-	-	-
Mean ± SD	23.5^{C} ±2.1	27.6^{A} ±1.5	17.0^{E} ±1.6	21.9^{D} ±1.7	17.4^{E} ±1.3	27.7^{A} ±1.5	23.2^{B} ±2.7	25.2^{A} ±1.2	12.8^{E} ±1.5	16.7^{D} ±2.1	15.3^{D} ±1.7	25.2^{A} ±1.2

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

Table 9. Effect of acetone soluble and acetone insoluble fractions of ethanolic extract of *Emblica officinalis* on distribution of eosinophils in percentage

S.No	Zero Day						Fifth Day					
	A_1	A_2	A_3	A_4	A_5	A_6	A_1	A_2	A_3	A_4	A_5	A_6
1	-	1	-	-	-	1	-	-	-	-	-	-
2	-	-	-	1	-	-	-	-	-	-	-	-
3	-	1	1	-	-	1	-	1	-	-	1	-
4	1	1	-	-	-	-	-	1	-	-	-	-
5	-	1	-	-	-	-	1	-	1	1	-	-
6	-	-	-	1	1	-	-	-	-	-	-	-
7	-	1	-	-	1	-	-	1	-	-	-	-
8	-	-	-	-	-	-	-	-	-	-	-	-
9	-	-	-	1	-	1	-	-	-	1	1	-
10	-	-	1	1	1	1	-	1	-	-	-	1
11	1	1	-	1	-	1	1	-	1	-	-	-
12	1	-	-	-	-	-	-	1	-	-	-	-

S.No	Twelfth Day						Nineteenth Day					
	A_1	A_2	A_3	A_4	A_5	A_6	A_1	A_2	A_3	A_4	A_5	A_6
1	-	1	-	-	-	-	-	-	-	-	-	-
2	-	-	-	-	-	-	-	-	1	-	-	-
3	1	-	1	-	-	1	1	1	-	-	-	-
4	-	-	-	-	1	-	-	-	-	-	-	-
5	-	1	-	1	-	-	-	-	-	1	1	-
6	-	-	-	-	-	-	-	1	-	-	-	-
7	-	-	-	-	-	1	-	-	-	-	-	-
8	-	1	-	1	1	-	-	-	-	-	-	-
9	-	1	-	1	-	-	-	-	-	-	-	-
10	1	-	-	-	-	-	-	-	-	-	-	-
11	1	1	1	-	1	-	-	-	-	-	-	-
12	-	-	1	1	-	1	-	-	-	-	-	-

Table 10. Effect of acetone soluble and acetone insoluble fractions of ethanolic extract of *Emblica officinalis* on distribution of monocytes in percentage

S.No	Zero Day						Fifth Day					
	A_1	A_2	A_3	A_4	A_5	A_6	A_1	A_2	A_3	A_4	A_5	A_6
1	1	-	-	-	1	-	1	-	-	-	1	-
2	1	-	-	-	-	-	1	-	1	-	-	1
3	-	-	-	-	1	-	-	1	-	1	-	1
4	-	1	-	1	-	1	-	-	1	-	-	-
5	-	-	1	-	-	-	-	-	-	-	1	-
6	1	-	-	-	-	-	-	-	1	-	1	-
7	1	-	-	-	1	1	-	-	-	-	-	-
8	-	1	1	1	-	-	-	-	-	1	1	-
9	1	-	-	1	-	-	1	1	1	-	-	1
10	-	-	1	1	1	1	-	-	1	-	1	1
11	1	-	1	-	1	1	-	-	-	1	-	-
12	1	-	1	-	-	-	-	-	1	1	1	-

S.No	Twelfth Day						Nineteenth Day					
	A_1	A_2	A_3	A_4	A_5	A_6	A_1	A_2	A_3	A_4	A_5	A_6
1	1	-	-	-	1	1	1	-	1	-	-	-
2	1	-	-	-	-	-	1	-	-	1	-	-
3	-	-	-	1	-	-	-	1	1	-	1	1
4	-	-	1	1	-	-	-	-	-	1	1	1
5	-	-	-	-	1	-	-	-	-	-	-	-
6	-	-	-	-	-	-	-	-	1	-	1	-
7	-	-	1	-	1	-	-	-	-	-	-	-
8	1	-	1	1	-	-	-	-	-	-	-	-
9	1	-	1	-	-	-	-	-	-	-	-	-
10	1	-	1	-	-	1	-	-	-	-	-	-
11	-	1	-	-	-	-	-	-	-	-	-	-
12	1	-	-	1	1	-	-	-	-	-	-	-

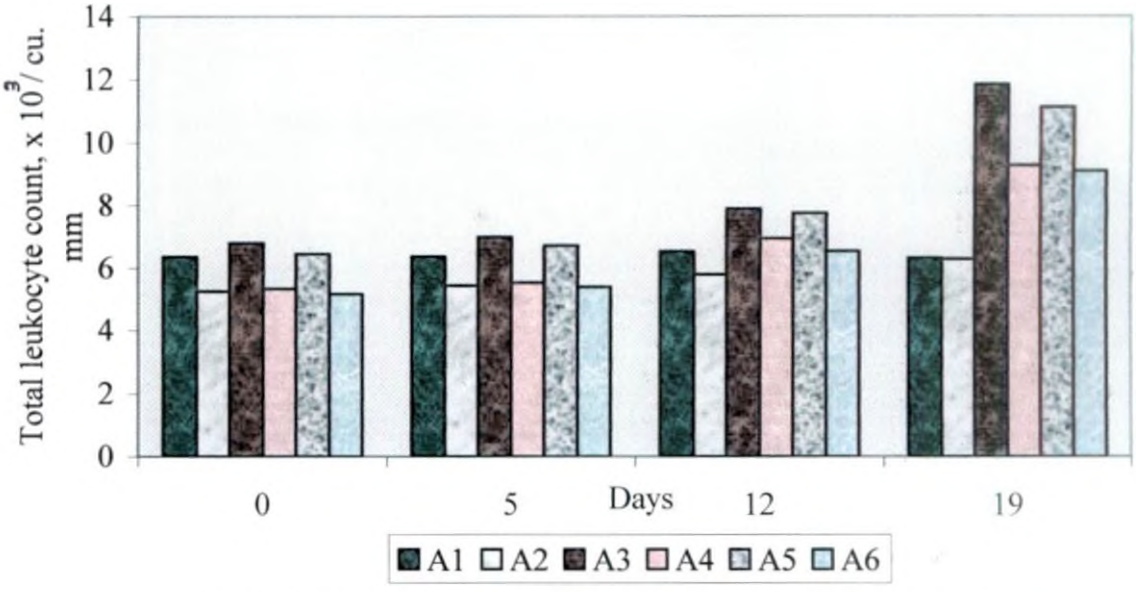


Fig. 4. Effect of acetone soluble and acetone insoluble fractions of ethanolic extract of *Emblica officinalis* on total leukocyte count

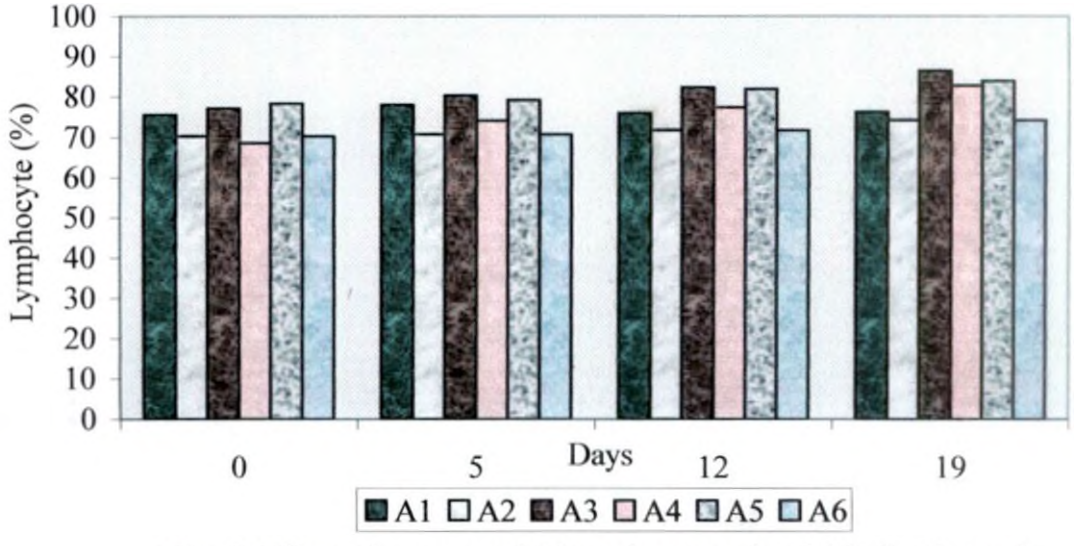


Fig. 5. Effect of acetone soluble and acetone insoluble fractions of ethanolic extract of *Emblica officinalis* on percentage distribution of Lymphocytes

4.2.2.2.2 Neutrophils

Mean percentage distribution of neutrophils in drug/vehicle administration groups is presented in Table 8.

The percentage distribution of neutrophils of the mice in groups A_2, A_4 and A_6 showed a significant ($P<0.05$) increase on day zero and day five of the experiment than their control groups. But on 12^{th} and 19^{th} day, only groups A_2 and A_6 showed a significant increase and group A_4 showed a significantly lower value than their control groups.

A significant decrease was noticed in groups A_3 and A_5 on day 12 and 19 of the experiment and only in A_3 on the day five of the experiment. On day zero, both A_3 and A_5 showed no significant difference.

The mean percentage distributions of neutrophils in groups A_1, A_2, A_3, A_4, A_5 and A_6 on day 19 are 23.2±2.71, 25.2±1.20, 12.8±1.47, 16.7±2.06, 15.3±1.75 and 25.2±1.17 respectively.

4.2.2.2.3 Eosinophils, Monocytes

The percentage distribution of eosinophils and monocytes were recorded on days 0, 5, 12 and 19 of drug/vehicle administration. The data is presented in the Table 9 and 10.

4.2.3 Biochemical Parameters

4.2.3.1 *Serum Total Protein*

The individual and mean values of serum protein concentration of drug treated groups and control groups on days 0, 5, 12 and 19 of experiment are recorded and it is given in the Table 11. The mean values are graphically presented in Fig. 6.

Table 11. Effect of acetone soluble and acetone insoluble fractions of ethanolic extract of *Emblica officinalis* on serum total protein in grams per decilitre

SI.No	Zero day						5th day					
	A_1	A_2	A_3	A_4	A_5	A_6	A_1	A_2	A_3	A_4	A_5	A_6
1	3.75	2.52	3.52	3.02	3.65	2.24	3.78	2.55	3.60	3.20	3.98	2.75
2	3.58	2.24	3.65	2.46	3.69	2.54	3.58	2.25	3.75	2.90	3.80	2.72
3	3.96	2.68	3.69	2.54	3.86	2.65	3.98	2.70	3.80	2.95	3.80	2.80
4	4.02	2.54	3.96	2.95	3.69	2.68	4.02	2.60	4.02	3.25	3.72	2.90
5	3.98	3.02	3.75	2.86	3.75	2.49	3.98	3.02	3.82	3.22	3.78	2.95
6	3.83	2.75	4.02	2.78	4.05	3.02	3.64	2.75	4.04	3.18	4.03	2.98
7	3.76	2.54	3.99	2.80	3.98	2.26	3.74	2.75	4.00	3.25	4.00	3.15
8	3.96	2.70	3.69	2.92	3.76	2.55	3.95	2.72	3.84	3.32	3.80	3.22
9	4.01	2.52	3.73	2.55	3.69	2.68	3.99	2.60	3.92	3.18	3.74	3.05
10	3.52	2.77	3.52	2.85	3.87	2.98	3.49	2.75	3.70	3.28	3.82	2.92
11	3.58	2.99	3.68	2.98	3.65	2.48	3.57	2.90	3.74	3.24	3.68	3.06
12	3.98	2.76	3.96	2.47	3.68	2.65	4.01	2.72	4.06	2.95	3.74	2.85
Mean ± SD	3.83^{A} ±0.19	2.67^{B} ±0.22	3.76^{A} ±0.18	2.77^{B} ±0.21	3.78^{A} ±0.13	2.60^{B} ±0.24	3.81^{A} ±0.20	2.69^{D} ±0.19	3.86^{A} ±0.15	3.16^{B} ±0.14	3.82^{A} ±0.12	2.95^{C} ±0.16

SI.No	12th day						19th day					
	A_1	A_2	A_3	A_4	A_5	A_6	A_1	A_2	A_3	A_4	A_5	A_6
1	3.80	2.65	4.03	3.80	4.02	3.80	3.75	3.30	4.90	4.10	4.30	4.00
2	3.59	2.42	4.12	3.63	4.00	3.59	3.85	3.25	4.60	4.00	4.25	3.89
3	4.03	2.86	4.10	3.75	4.00	3.63	3.70	3.40	5.40	4.05	4.15	3.85
4	4.05	2.72	4.16	3.80	4.10	3.80	3.80	3.30	5.50	3.95	4.30	3.85
5	4.00	3.05	4.16	3.85	3.96	3.80	3.90	3.60	5.20	4.00	4.12	3.90
6	3.63	2.80	4.25	3.75	4.10	3.64	3.83	3.40	4.60	4.20	4.20	4.05
7	3.62	3.02	4.15	3.74	3.95	3.63	-	-	-	-	-	-
8	4.05	2.79	4.25	3.78	4.03	3.81	-	-	-	-	-	-
9	4.07	2.85	4.10	3.65	4.03	3.62	-	-	-	-	-	-
10	4.02	2.71	4.17	3.85	4.09	3.82	-	-	-	-	-	-
11	3.81	2.66	4.02	3.65	4.11	3.64	-	-	-	-	-	-
12	3.59	2.42	4.11	3.77	4.01	3.81	-	-	-	-	-	-
Mean ± SD	3.86^{B} ±0.20	2.75^{D} ±0.20	4.14^{A} ±0.07	3.75^{BC} ±0.07	4.03^{A} ±0.05	3.72^{C} ±0.10	3.80^{D} ±0.07	3.38^{E} ±0.13	5.03^{A} ±0.40	4.05^{BC} ±0.09	4.22^{B} ±0.07	3.92^{CD} ±0.08

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

The serum protein concentration of the mice in groups A_2, A_4 and A_6 showed a significantly lower value (immunosuppressed with dexamethasone before the start of the experiment) on day zero and day five of the experiment than their control groups, but on 12^{th} day groups A_2 and A_6 only showed such a significant decrease. On 19^{th} day, only A_2 was significantly lower where as A_4 showed a significant increase and A_6 showed no significant difference than their control groups.

There was no significant difference noticed in the groups A_3 and A_5 on the zero and 5^{th} day of the experiment but on 12^{th} and 19^{th} day, they showed a significant ($P<0.05$) increase than their control groups.

The mean serum total protein values in groups A_1, A_2, A_3, A_4, A_5 and A_6 on day 19 are 3.80±0.07, 3.38±0.13, 5.03±0.40, 4.05±0.09, 4.22±0.07 and 3.92±0.08 respectively.

4.2.3.2 *Globulin*

The individual and mean values of globulin concentration of drug treated groups and control groups on days 0, 5, 12 and 19 of experiment are recorded and it is presented in the Table 12 and Fig. 7.

The serum globulin concentration of the mice in groups A_2, A_4 and A_6 showed a significant decrease (immunosuppressed with dexamethasone before the start of the experiment) on day zero and five of the experiment than their control groups. But on 12^{th} day, groups A_2 and A_6 only showed such a significantly lower value. On 19^{th} day only A_2 was significantly lower where as A_4 showed a significantly higher value and A_6 showed no significant difference than their control groups.

Table 12. Effect of acetone soluble and acetone insoluble fractions of ethanolic extract of *Emblica officinalis* on serum globulin in grams per decilitre

SI.No	Zero day						5th day					
	A_1	A_2	A_3	A_4	A_5	A_6	A_1	A_2	A_3	A_4	A_5	A_6
1	1.55	1.0	1.46	1.17	1.52	0.90	1.56	1.01	1.66	1.32	1.65	1.13
2	1.49	0.90	1.52	0.96	1.53	1.01	1.49	0.90	1.55	1.20	1.57	1.10
3	1.62	1.05	1.53	1.01	1.58	1.04	1.63	1.05	1.59	1.22	1.57	1.14
4	1.65	1.01	1.63	1.14	1.53	1.05	1.65	1.03	1.65	1.32	1.54	1.19
5	1.64	1.17	1.58	1.12	1.55	0.98	1.64	1.17	1.59	1.32	1.56	1.21
6	1.48	1.09	1.65	1.08	1.65	1.18	1.48	1.09	1.66	1.31	1.67	1.21
7	1.55	1.01	1.64	1.08	1.64	0.91	1.54	1.07	1.65	1.33	1.66	1.28
8	1.63	1.06	1.52	1.13	1.55	1.01	1.63	1.07	1.60	1.35	1.56	1.32
9	1.14	1.00	1.57	1.02	1.53	1.05	1.63	1.05	1.62	1.31	1.55	1.26
10	1.47	1.10	1.46	1.12	1.60	1.17	1.46	1.10	1.54	1.34	1.58	1.18
11	1.49	1.17	1.52	1.16	1.52	0.98	1.49	1.15	1.56	1.33	1.52	1.26
12	1.63	1.09	1.63	0.96	1.53	1.04	1.63	1.07	1.68	1.20	1.55	1.15
Mean ± SD	1.52^{A} ±0.14	1.05^{B} ±0.07	1.56^{A} ±0.07	1.08^{B} ±0.07	1.56^{A} ±0.05	1.03^{B} ±0.09	1.57^{A} ±0.07	1.06^{C} ±0.07	1.61^{A} ±0.05	1.30^{B} ±0.06	1.58^{A} ±0.05	1.20^{B} ±0.07

SI.No	12th day						19th day					
	A_1	A_2	A_3	A_4	A_5	A_6	A_1	A_2	A_3	A_4	A_5	A_6
1	1.58	1.03	1.68	1.58	1.67	1.56	1.55	1.20	2.10	1.70	1.80	1.65
2	1.49	0.95	1.72	1.50	1.68	1.49	1.59	1.25	2.00	1.70	1.77	1.62
3	1.65	1.10	1.72	1.55	1.68	1.50	1.52	1.60	2.30	1.69	1.75	1.59
4	1.68	1.05	1.74	1.60	1.70	1.56	1.55	1.60	2.30	1.65	1.85	1.60
5	1.66	1.18	1.74	1.57	1.65	1.55	1.60	1.40	2.25	1.67	1.76	1.62
6	1.50	1.11	1.78	1.55	1.70	1.50	1.58	1.30	2.00	1.75	1.78	1.65
7	1.50	1.17	1.74	1.55	1.64	1.49	-	-	-	-	-	-
8	1.68	1.10	1.78	1.57	1.68	1.56	-	-	-	-	-	-
9	1.67	1.10	1.71	1.50	1.67	1.49	-	-	-	-	-	-
10	1.66	1.05	1.74	1.59	1.70	1.57	-	-	-	-	-	-
11	1.59	1.04	1.68	1.50	1.71	1.50	-	-	-	-	-	-
12	1.49	0.96	1.72	1.57	1.67	1.56	-	-	-	-	-	-
Mean ± SD	1.60^{C} ±0.08	1.07^{E} ±0.07	1.73^{A} ±0.03	1.55^{CD} ±0.03	1.68^{B} ±0.02	1.53^{D} ±0.03	1.57^{D} ±0.03	1.39^{E} ±0.17	2.16^{A} ±0.14	1.69^{BC} ±0.03	1.79^{B} ±0.04	1.62^{CD} ±0.02

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

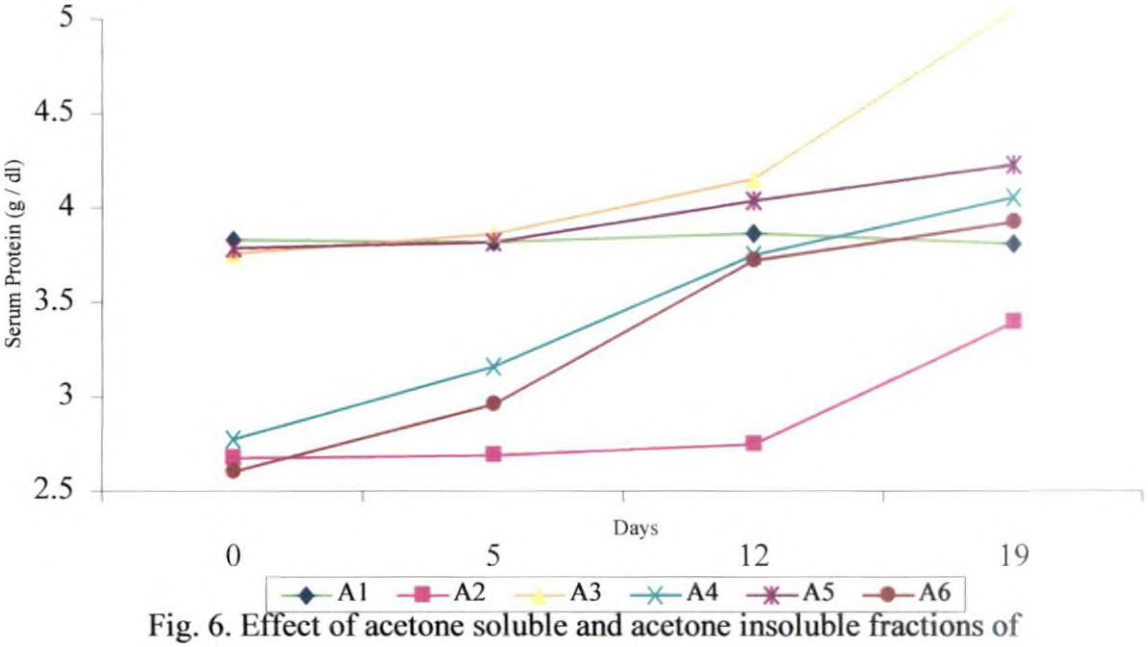


Fig. 6. Effect of acetone soluble and acetone insoluble fractions of ethanolic extract of *Emblica officinalis* on total serum protein

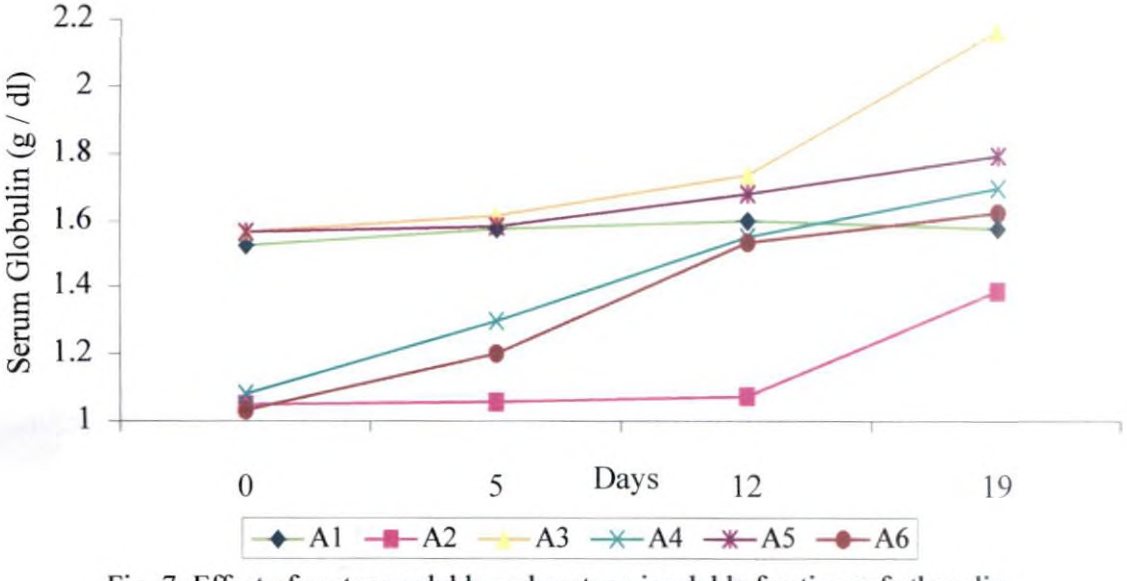


Fig. 7. Effect of acetone soluble and acetone insoluble fractions of ethanolic extract of *Emblica officinalis* on serum globulin

There was no significant difference in the groups A_3 and A_5 on the zero and 5th day of the experiment but on 12th and 19th day, significant ($P<0.05$) increase was noticed than their control groups.

The mean serum globulin values in groups A_1, A_2, A_3, A_4, A_5 and A_6 on day 19 are 1.57±0.03, 1.39±0.17, 2.16±0.14, 1.69±0.03, 1.79±0.04 and 1.62±0.02 respectively.

4.2.3.3 *Albumin-Globulin Ratio*

The individual and mean values of albumin-globulin ratio of drug treated groups and control groups on days 0, 5, 12 and 19 of experiment are calculated and it is given in the Table 13. The mean values are graphically presented in Fig. 8.

The serum albumin-globulin ratio of the mice in groups A_2, A_4 and A_6 showed a significantly higher value on day zero. On day 5 and 12, only groups A_2 and A_6 showed such a significantly higher value. On 19th day, only A_2 was significantly higher, where as A_4 and A_6 showed no significant difference than their control groups.

There was no significant difference noticed in the groups A_3 and A_5 on zero and 5th day of the experiment but on 12th and 19th day significant ($P<0.05$) decrease was noticed than their control groups. The mean value recorded on day 19 of the experiment in the groupsA_1, A_2, A_3, A_4, A_5 and A_6 are 1.43±0.01, 1.63±0.07, 1.32±0.02, 1.40±0.01, 1.36±0.02 and 1.42±0.01 respectively.

4.2.4 Enzymatic Parameters

4.2.4.1 *Superoxide Dismutase*

The superoxide dismutase level was measured on days 12 and 19 of the experiment and their individual and mean values are given in the Table 14. The mean value is presented graphically in Fig. 9.

Table 13. Effect of acetone soluble and acetone insoluble fractions of ethanolic extract of *Emblica officinalis* on serum albumin-globulin ratio

SI.No	Zero day						5th day					
	A_1	A_2	A_3	A_4	A_5	A_6	A_1	A_2	A_3	A_4	A_5	A_6
1	1.41	1.52	1.41	1.58	1.40	1.48	1.42	1.52	1.40	1.42	1.41	1.43
2	1.40	1.48	1.40	1.56	1.41	1.51	1.40	1.50	1.42	1.42	1.42	1.47
3	1.44	1.55	1.41	1.51	1.44	1.55	1.44	1.57	1.39	1.42	1.42	1.46
4	1.47	1.51	1.43	1.59	1.41	1.55	1.44	1.52	1.44	1.46	1.42	1.44
5	1.43	1.58	1.37	1.55	1.41	1.54	1.43	1.58	1.40	1.44	1.42	1.44
6	1.47	1.52	1.44	1.57	1.45	1.56	1.45	1.52	1.43	1.43	1.43	1.46
7	1.42	1.51	1.43	1.59	1.43	1.48	1.43	1.57	1.42	1.44	1.41	1.46
8	1.43	1.55	1.41	1.58	1.43	1.52	1.42	1.54	1.40	1.46	1.44	1.44
9	1.44	1.52	1.38	1.50	1.41	1.55	1.45	1.48	1.42	1.43	1.41	1.42
10	1.39	1.52	1.41	1.54	1.42	1.55	1.39	1.50	1.40	1.45	1.42	1.47
11	1.40	1.56	1.42	1.57	1.40	1.53	1.40	1.52	1.40	1.44	1.42	1.43
12	1.44	1.53	1.43	1.57	1.41	1.55	1.46	1.54	1.42	1.42	1.41	1.48
Mean ± SD	1.43^{C} ±0.03	1.53^{B} ±0.03	1.41^{C} ±0.02	1.56^{A} ±0.03	1.42^{C} ±0.01	1.53^{B} ±0.03	1.43^{CD} ±0.02	1.53^{A} ±0.03	1.41^{D} ±0.02	1.44^{C} ±0.02	1.42D ±0.00	1.45^{B} ±0.02

SI.No	12th day						19th day					
	A_1	A_2	A_3	A_4	A_5	A_6	A_1	A_2	A_3	A_4	A_5	A_6
1	1.41	1.57	1.40	1.41	1.40	1.44	1.42	1.76	1.33	1.41	1.39	1.42
2	1.41	1.54	1.39	1.42	1.38	1.41	1.42	1.60	1.30	1.38	1.37	1.40
3	1.44	1.60	1.38	1.42	1.38	1.42	1.43	1.61	1.35	1.40	1.37	1.42
4	1.41	1.59	1.39	1.44	1.41	1.44	1.45	1.64	1.34	1.39	1.33	1.41
5	1.41	1.58	1.39	1.41	1.40	1.45	1.44	1.57	1.31	1.40	1.34	1.41
6	1.42	1.52	1.39	1.42	1.41	1.43	1.42	1.61	1.30	1.40	1.36	1.45
7	1.41	1.58	1.38	1.41	1.41	1.44	-	-	-	-	-	-
8	1.41	1.54	1.39	1.41	1.40	1.44	-	-	-	-	-	-
9	1.44	1.59	1.40	1.43	1.41	1.43	-	-	-	-	-	-
10	1.42	1.58	1.40	1.42	1.41	1.43	-	-	-	-	-	-
11	1.40	1.56	1.39	1.43	1.40	1.43	-	-	-	-	-	-
12	1.41	1.52	1.39	1.41	1.40	1.44	-	-	-	-	-	-
Mean ± SD	1.42^{C} ±0.01	1.56^{A} ±0.03	1.39^{D} ±0.01	1.42^{C} ±0.01	1.40^{D} ±0.01	1.43^{B} ±0.01	1.43^{B} ±0.01	1.63^{A} ±0.07	1.32^{D} ±0.02	1.40^{BC} ±0.01	1.36^{C} ±0.02	1.42^{B} ±0.01

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

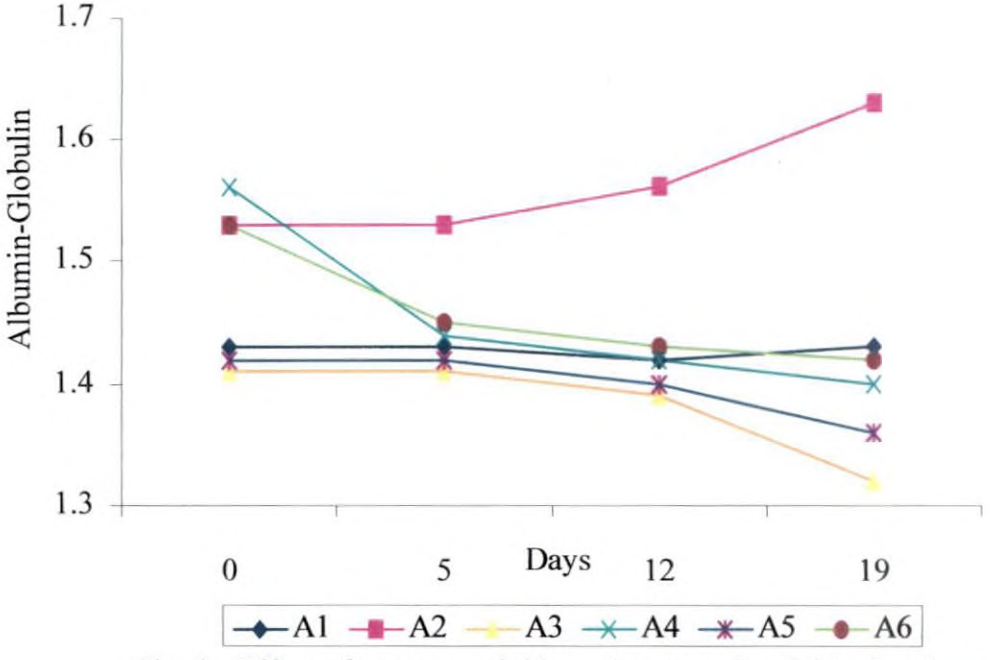


Fig. 8. Effect of acetone soluble and acetone insoluble fractions of ethanolic extract of *Emblica officianlis* on albumin-globulin ratio

The superoxide dismutase level in groups A_2, A_4 and A_6 showed a significantly lower value than their control groups, whereas the group A_3 showed a significantly ($P<0.05$) higher values and group A_5 showed no significant difference on 12^{th} day of the experiment than their control groups. The superoxide dismutase level in groups A_3 and A_5 showed a significant ($P<0.05$) increase and groups A_2, A_4 and A_6 showed a significantly lower values on 19^{th} day of the experiment than their control groups.

The mean values of the groups A_1, A_2, A_3, A_4, A_5 and A_6 on day 19 are 5.67±0.67, 4.56±0.10, 6.40±0.09, 5.34±0.07, 6.18±0.20 and 5.14±0.17 respectively

4.2.4.2 *Catalase*

The catalase level was measured on days 12 and 19 of the experiment and their individual and mean values are given in the Table 15. The mean value is presented graphically in Fig. 10.

The catalase level in groups A_2, A_4 and A_6 showed a significantly lower value than their control groups, whereas group A_3 showed a significantly ($P<0.05$) higher values and group A_5 showed no significant difference on 12^{th} day of the experiment than their control groups. The catalase level in groups A_3 and A_5 showed a significant ($P<0.05$) increase. However, groups A_2 and A_6 showed significantly lower values on day 19 of the experiment than their control groups. Group A_4 showed no significant difference on 19^{th} day of the experiment.

The mean values of groups A_1, A_2, A_3, A_4, A_5 and A_6 are 6.14±0.08, 5.34±0.09, 7.16±0.12, 6.03±0.11, 6.80±0.09 and 5.68±0.34 respectively.

Table 14. Effect of acetone soluble and acetone insoluble fractions of ethanolic extract of *Emblica officinalis* on quantity of superoxide dismutase in units per milligram of protein

S.No	12th day						19th day					
	A_1	A_2	A_3	A_4	A_5	A_6	A_1	A_2	A_3	A_4	A_5	A_6
1	5.50	4.28	5.85	4.84	5.78	4.55	5.55	4.56	6.38	5.32	6.08	5.02
2	5.65	4.41	5.68	4.75	5.65	4.68	5.65	4.64	6.42	5.26	6.24	5.24
3	5.73	4.36	5.81	4.89	5.81	4.64	5.77	4.58	6.46	5.34	6.54	5.01
4	5.71	4.44	5.96	4.92	5.77	4.78	5.79	4.76	6.29	5.47	5.96	5.38
5	5.62	4.31	5.89	5.02	5.74	4.69	5.61	4.59	6.54	5.31	6.12	4.96
6	5.69	4.22	5.94	4.45	5.82	4.68	5.67	4.44	6.32	5.37	6.13	5.25
Mean ± SD	5.65^{B} ±0.08	4.34^{E} ±0.08	5.86^{A} ±0.10	4.81^{C} ±0.20	5.76^{AB} ±0.06	4.67^{D} ±0.07	5.67^{C} ±0.09	4.56^{F} ±0.10	6.40^{A} ±0.09	5.34^{D} ±0.07	6.18^{B} ±0.20	5.14^{E} ±0.17

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

Table 15. Effect of acetone soluble and acetone insoluble fractions of ethanolic extract of *Emblica officinalis* on quantity of catalase in units per assay mixture

S.No	12th day						19th day					
	A_1	A_2	A_3	A_4	A_5	A_6	A_1	A_2	A_3	A_4	A_5	A_6
1	6.25	5.10	6.48	5.50	6.42	5.40	6.21	5.32	6.98	5.95	6.75	5.98
2	5.90	5.05	6.32	5.45	6.35	5.35	6.05	5.48	7.12	6.04	6.86	5.75
3	6.22	5.18	6.52	5.75	6.25	5.25	6.12	5.22	7.21	6.18	6.81	5.82
4	6.18	5.11	6.54	5.65	6.26	5.28	6.25	5.31	7.07	6.12	6.66	5.76
5	6.19	5.12	6.54	5.60	6.18	5.09	6.15	5.38	7.24	5.96	6.92	5.75
6	6.21	5.13	6.47	5.50	6.09	5.32	6.06	5.32	7.31	5.90	6.82	5.01
Mean ± SD	6.16^{B} ±0.13	5.12^{E} ±0.42	6.48^{A} ±0.08	5.58^{C} ±0.11	6.26^{B} ±0.12	5.28^{D} ±0.11	6.14^{C} ±0.08	5.34^{E} ±0.09	7.16^{A} ±0.12	6.03^{C} ±0.11	6.80^{B} ±0.09	5.68^{D} ±0.34

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

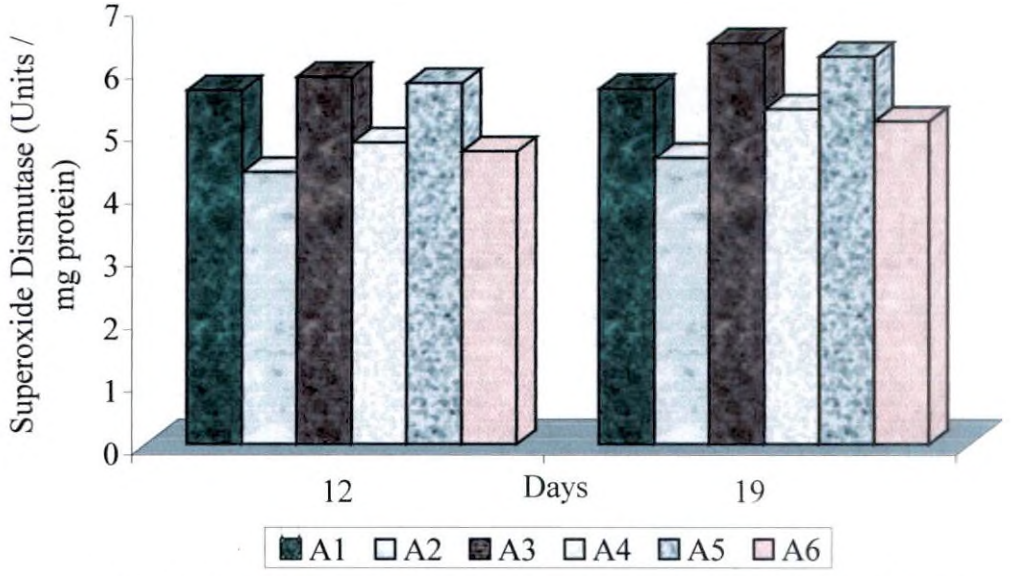


Fig. 9. Effect of acetone soluble and acetone insoluble fractions of ethanolic extract of *Emblica officianlis* on quantity of Superoxide Dismutase

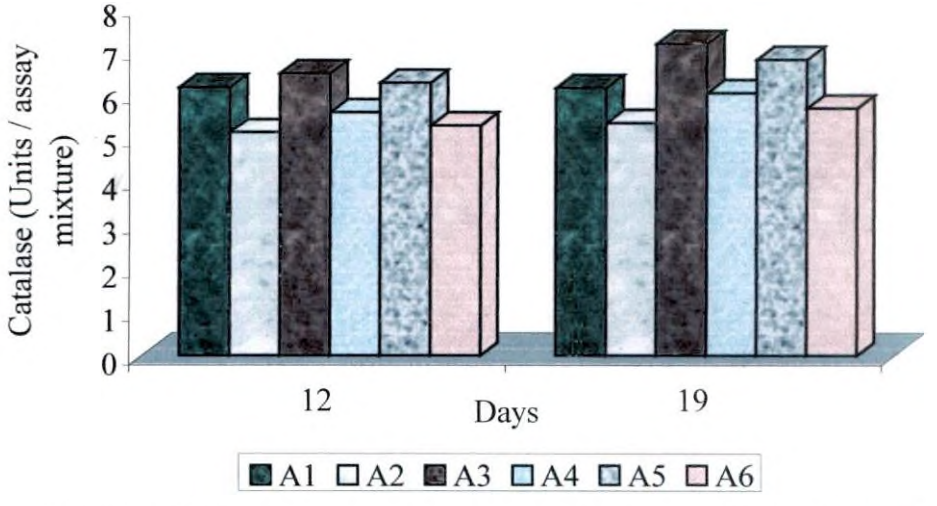


Fig. 10. Effect of acetone soluble and acetone insoluble fractions of ethanolic extract of *Emblica officianlis* on quantity of Catalase

4.2.5 Immunological Parameters

4.2.5.1 *Haemagglutination Titre*

Haemagglutination titre of sera from mice was taken on days 0, 5, 12 and 19 of experiment and is given in the Table 16. The maximum titre level observed during the experimental period was on day 19 in group A_3.

The mean values of the groups A_1, A_2, A_3, A_4, A_5 and A_6 on day 19 are 37.33±13.06, 37.33±13.06, 192.00±70.11, 53.33±16.53, 149.33±52.26 and 33.33±13.06 respectively.

Groups A_2, A_4 and A_6 (immunosuppressed with dexamethasone) showed significantly lower value and rest of the groups showed no significant difference than their control groups on zero day of treatment. On 12^{th} day of the experiment, groups A_3 and A_5 showed a significant ($P<0.05$) increase, while group A_2 showed significantly lower value. The rest of the groups showed no significant difference.

Groups A_3, A_4 and A_5 showed a significant ($P<0.05$) increase on 19^{th} day of the experiment, while rest of the groups showed no significant difference.

4.2.5.2 *Spleenic Cell Plaque forming Assay*

Numbers of spleenic plaque forming cells are recorded on 12^{th} and 19^{th} day of the experiment and their individual and mean values are given in the Table 17 and Fig. 11.

Number of plaque forming cells in groups A_2, A_4 and A_6 showed a significantly lower value than their control groups, where as the groups A_3 and A_5 showed a significantly ($P<0.05$) higher values on 12^{th} day of the experiment. Groups A_3, A_4 and A_5 showed a significantly higher value but significantly lower values were found in the group A_2. In group A_6, significant difference was not seen on 19^{th} day of the experiment.

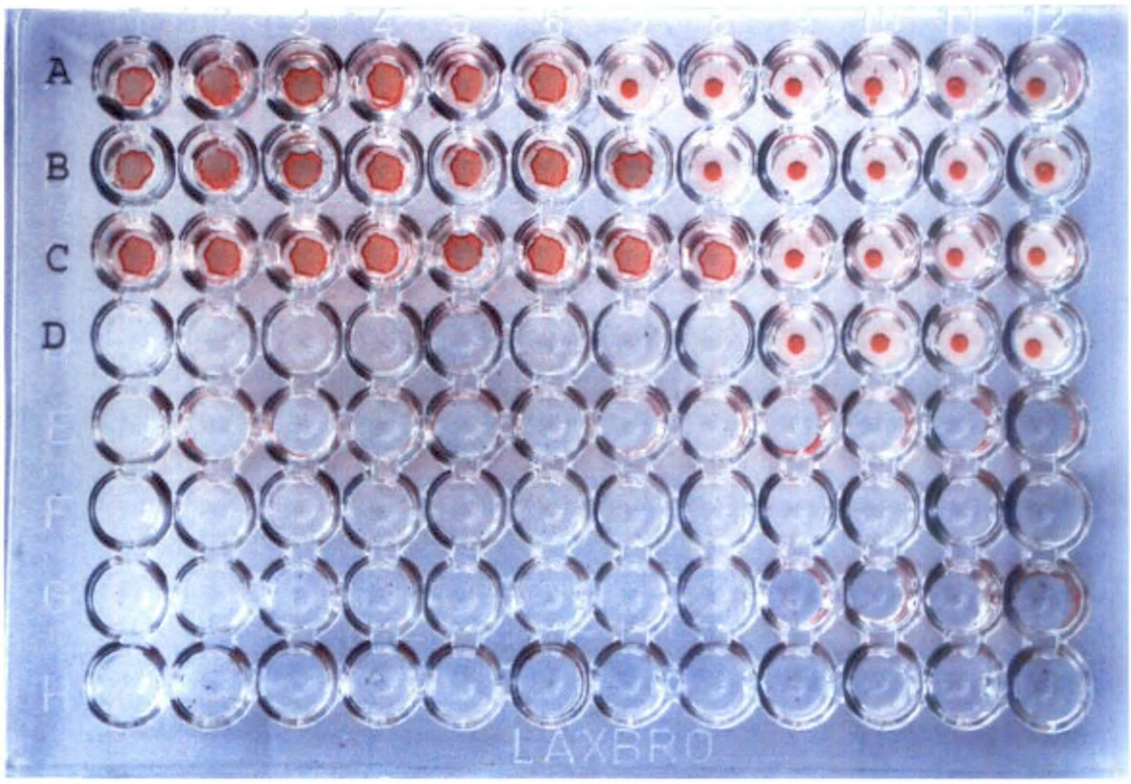


Plate 1. Haemagglutination test

(A) Control group
(B) Acetone insoluble fraction treated group
(C) Acetone soluble fraction treated group
(D) RBC control

Table 16. Effect of acetone soluble and acetone insoluble fractions of ethanolic extract of *Emblica officinalis* on Haemagglutination titre.

S.No	Zero day						12th day						19th day					
	A_1	A_2	A_3	A_4	A_5	A_6	A_1	A_2	A_3	A_4	A_5	A_6	A_1	A_2	A_3	A_4	A_5	A_6
1	2	2	4	2	4	2	32	16	128	16	64	16	32	32	256	64	128	32
2	4	2	2	2	2	2	32	16	64	32	64	16	32	32	128	64	128	32
3	4	2	4	2	2	2	64	4	128	16	128	32	64	32	256	32	256	64
4	2	2	4	2	4	2	32	16	64	32	64	16	32	64	128	64	128	32
5	4	2	2	4	4	4	32	16	128	32	128	16	32	32	128	64	128	32
6	4	4	4	2	2	2	32	4	128	32	64	16	32	32	256	32	128	32
7	4	2	4	2	4	2	32	16	64	32	64	16	-	-	-	-	-	-
8	2	2	2	2	2	4	32	16	128	16	128	16	-	-	-	-	-	-
9	4	2	4	4	4	2	64	4	64	32	64	16	-	-	-	-	-	-
10	2	2	2	2	4	2	32	16	128	16	64	32	-	-	-	-	-	-
11	4	2	4	2	4	2	32	16	64	32	64	16	-	-	-	-	-	-
12	2	2	4	2	4	2	32	16	128	32	64	32	-	-	-	-	-	-
Mean ±SD	3.17^A ±1.03	2.17^B ±0.58	3.33^A ±0.99	2.33^B ±0.78	3.33^A ±0.99	2.33^B ±0.78	37.33^B ±12.46	13.00^A ±5.43	101.3^C ±32.96	26.67^B ±7.08	80.00^D ±28.95	20.25^B ±7.14	37.33^B ±13.06	37.33^B ±13.06	192.0^D ±70.11	53.33^C ±16.53	149.0^D ±52.26	33.33^B ±13.06

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

Table 17. Effect of acetone soluble and acetone insoluble fractions of ethanolic extract of *Emblica officinalis* on number of plaque forming cells per 10^6 spleen cells.

SI.No	12^{th} day						19^{th} day					
	A_1	A_2	A_3	A_4	A_5	A_6	A_1	A_2	A_3	A_4	A_5	A_6
1	762	624	980	710	960	675	775	685	1120	820	1140	805
2	748	585	965	685	895	655	715	615	1085	808	965	809
3	712	605	915	728	905	685	760	625	1105	865	1038	721
4	755	612	973	722	875	705	695	678	1054	795	1054	768
5	768	610	970	682	862	692	786	680	1068	882	1032	708
6	786	590	950	696	912	660	775	695	1180	792	985	760
Mean ± SD	755.17^{C} ±24.80	604.33^{E} ±14.54	958.83^{A} ±23.71	703.83^{D} ±19.21	901.50^{B} ±34.20	678.67^{D} ±19.15	751.00^{D} ±37.12	663.00^{E} ±33.97	1102.00^{A} ±45.09	827.00^{C} ±37.76	1035.67^{B} ±61.32	761.83^{D} ±41.70

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

The mean values of the groups A_1, A_2, A_3, A_4, A_5 and A_6 on day 19 are 751.00±33.97, 663.00±33.97, 1102.00±45.09, 827.00±37.76, 1035.67±61.32 and 761.83±41.70 respectively.

4.2.5.3 *Bone Marrow Cellularity*

Total bone marrow cellularity was recorded on 12th and 19th day of the experiment and their individual and mean values are given in the Table 18 and Fig.12. The mean values of the groups A_1, A_2, A_3, A_4, A_5 and A_6 on day 19 are 10.76±0.31, 7.74±0.40, 13.58±0.27, 9.88±0.34, 12.71±0.42 and 8.61±0.30 respectively.

The bone marrow cellularity in groups A_2, A_4 and A_6 showed a significantly lower value, whereas the groups A_3 and A_5 showed a significantly ($P<0.05$) higher value on both 12th and 19th day of the experiment than their control groups.

4.2.5.4 *Delayed Type of Hypersensitivity*

Delayed Type of Hypersensitivity 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. The increase in footpad thickness of the treatment and control groups on day 12 and day 19 are presented in Table 19 and Fig. 13.

The increase in thickness of the foot pad in groups B_3, B_4, B_5 and B_6 showed a significantly ($P<0.05$) higher value and group B_2 showed a significantly lower value than their control groups on both 12th and 19th day of the experiment.

4.2.5.5 *Macrophage Migration Index (MMI)*

Macrophages were isolated from peritoneal washing of drug / treatment groups A_1, A_2, A_3, A_4, A_5 and A_6 without red cell contamination. On an average 90 percent cells were found to be viable by dye exclusion method. The

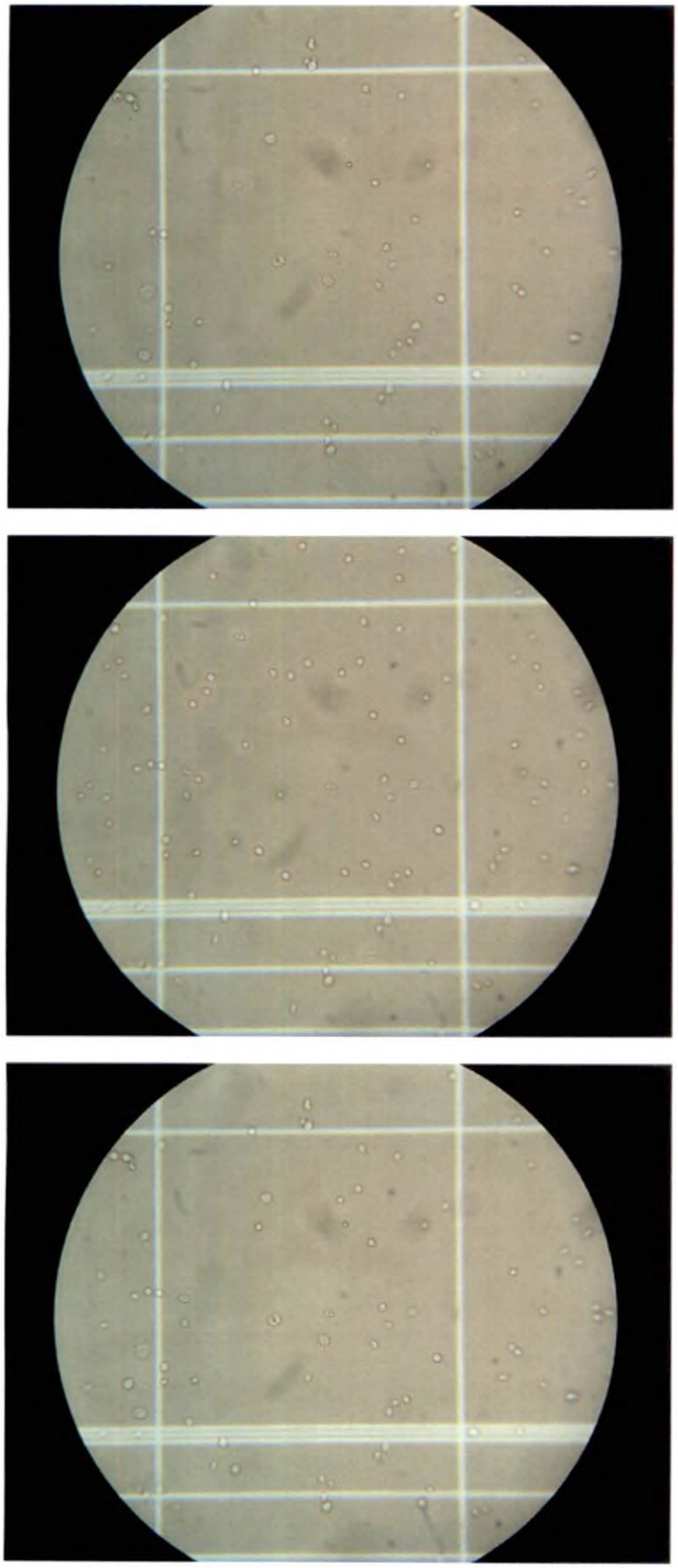


Plate 2. Bone marrow cellularity

(A) Control group
(B) Acetone soluble fraction treated group
(C) Acetone insoluble fraction treated group

Table 18. Effect of acetone soluble and acetone insoluble fractions of ethanolic extract of *Emblica officinalis* on bone marrow cellularity in million cells per femur

S.No	12th day						19th day					
	A_1	A_2	A_3	A_4	A_5	A_6	A_1	A_2	A_3	A_4	A_5	A_6
1	10.50	6.42	13.10	8.90	12.25	7.50	10.80	7.42	14.05	9.75	13.12	8.65
2	10.85	6.82	12.85	8.12	12.05	8.26	10.88	7.28	13.50	10.10	12.95	8.87
3	9.50	6.96	12.82	8.65	11.58	7.18	10.20	7.69	13.48	9.60	12.68	8.41
4	10.85	6.28	13.26	7.86	12.26	7.26	10.92	7.75	13.50	9.92	12.18	8.41
5	10.23	7.12	13.48	8.67	13.12	8.22	11.10	8.42	13.26	10.41	13.10	9.05
6	10.42	6.85	13.32	7.92	11.96	7.29	10.68	7.90	13.68	9.48	12.25	8.29
Mean ± SD	10.39^{C} ±0.50	6.74^{F} ±0.32	13.14^{A} ±0.26	8.35^{D} ±0.44	12.20^{B} ±0.51	7.62^{E} ±0.50	10.76^{C} ±0.31	7.74^{F} ±0.40	13.58^{A} ±0.27	9.88^{D} ±0.34	12.71^{B} ±0.42	8.61^{E} ±0.30

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

Table 19. Effect of acetone soluble and acetone insoluble fractions of ethanolic extract of *Emblica officinalis* on delayed hypersensitivity reaction in millimetre

S.No	12th day						19th day					
	B_1	B_2	B_3	B_4	B_5	B_6	B_1	B_2	B_3	B_4	B_5	B_6
1	0.5	0.40	1.40	0.55	1.40	0.50	0.60	0.50	1.80	0.90	1.70	0.90
2	0.4	0.30	1.50	0.55	1.45	0.55	0.55	0.40	1.95	0.90	1.90	0.80
3	0.5	0.40	1.60	0.70	1.50	0.60	0.65	0.50	2.10	1.10	1.70	0.80
4	0.4	0.37	1.32	0.80	1.30	0.80	0.50	0.40	1.80	1.20	1.90	1.10
5	0.5	0.30	1.20	0.75	1.20	0.70	0.55	0.50	1.70	1.20	1.70	1.10
6	0.45	0.40	1.20	0.80	1.10	0.70	0.60	0.50	1.70	1.10	1.60	1.00
Mean ± SD	0.46^{C} ±0.45	0.36^{C} ±0.05	1.37^{A} ±0.16	0.69^{B} ±0.12	1.32^{A} ±0.15	0.64^{B} ±0.11	0.58^{C} ±0.05	0.47^{C} ±0.05	1.84^{A} ±0.16	1.07^{B} ±0.14	1.76^{A} ±0.12	0.95^{B} ±0.14

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

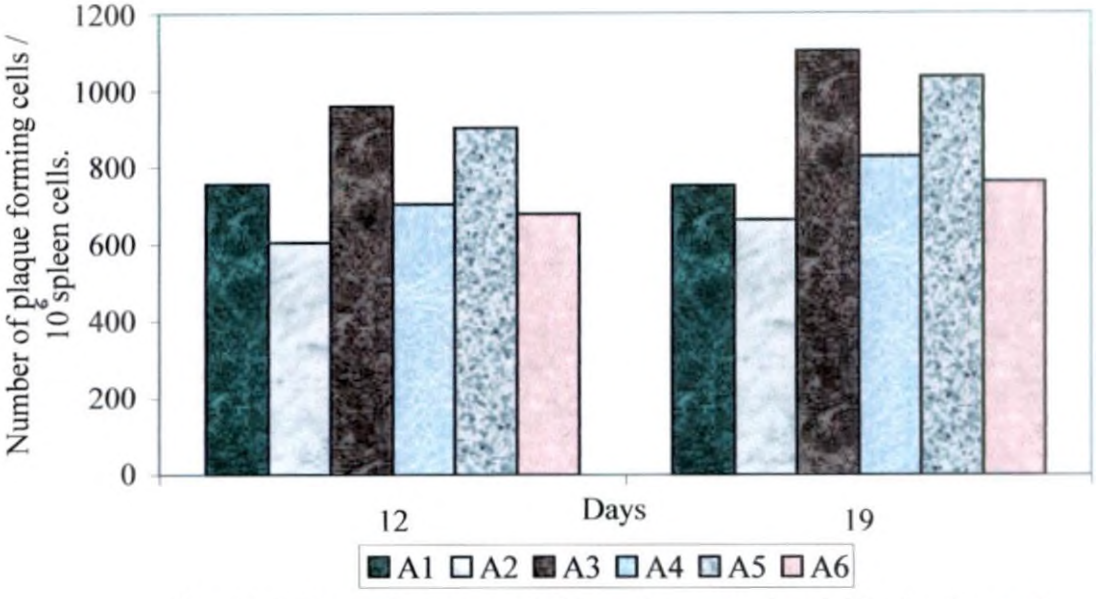


Fig. 11. Effect of acetone soluble and acetone insoluble fractions of ethanolic extract of *Emblica officinalis* on plaque forming cells

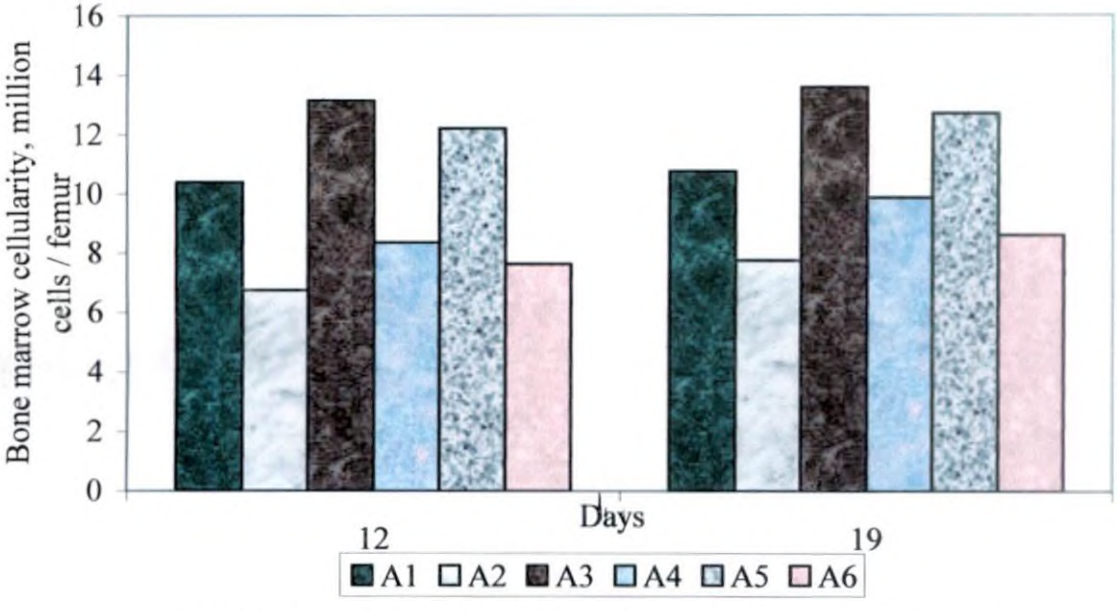


Fig. 12. Effect of acetone soluble and acetone insoluble fractions of ethanolic extract of *Emblica officinalis* on bone marrow cellularity

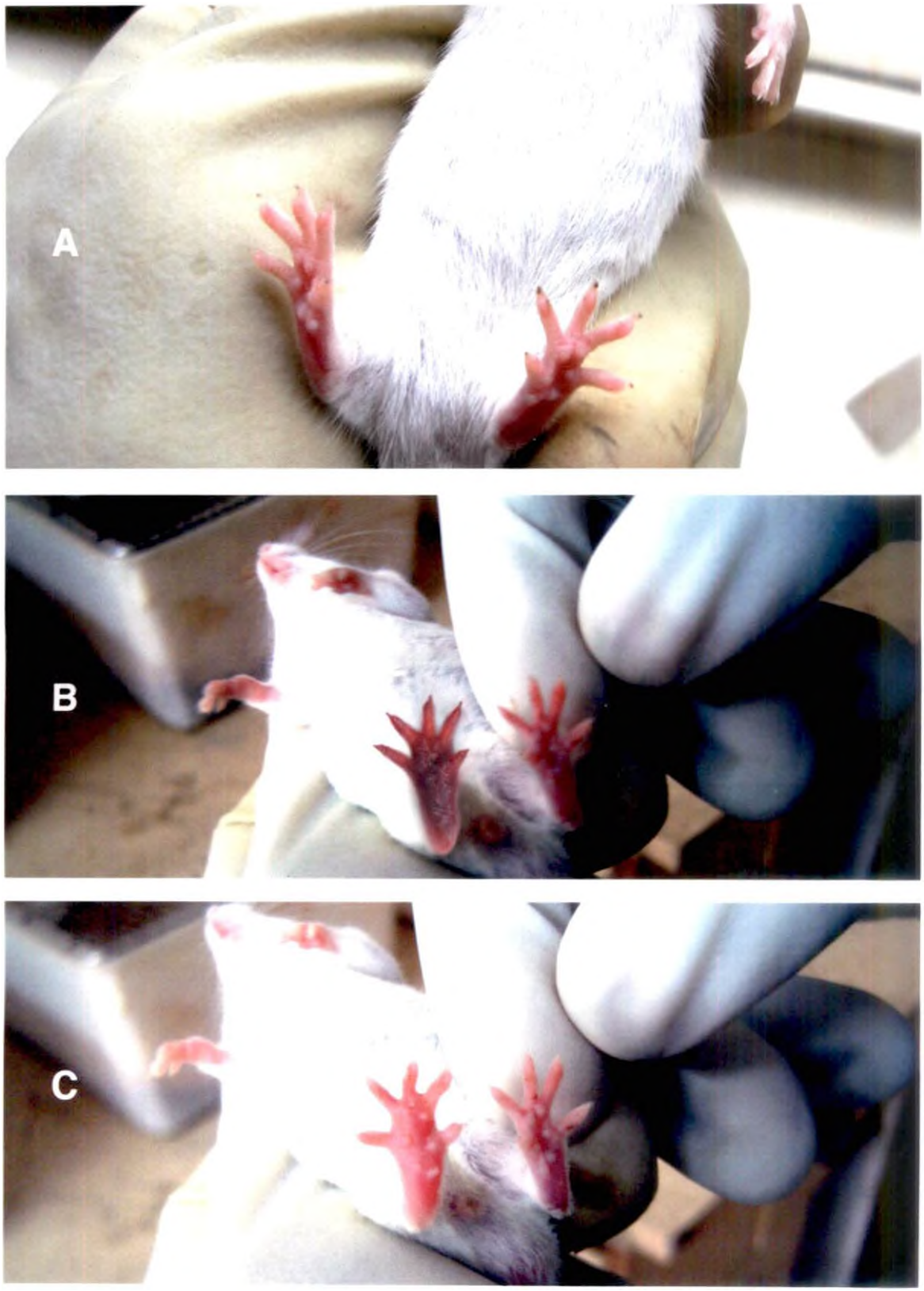


Plate 3. Delayed hypersensitivity reaction on footpad of mice

(A) Control group

(B) Acetone soluble fraction treated group

(C) Acetone insoluble fraction treated group

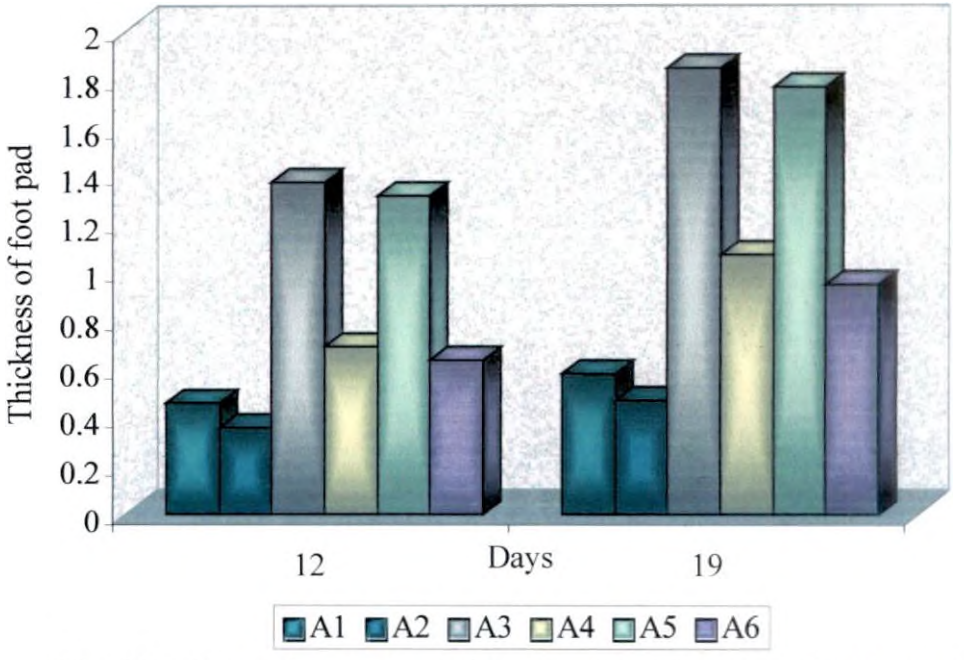


Fig. 13. Effect of acetone soluble and acetone insoluble fractions of ethanolic extract of *Emblica officinalis* on delayed hypersensitivity reaction

macrophage suspension prepared at a concentration of 60-80 X 10^6 cells per ml did not show clumping or reduction in viability.

Macrophages 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. The diameter of the area was measured at different angles viewing through a magnifying lens.

Macrophage migration area of all mice was taken on day 12 and 19 of drug / vehicle administration. The individual and mean values of the macrophage migration area of all the groups are given in the Table 20 and Fig. 14.

The macrophage migration area of the groups A_3 and A_5 showed significantly ($P<0.05$) higher value, while others showed no significant difference than their control groups on 12^{th} day of treatment. On 19^{th} day of the experiment, groups A_3, A_4 and A_5 showed a significant ($P<0.05$) increase, where as groups A_2 and A_6 showed no significant difference than their control groups.

The maximum MMI level was noticed in the group A_3 on 19^{th} day of the experiment as presented in the Table 21 and Fig. 15. The mean MMI values obtained from groups A_2, A_3, A_4, A_5 and A_6 on 12^{th} day were 3.92±0.60, 7.28±0.55, 4.91±0.15, 6.98±0.45 and 4.32±0.46 and on 19^{th} day were 4.69±0.46, 9.15±0.56, 5.93±0.30, 7.07±0.44 and 5.03±0.16.

4.2.5.6 *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 that can be detected through an assay based on the reduction of NBT. NBT readings of the control and treatment group are presented in Table 22 and Fig. 16.

NBT values of the groups A_3 and A_5 showed significantly ($P<0.05$) higher value, while groups A_2 and A_6 showed significantly lower value and group A_4

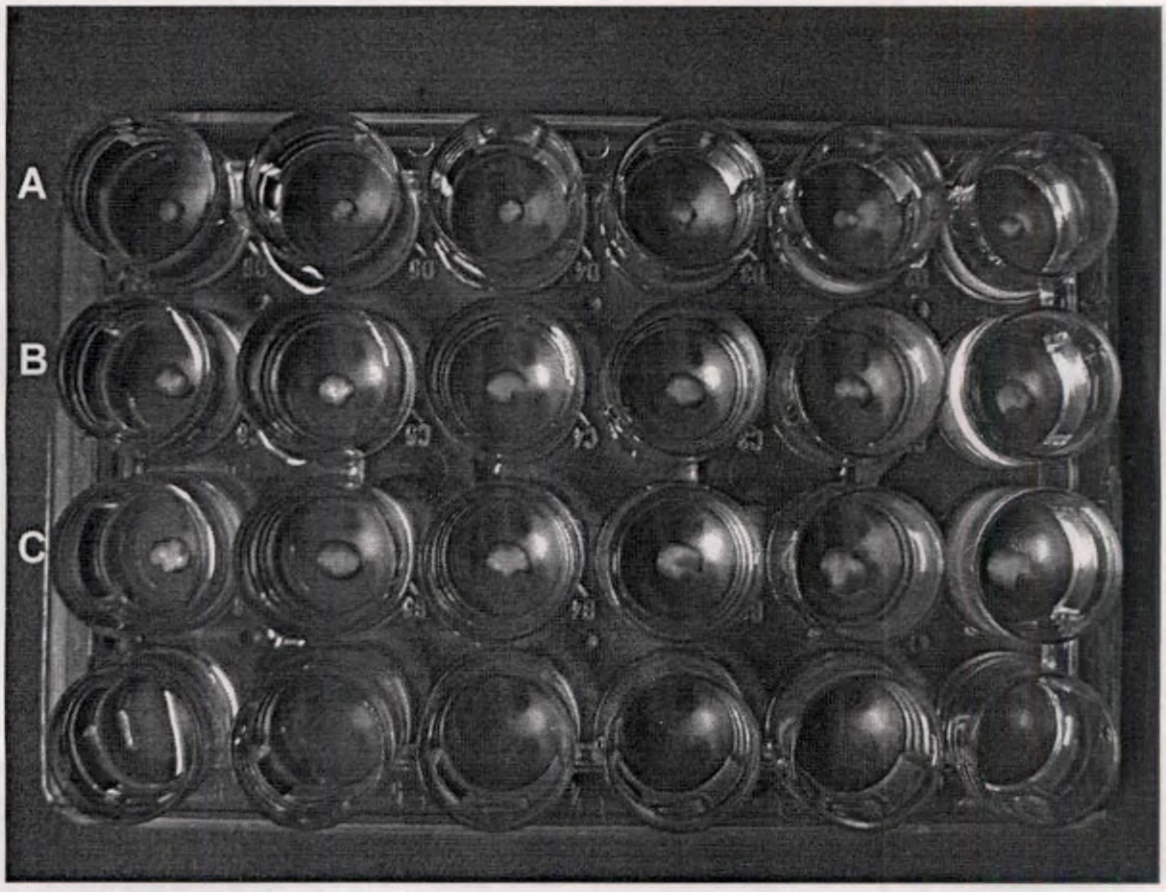


Plate 4. Macrophage migration test

(A) Control group

(B) Acetone insoluble fraction treated group

(C) Acetone soluble fraction treated group

Table 20. Effect of acetone soluble and acetone insoluble fractions of ethanolic extract of *Emblica officinalis* on macrophage migration area in square millimetre

S.No	12th day						19th day					
	A_1	A_2	A_3	A_4	A_5	A_6	A_1	A_2	A_3	A_4	A_5	A_6
1	5.50	4.82	7.75	4.85	7.50	4.85	6.15	5.14	9.75	5.65	7.75	5.05
2	6.25	3.75	8.15	4.75	7.45	4.10	5.30	4.60	9.62	6.25	7.15	4.95
3	4.35	3.50	7.15	5.10	7.20	3.75	4.25	4.25	8.75	5.90	7.07	4.85
4	3.48	4.25	6.72	4.75	6.50	4.22	4.10	4.50	9.58	5.50	6.45	5.15
5	5.00	4.10	6.85	4.96	6.60	4.25	5.05	5.35	8.50	6.20	6.75	5.28
6	4.25	3.10	7.07	5.05	6.65	4.75	4.30	4.28	8.70	6.10	7.25	4.92
Mean ± SD	4.80^{BC} ±0.99	3.92^{C} ±0.60	7.28^{A} ±0.55	4.91^{B} ±0.15	6.98^{A} ±0.45	4.32^{B} ±0.41	4.86^{D} ±0.79	4.69^{D} ±0.46	9.15^{A} ±0.26	5.63^{C} ±0.31	7.07^{B} ±0.44	5.03^{D} ±0.16

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

Table 21. Effect of acetone soluble and acetone insoluble fractions of ethanolic extract of *Emblica officinalis* on macrophage migration index

SI.No	12th day					19th day				
	A_2	A_3	A_4	A_5	A_6	A_2	A_3	A_4	A_5	A_6
1	4.82	7.75	4.85	7.50	4.85	5.14	9.75	5.65	7.75	5.05
2	3.75	8.15	4.75	7.45	4.10	4.60	9.62	6.25	7.15	4.95
3	3.50	7.15	5.10	7.20	3.75	4.25	8.75	5.90	7.07	4.85
4	4.25	6.72	4.75	6.50	4.22	4.50	9.58	5.50	6.45	5.15
5	4.10	6.85	4.96	6.60	4.25	5.35	8.50	6.20	6.75	5.28
6	3.10	7.07	5.05	6.65	4.75	4.28	8.70	6.10	7.25	4.92
Mean ± SD	3.92^{C} ±0.60	7.28^{A} ±0.55	4.91^{B} ±0.15	6.98^{A} ±.045	4.32^{C} ±0.41	4.69^{D} ±0.46	9.15^{A} ±0.56	5.93^{C} ±0.30	7.07^{B} ±0.44	5.03^{D} ±0.16

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

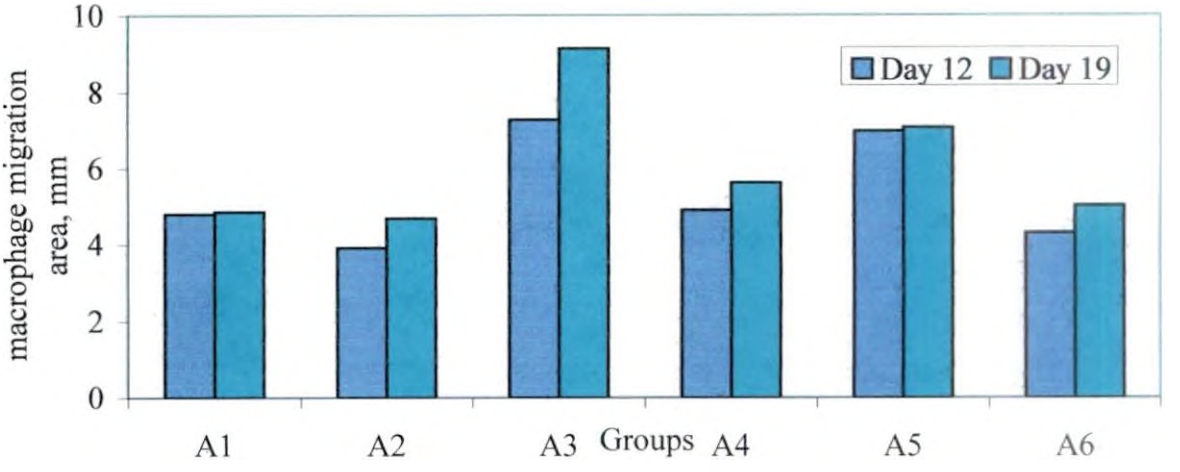


Fig. 14. Effect of acetone soluble and acetone insoluble fractions of ethanolic extract of *Emblica officinalis* on macrophage migration area

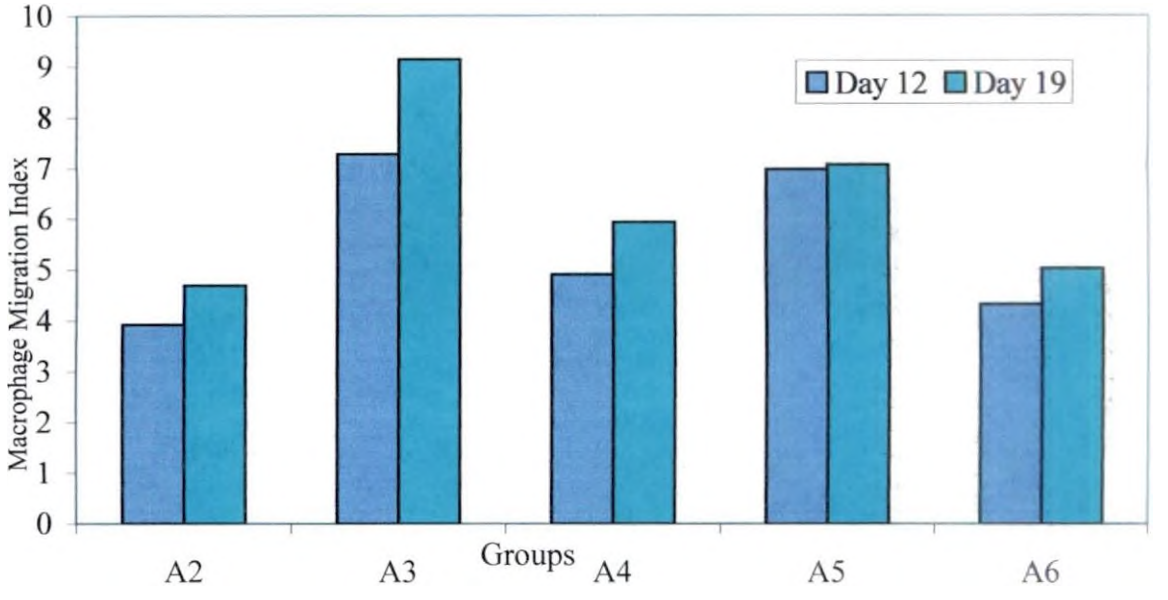


Fig. 15. Effect of acetone soluble and acetone insoluble fractions of ethanolic extract of *Emblica officinalis* on macrophage migration index

Table 22. Effect of acetone soluble and acetone insoluble fractions of ethanolic extract of *Emblica officinalis* on nitroblue tetrazolium dye reduction test

S.No	12th day						19th day					
	A_1	A_2	A_3	A_4	A_5	A_6	A_1	A_2	A_3	A_4	A_5	A_6
1	0.275	0.225	0.450	0.270	0.380	0.270	0.315	0.280	0.520	0.320	0.430	0.305
2	0.310	0.210	0.440	0.285	0.320	0.285	0.345	0.295	0.545	0.360	0.410	0.332
3	0.325	0.205	0.410	0.250	0.320	0.240	0.368	0.300	0.510	0.355	0.395	0.351
4	0.295	0.200	0.425	0.272	0.310	0.226	0.339	0.294	0.525	0.325	0.407	0.325
5	0.282	0.208	0.398	0.286	0.340	0.242	0.332	0.314	0.490	0.348	0.440	0.318
6	0.285	0.205	0.405	0.252	0.335	0.252	0.305	0.287	0.508	0.339	0.424	0.320
Mean ± SD	0.30^{C} ±0.02	0.21^{E} ±0.01	0.42^{A} ±0.02	0.27^{CD} ±0.02	0.33^{B} ±0.02	0.25^{D} ±0.02	0.33^{C} ±0.02	0.30^{D} ±0.01	0.52^{A} ±0.01	0.34^{C} ±0.02	0.42^{B} ±0.02	0.33^{C} ±0.02

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

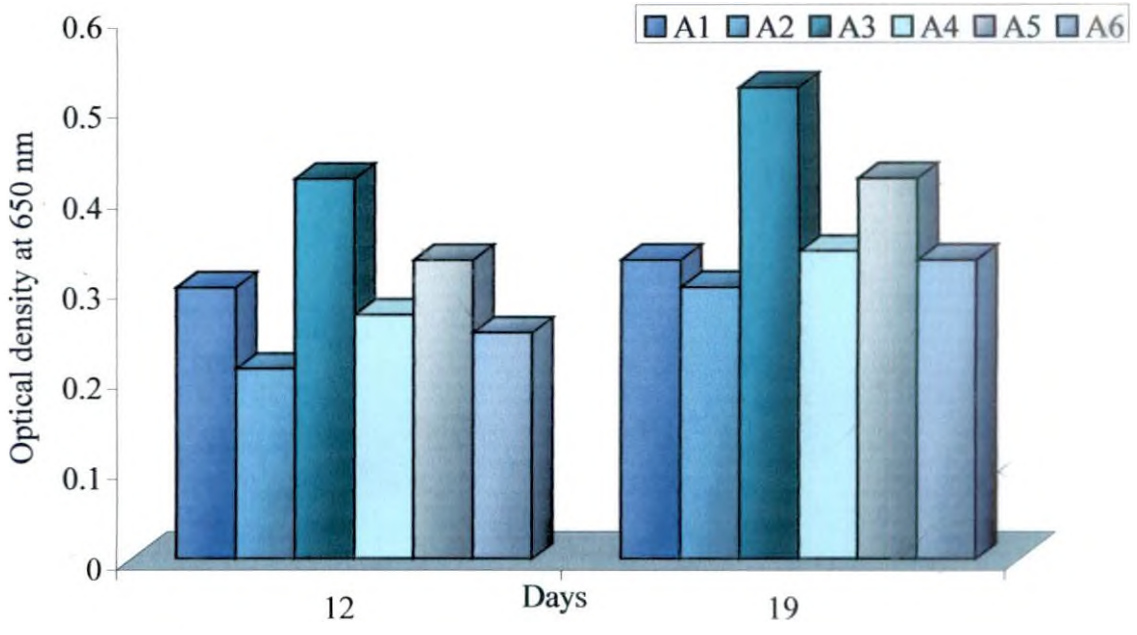


Fig. 16. Effect of acetone soluble and acetone insoluble fractions of ethanolic extract of *Emblica officinalis* on nitroblue tetrazolium dye reduction test

showed no significant difference than their control groups on 12^{th} day of treatment. On 19^{th} day of the experiment, groups A_3 and A_5 showed a significant ($P<0.05$) increase while group A_2 showed significantly lower value and no significant difference was found in the groups A_4 and A_6 than their control groups.

The mean NBT values obtained from groups A_1, A_2, A_3, A_4, A_5 and A_6 on 12^{th} day were 0.30±0.02, 0.21±0.01, 0.42±0.02, 0.27±0.02, 0.33±0.02 and 0.25±0.02 and on 19^{th} day were 0.33±0.02, 0.30±0.01, 0.52±0.01, 0.34±0.02, 0.42±0.02 and 0.33±0.02

Discussion

5. DISCUSSION

The survival of an animal depends on the successful body defence against microbial invasions. Immune deficiencies have been demonstrated following several injuries like thermal, trauma, sepsis etc., (Rao *et al.*, 1994). These deficiencies can predispose patients to potentially lethal infections. The development of antibacterials has revolutionized the treatment of bacterial diseases and eventual complete control of bacterial diseases was predicted. But, development of antibiotic resistance in microbes and immuno-deficiency in patients make even high-dose antibiotic therapy incapable of eradicating the infective microorganisms.

An attempt to overcome this problem has been made by introducing the concept of prohost therapy (Hadden, 1982). This approach aims to boost immune defenses against infections by administering drugs. Several natural and synthetic substances have been used to stimulate the non-specific host resistance against infections. But, these are too expensive and not without side effects.

Medicinal plants have been used to cure human illness since time immemorial and every country has lists of herbal remedies for the treatment of various disease conditions. Some of these drugs are believed to promote positive health and maintain resistance against infection by re-establishing body equilibrium (Homeostasis) and conditioning the body tissues (Kapil and Sharma, 1997).

Immunomodulation by pharmacological manipulations as a therapy for revitalizing the suppressed immune system is under extensive trials. Of late, large numbers of medicinal plants are being tested for the immunomodulating effects as an alternative drug, being cheap, cost-effective, non-toxic and scientifically sustainable.

Emblica officinalis is one of the principal ingredients in various ayurvedic preparations used as preventive/curative/health restorative agents. Rani (2003)

found that the aqueous and alcoholic extract of *Emblica officinalis* fruit pulp produced an immunstimulant activity in mice. There is a growing interest in identifying and characterizing the natural compounds with immunomodulatory activity ever since they have been suggested in modern medicine, With this in mind, the study was undertaken to evaluate the immunomodulatory potential of acetone soluble and acetone insoluble fractions of ethanolic extract of *Emblica officinalis*.

Mice were administered with acetone soluble and acetone insoluble fractions of ethanolic extract of *Emblica officinalis* at the dose rate of 200 mg per kg body weight in immunocompetent as well as in immunosuppressed animal (Immune system of the animal was suppressed by the administration of dexamethasone at the dose rate of 0.75 mg per kg body weight intra peritoneally for seven days). Immunomodulatory status was assessed by various physiological, haematological, biochemical, enzymatic and immunological parameters like body weight, relative organ weight, total leukocyte count, differential leukocyte count, total serum protein, serum globulin, albumin-globulin ratio, superoxide dismutase level, catalase level, haemagglutination titre, Jerene's plaque forming assay, bone marrow cellularity, delayed type of hypersensitivity test, macrophage migration index and nitro blue tetrazolium dye reduction test.

5.1 SCREENING OF ACETONE SOLUBLE AND ACETONE INSOLUBLE FRACTIONS OF ETHANOLIC EXTRACT OF *Emblica officinalis* FOR ACTIVE PRINCIPLES.

The phytochemical study on acetone soluble and acetone insoluble fraction of ethanolic extract of dried *Emblica officinalis* fruit pulp revealed that no detectable level of steroids and alkaloids are present in them. Diterpenes and triterpenes were found to be present in acetone soluble fraction. Tannins, phenolic compounds, flavonoids, glycosides, and saponins were found to be present in acetone insoluble fraction.

Phenolic compound embraces a wide range of plant substances ranging from simple monocyclic compound to complex polyphenolic compounds. Among the naturally occurring phenolic compounds, flavonoids form the largest group. Flavonoids are used as antioxidants, stress modifiers, antiviral, anticarcinogenic and antiallergic agents. Some flavonoids may be found in association with sugars and are called flavonoid glycosides. These are demonstrated to have anti-inflammatory, antiallergic, antithrombotic and vasoprotective properties. Tannins are polyphenolic compounds that are used as astringent, antiseptic and immunomodulatory agent. It can be seen that phenols act as a parent group of many phytochemicals, which are commonly used as antioxidants. Saponins are glycoside compounds and are used as antioxidants, immunomodulatory agents, regulators of cell proliferation and in the treatment of cytotoxicity of cancer cells. Terpenoids form the largest group of plant product, which are derived biosynthetically from the molecule of isoprene. They are categorized as monoterpenoids, diterpenoids, triterpenoids, and sesquiterpenoids and are used as antioxidants and immunomodulatory agents.

Jose *et al.* (1997) have observed that *Emblica officinalis* fruit contain several polyphenolic compounds including tannins. Zhang *et al.* (2001) isolated ellagitannins, phyllanemblins along with 30 known tannins and six new phenolic compounds from *Phyllanthus emblica* fruit. Bhattacharya *et al.* (1999) have found that the ascorbic acid in amla fruit is conjugated to gallic acid and reducing sugars, forming a tannoid complex that is more stable. Khandelwal *et al.* (2002) have reported that *Emblica officinalis* fruit contain 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, no significant increase in body weight was noticed on day 12^{th} of the experiment in any of the groups. In immunocompetent animals treated with acetone soluble fraction (group A_3), a significant increase in body weight on 19^{th} day was noticed (Table 2). All other groups showed a tendency to increase in body weight on 19^{th} day, though not significant. The acetone soluble fraction treated immunocompetent group produced a significantly higher spleen and liver weight on both 12^{th} and 19^{th} day of the experiment (Table 3, 5), while acetone insoluble fraction treated immunocompetent group produced a significantly higher spleen and liver weight only on 19^{th} day. Though there was no significant increase in body weight and organ weights in immunosuppressed animals, there is a tendency of gradual gain in body weight and organ weights from zero day to nineteenth day. This indicates that both acetone soluble and acetone insoluble fraction treated groups increased both body weight and organ weights in normal as well as in immunosuppressed animals. An increase in weight of immune organs in mice pretreated with cyclophosphamide (Immunosuppressant drug) was also noticed, when administered with total saponin extracted from *Gynostemma pentaphylla* (Zhang et al., 1990). There was no such an increase in kidney weight through out the experiment.

The observation in the present study is in agreement with the observation of Gulati *et al.* (1995) and Sajitha (2002), that an increase in body weight was recorded in rats having liver damage, when they are administered with extracts of *Phyllanthus emblica* fruit pulp. Polyherbal preparations like "Septilin" and "Brahma Rasayana" that contain *Emblica officinalis* as a major component also showed an increase in body weight (Sharma and Ray, 1997; Rekha *et al.*, 1998). The increase in body weight after administration of *Emblica officinalis* could be due to better feed utilization, as this agent was found to regulate the gastric function and also possess hepatoprotective activity (Xia *et al.*, 1997). A

significant increase in the relative weight of lymphoid organs like spleen and thymus was also noted on administration of immunostimulants like curcumin, *Trigonella foenum-graecum* (Antony *et al.*, 1999; Hafeez *et al.*, 2003). All the above reports support the present findings.

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) that 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 an increase in immunocompetency in the treated animals (Tizard, 2004; Lydyard *et al.*, 2003).

5.2.2 Haematological Parameters

A consistent increase in total WBC count was noted in the present study in all the groups, though not significant. However, a significant increase was noted on days 5, 12 and 19 in immunocompetent animals (groups A_3 and A_5), when administered with acetone soluble and insoluble fractions. In immunosuppressed animals (groups A_2, A_4 and A_6), there was not much increase till day 12. On day 19, significantly higher values were obtained in groups A_4 and A_6. This indicates that both acetone soluble and acetone insoluble fractions produced a significant increase in total WBC count on 19^{th} day in both immunocompetent and immunosuppressed animals. The highest value was observed on day 19 in mice fed with acetone soluble fraction. Groups fed with acetone soluble fraction produced a higher WBC count than groups fed with acetone insoluble fraction.

In immunocompetent animals (groups A_3 and A_5), both acetone soluble and acetone insoluble fractions showed a significant increase in percentage distribution of lymphocytes on 12^{th} and 19^{th} day, where as in immunosuppressed animals, only acetone soluble fraction (A_4) could produce a significant increase

on 19th day. Hence, on 19th day, percentage distribution of lymphocytes were significantly increased in both immunocompetent as well as in immunosuppressed animals that was fed with acetone soluble fraction.

The increased total WBC count and percentage of lymphocyte distribution observed in normal as well as in immunosuppressed animals in the present findings are in agreement with the observation of Rekha *et al.* (1998), who showed that a preparation made from *Emblica officinalis* stimulated proliferation of WBC in normal and cyclophosphamide treated groups. This is also in agreement with the findings of Sairam *et al.* (2003), who observed the ameliorative effect of amla on immunosuppression induced by chromium on lymphoid organ. Similarly increase in total WBC count of mice was observed by Raphael and Kuttan (2003), when naturally occurring triterpenoid compounds such as glycyrrhizic acid, ursolic acid, oleanolic acid, and nomilin were administered in mice. The administration of septilin, a polyherbal formulation containing *Emblica officinalis* also increased the WBC count (Kumar *et al.*, 1992; Sharma and Ray, 1997).

Glycosides isolated from *Formosana hayata* (Chiang *et al.*, 1992) and sesquiterpene glycosides extracted from *Dendrobium nobile* (Zhao *et al.*, 2001) also caused proliferative responses of mouse lymphocytes to concanavalin A and augmentation of mouse granulocyte / macrophage colony forming cells in *in vitro* conditions. The lymphocyte leukocytosis might be due to 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). However, flavonoids isolated from *Syzygium samarangense* showed inhibitory potency on peripheral blood mononuclear cells proliferation (Kuo *et al.*, 2004) and that may be the cause for the lesser total WBC count in acetone insoluble fraction treated group than the acetone soluble fraction treated group because flavonoids are present in acetone insoluble fraction.

5.2.3 Biochemical Parameters

The administration of acetone soluble and acetone insoluble fraction of extracts of *Emblica officinalis* produce a significant increase in total serum protein as well as serum globulin concentration on 12^{th} and 19^{th} day in immunocompetent animals (groups A_3 and A_5). A significant decrease in albumin-globulin ratio was also noticed during the same period in these groups. In immunosuppressed animals that were administered with acetone soluble fraction (group A_4), a significant increase was noticed on 19^{th} day only. The highest value of total serum protein and serum globulin was observed on day 19 in mice fed with acetone soluble fraction (Tables 11 and 12). Both fractions increased total serum protein as well as serum globulin in normal as well as in immunosuppressed animals in all the groups, though not significant. These results are supported by the observations made by Plohmann *et al.* (1997) that an increase in the level of the total serum protein as well as serum globulin in murines fed with triterpenoid saponins of *Solidago virgaurea L.* and *Helianthus annuus L.* The extract of *Trichopus zeylanicus* plant also produced the same results in mice (Subramoniam *et al.*, 2000). An increase in serum globulin concentration was also noticed by Anilkumar and Rajan (1986) after treatment with a synthetic immunostimulant, Levamisole in kids.

Serum protein consists of hundreds of protein with a wide range of structures and functions. 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 (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.* The mechanism of action by which the extract of Emblica results in an increased Ig level is yet to be studied. This may be due to enhanced responsiveness to B lymphocyte subset to the stimuli.

5.2.4 Enzymatic Parameters

During the function of the immune system such as in phagocytosis, reactive oxygen and nitrogen species are generated. The migration of leukocytes at an infected site results in phagocytosis with the release of enzymes (antioxidant enzymes) and cytokines from both macrophages and neutrophils. During the patho-physiological conditions, reactive oxygen and nitrogen species gets enhanced resulting in various diseased states. This may be effectively neutralized by enhancing the cellular defences, in the form of antioxidant enzymes mainly superoxide dismutase, catalase and myeloperoxidase (Devasagayam and Sainis, 2002). So immunomodulation can go hand in hand with antioxidant activity.

The superoxide dismutase and catalase level in both the acetone soluble and acetone insoluble fraction treated groups showed significant increase on 19^{th} day. On 12^{th} day, only acetone soluble fraction produced a significant increase in immunocompetent animals (groups A_3 and A_5), while in immunosuppressed animals (groups A_4 and A_6) there was no significant increase. A tendency to increase the level of both enzymes from 12^{th} to 19^{th} day is seen in immunosuppressed animals also. The results indicate that both the fractions could increase the level of these antioxidant enzymes in normal as well as in immunosuppressed animals, though not significant in all the animals (Table 14, 15 and Fig. 9, 10). The acetone soluble fraction treated groups produced a significantly higher superoxide dismutase and catalase level than the acetone insoluble fraction treated groups on both day 12 and 19 of the experiment. Similiar increase in antioxidant enzyme activity was observed by Bhattacharya *et al.* (1999), when mice was fed with the tannoid principles found in the *Emblica officinalis* fruit pulp. Devasagayam and Sainis (2002) also showed that natural compounds from medicinal plants are having antioxidant and immunomodulatory activities.

5.2.5 Immunological Parameters

5.2.5.1 *Haemagglutination Titre*

The present study showed a significant increase in antibody titre in both acetone soluble and acetone insoluble fraction treated immunocompetent animals on 12^{th} as well as on 19^{th} day of the experiment. Though not significantly higher, a consistent increase in antibody titre was noticed in immunosuppressed animals also from day zero to day nineteen. The highest antibody titre was noticed in acetone soluble fraction treated group on 19^{th} day of the experiment. Similar increase in antibody titre was observed by Raphael and Kuttan (2003), when mice was fed with naturally occurring triterpenoid compounds such as glycyrrhizic acid, ursolic acid, oleanolic acid, and nomilin. Lobanok *et al.* (2003) also observed a similar results, when phenolic compounds extracted from the submerged mycelium of *Lentinus edodes* was administered in mice.

The current observations are supported by the reports of Rao *et al.* (1987) that addition of crude saponin preparations to mouse spleen cell cultures produced proliferation of B cells and thereby the antibody titre. Sasaki *et al.* (1998) also noticed an enhanced antigen-specific serum immunoglobulin G2a (IgG2a) level, on immunization with a DNA vaccine encoding HIV-1 containing QS-21 saponin adjuvant in murines. Immue-21, a polyherbal product containing *Emblica officinalis* also significantly potentiated the humoral immunity in rabbits, as evidenced by an increase in antibody titre (De *et al.*, 1998). Similarly septilin (Amla containing patent preparation) also increased humoral immunity in mice (Kumar *et al.,* 1992). The flavonoid fraction of *Tephrosia purpurea* significantly inhibited sheep erythrocyte specific haemagglutination antibody titre in mice (Damre *et al.*, 2003) and this may be the reason for the lowered humoral response in acetone insoluble fraction treated groups in the present study because flavonoids are detected only in acetone insoluble fraction.

Sharma *et al.* (1994) also found an increase in the antibody titre in mice, when administered with *Picrorrhiza kurroa* and he suggested that enhanced responsiveness of macrophages and T and B lymphocyte subsets are involved in the antibody synthesis. 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.

5.2.5.2 *Spleenic Cell Plaque forming Assay*

Plaque forming cells are also called as antibody producing cells. The antibody diffuse from lymphocytes and combine with antigen on the surface of adjacent red cells and in the presence of complement, the red cell coated with antibody will lyse thereby gives a clear plaque around each antibody producing cell. Increased number of spleenic plaque forming cells closely correlated with the enhanced antibody titre. This may be due to the stimulation of the spleen to generate more antibody producing cells.

Significantly higher values were recorded in acetone soluble and acetone insoluble fraction treated immunocompetent animals (groups A_3 and A_5) on both 12^{th} and 19^{th} day of the experiment. Even though not significant, all the groups (both immunocompetent and immunosuppressed animals) showed an increase in number of plaque forming cells from day twelve to day nineteen (Table 17 and Fig.11). It indicates that both the fractions increased the number of plaque forming cells and thereby increased the humoral immunity from day twelve to day nineteen in immunocompetent as well as in immunosuppressed animals. The highest numbers of plaque forming cells were observed in acetone soluble fraction treated group on day 19. Similiar increased number of plaque forming cells in the spleen of mice was observed by Plohmann *et al.* (1997), when mice was administered with triterpenoid saponins of *Solidago virgaurea* L. and *Helianthus annuus* L. The extract of *Trichopus zeylanicus* also showed an increase in number of plaque forming cells in mice (Subramoniam *et al.*, 2000).

Immunizations with a DNA vaccine encoding HIV-1 containing QS-21 saponin adjuvant also consistently enhanced antigen-specific cytolytic activity of splenocytes and delayed type hypersensitivity reaction in mice (Sasaki *et al.*, 1998).

Increased number of plaque forming cells were also observed by Raphael and Kuttan (2003), when mice was administered with naturally occurring triterpenoid compounds such as glycyrrhizic acid, ursolic acid, oleanolic acid, and nomilin. In the present study also, the increased number of plaque forming cells observed can be attributed to the occurrence of triterpenoid compounds in acetone soluble fraction.

5.2.5.3 *Bone Marrow Cellularity*

Bone marrow consists of mostly haematopoietic cells and fat cells filling the available space. In all mammals, bone marrow is the haematopoietic organ serving as the source of all blood cells including lymphocytes. Haematopoietic stem cells in bone marrow are continuously being stimulated to produce differentiated granulocytes, erythrocytes and platelets. Increased bone marrow cellularity correlates with the increased blood cell counts.

Significantly higher numbers of cells were recorded in acetone soluble and acetone insoluble fraction treated immunocompetent animals (groups A_3 and A_5) on both 12^{th} and 19^{th} day of the experiment (Table 18 and figure 12). Moreover, all the groups showed an increase in the cellularity of the bone marrow even though not significant and it indicates that both the fractions increased the cellularity of the bone marrow from day twelve to day nineteen in normal as well as in immunosuppressed animals. The highest cellularity of the bone marrow was observed in acetone soluble fraction treated group on day 19. Similarly, increased bone marrow cellularity and alpha-esterase positive cells were observed by Raphael and Kuttan (2003), when mice was administered with naturally occurring triterpenoid compounds (found in acetone soluble fraction)

such as glycyrrhizic acid, ursolic acid, oleanolic acid, and nomilin. A similar observation was also found by Yuvaraj and Ramnath (2004), when they administered a synthetic immunostimulant, Levamisole in mice.

Higher number of bone marrow cells observed in acetone soluble fraction might be due to the presence of triterpenes and diterpenes. This is supported by the observation of Plohmann *et al.* (1997) that triterpenoid saponins of *Solidago virgaurea* L. and *Helianthus annuus* L. increased bone marrow cellularity in mice.

5.2.5.4 *Delayed Type of Hypersensitivity (DTH)*

In the delayed type of hypersensitivity reaction, macrophages and T lymphocyte play the major part. It has been suggested that primary contact with antigen followed by one or two week induction phase result in activation of T-helper cells. When the same antigen is reintroduced, the reacting cells secrete cytokines such as monocyte chemotactic factor that recruit and activate macrophages and other non-specific inflammatory cells. Macrophage thus becomes the principal effector cell in DTH. These events lead to a localized inflammatory response, resulting in damage to the invading agent. Thus the increased DTH response obtained in the present study revealed that Emblica might enhance the cell-mediated immune response by favouring these processes.

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, significantly higher values were recorded in acetone soluble and acetone insoluble fractions treated immunocompetent animals (groups B_3 and B_5) alone on 12^{th} day. On 19^{th} day, there was a significant increase in both immunocompetent and immunosuppressed animals (groups B_4 and B_6). It indicates that even in immunosuppressed animals, Emblica extract can increase cell-mediated immunity in mice, if it is administered continuously for at least nineteen days.

The maximum DTH response was seen in acetone soluble fraction treated animal on day 19 of the experiment (Table 19 and Fig.13). Similar result was observed, when isopimarane diterpene isolated from *Euphorbia ebracteolata Hayata* was administered in sensitized mice by Xu *et al.* (2000). Triterpene saponins isolated from *Astragalus species* also increased cell-mediated immunity by activating T cells in *in vitro* conditions (Yesilada *et al.*, 2005).

Allan *et al.* (1999) showed that oral vaccination with the immune stimulating complexes containing the saponin adjuvant, 'Quil A' activated T cell and thereby stimulation of production of IL-2.

Sharma and Ray (1997) have also reported that "septilin" containing *Emblica officinalis* as one of its components facilitated the footpad thickness response to SRBC in sensitized mice. The increase in DTH response as evidenced by increased footpad thickness has been demonstrated in other immunostimulatory plants like *Azadirachta indica* and *Centella asiatica* (Sen *et al.*, 1992; Hafeez *et al.*, 2003).

The flavonoid fraction of *Tephrosia purpurea* significantly inhibited sheep red blood cells induced delayed type of hyersensitivity reactions in mice (Damre *et al.*,2003), which might be the cause for a lesser DTH response in acetone insoluble fraction treated group in the present study also because flavonoids are present in acetone insoluble fraction.

5.2.5.5 *Macrophage Migration Index (MMI)*

In the present study, Significantly higher values were recorded in the acetone soluble and acetone insoluble fraction treated immunocompetent animals (groups A_3 and A_5) alone on 12^{th} day. On 19^{th} day, there was a significant increase in immunocompetent as well as in immunosuppressed animals that were administered with acetone soluble fraction. It reveals that both the fractions of the *Emblica officinalis* fruit pulp can increase the macrophage migration area in immunocompetent as well as in immunosuppressed animals, if we feed the

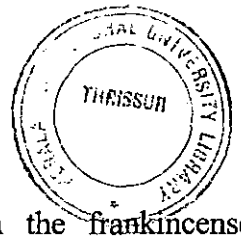
fractions continuously for at least 19 days. Moreover all the groups showed a progressive increase in the migration area, though not significant, from twelfth to nineteenth day. The maximum migration area was seen in acetone soluble fraction treated animal on day 19 of the experiment. The same observation was made by Dahanukar *et al.* (2000) and Devasagayam and Sainis (2002) in their experiment. They noticed a stimulation of macrophages in mice by administration of extract of *Emblica officinalis* fruit pulp. De *et al.* (1998) and Chatterjee (2001) also suggested the mechanism of immune protection offered by 'Septilin' and 'Immue-21' containing Emblica could be due to increase in number of activated macrophages.

The result obtained in our studies was in close relation to the findings that activity and proliferation of macrophages was enhanced by oral vaccination with immune stimulating complexes containing the saponin adjuvant, 'Quil A' in humans (Allan *et al.*, 1999) and by hydrolysable tannins like ellagitannins, gallotannins in mice (Kolodziej *et al.*, 2001).

Agarwal *et al.* (1994) and Puri *et al.* (1994) have evaluated the immunostimulatory potential of Pippali rasayana and *Nyctanthes arbor-tristis* using MMI as an index of immune status in mice and concluded that these induced a significant increase in MMI.

Peritoneal macrophages 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. Macrophage Migration Index appears to be a close correlate of macrophage activation and possibly also of the status of cell mediated immune response (Saxena *et al.*, 1991).

In the present study, the acetone soluble fraction produced a higher macrophage migration area than the acetone insoluble fraction, which was supported by the observation of Mikhaeil *et al.* (2003) that monoterpenes,



sesquiterpenes and diterpenes isolated form the frankincense essential oil produced a significant macrohage proliferation in *in vitro* conditions. However, flavonoids (Present in acetone insoluble fraction), mainly derivatives of flavone and flavonol have suppressive effects on proliferation of macrophages (Namgoong *et al.*, 1994).

Macrophages play a major role in non-specific 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 tumour cells also (Kapil and Sharma, 1997).

5.2.5.6 *Nitro Blue Tetrazolium Dye Reduction Test (NBT test)*

Phagocytosis of particles by macrophage is usually accompanied by a burst of oxidative metabolism that results 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 activity of the macrophages (Park *et al.*, 1968). NBT test is routinely being employed for assay of respiratory burst activity viz., granulocytes and macrophages.

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

The present study showed that both the acetone soluble and acetone insoluble fraction treated groups in immunocompetent animals (groups A_3 and A_5) produced an increase in NBT dye reduction test values on day 12 and day 19 of the experiment than the control groups where as no such significantly higher values were observed in immunosuppressed animals (groups A_2 and A_6). However, both the fractions showed a tendency to increase the NBT values in immunocompetent as well as in immunosuppressed animals from zero day to nineteenth day. The highest value was seen in the mice administered with acetone soluble fraction alone on the 19th day of the experiment (Table 22 and Fig.16). This indicates that both the fractions can increase the activity and phagocytosis of macrophages, if we administer continuously for at least 19 days.

The above observation was supported by the reports that enhanced production of IL-1 beta, IL-2 and TNF-alpha from monocytes and macrophages was observed in Cuphiin D1, macrocyclic hydrolyzable tannin isolated from *Cuphea hyssopifolia* (Wang *et al.*, 2002) and in polyphenols extracted from the *Epigallocatechin gallate* (Kazuto *et al.*, 2001) in *in vitro* conditions. Antony *et al.* (1999) have also demonstrated the increased macrophage phagocytic activity of *Curcuma longa* in mice after immunization with SRBC.

In the present study, the acetone soluble fraction produced a higher NBT reduction values than the acetone insoluble fraction and the result is supported by the observation that triterpenoid saponins of *Solidago virgaurea* L. and *Helianthus annuus* L. produced a significant induction of cytotoxic macrophages and a TNF alpha release from murine macrophages (Plohmann *et al.*, 1997). Conversely, Benencia *et al.* (1995) and Nores *et al.* (1997) have showed that *Cedrela tubiflora* leaves that have flavonoids in it, produced a diminution of both phagocytosis and respiratory burst activity with the help of NBT reduction test.

Many active principles of the plants were used in traditional medicine to stimulate the immune system. Some of the active fractions stimulate both humoral and cell mediated immunity, while others activate only cellular

components of the immune system. The present study establishes a strong immunostimulant activity of acetone soluble fraction of *Emblica officinalis* than acetone insoluble fraction of *Emblica officinalis*.

Diterpenes and Triterpenes, which are found in acetone soluble fraction possessed immunostimulant activity (Raphael and Kuttan, 2003). Tannins, saponins, flavonoids, glycosides and phenolic compounds, which are found in the acetone insoluble fraction, also have immunostimulant activity (Wang *et al.*, 2002; Sasaki *et al.*, 1998; Chiang et al., 1992; Kazuto *et al.*2001).

Although immunomodulating action of flavonoids of *Emblica officinalis* is yet to be studied, flavonoids (found in acetone insoluble fraction) of many other plants are found to have immunosuppressive actions (Damre *et al.*, 2003; Namgoong *et al.*, 1994). And it may be presumed that the immunosuppressive action of flavonoids are responsible for the lowered immunostimulant action of the acetone insoluble fraction than the acetone soluble fraction and further study is needed to evaluate the immunomodulatory action of flavonoids of *Emblica officinalis*.

Summary

6. SUMMARY

The present study was undertaken to evaluate the immunomodulatory potential of acetone soluble and acetone insoluble fractions of ethanolic extract of *Emblica officinalis* fruit pulp extracts in mice and also to assess the active principles present in each fraction. One hundred and forty four male Swiss Albino mice weighing between 15 g to 30 g and two to three months of age were divided into two groups of seventytwo each. One group was again divided into six subgroups namely A_1, A_2 A_3, A_4, A_5 and A_6 and the another group was also divided into six subgroups namely B_1, B_2 B_3, B_4, B_5 and B_6. Groups A_1 and B_1 were administered with vehicle alone, A_2 and B_2 were administered with dexamethasone, A_3 and B_3 were administered with acetone soluble fraction, A_4 and B_4 were administered with dexamethasone and acetone soluble fraction, A_5 and B_5 were administered with acetone insoluble fraction and A_6 and B_6 were administered with dexamethasone and acetone insoluble fraction. Dexamethasone was administered at the dose rate of 0.75 mg per kg intra peritoneally for seven days before the start of the experiment to suppress the immune system of the animal. Acetone soluble and acetone insoluble fractions were administered at the dose rate of 200 mg per kg body weight orally for 19 days.

The phytochemical study on acetone soluble and acetone insoluble fraction of ethanolic extract of dried *Emblica officinalis* fruit pulp revealed that no detectable level of steroids and alkaloids are present in them. Diterpenes and triterpenes were found to be present in acetone soluble fraction. Tannins, phenolic compounds, flavonoids, glycosides, and saponins were found to be present in acetone insoluble fraction.

One group of mice were used for evaluating the body weight, weight of the organs like spleen, liver and kidney, total and differential leukocyte count, total serum protein, serum globulin, albumin globulin ratio, Haemagglutination (HA) antibody titre, Jerene's plaque forming assay, enzymes like superoxide dismutase and catalase, bone marrow cellularity, macrophage migration index (MMI) and nitro

blue tetrazolium (NBT) dye reduction test and another group of mice was used to assess delayed type of hypersensitivity.

A gradual increase in body weight was noted in immunocompetent as well as in immunosuppressed animals during the experimental period. Acetone soluble fraction treated immunocompetent animals only showed a significant increase on 19^{th} day of the experiment. Significantly higher spleen weight on both 12^{th} and 19^{th} day of the experiment was observed in immunocompetent animals treated with acetone soluble and acetone insoluble fractions. A significant increase in liver weight was seen in immunocompetent animals treated with acetone soluble and acetone insoluble fractions only on 19^{th} day. However, acetone soluble fraction produced such an increase even on 12^{th} day. There was no such significant increase noticed in kidney weight in any of the groups. But a tendency to increase in weights of spleen, liver and kidney was noticed in immunocompetent as well as in immunosuppressed animals through out the experiment.

Haematological studies showed a significant increase in total WBC count in acetone soluble and acetone insoluble fractions treated immunocompetent animals on 5^{th}, 12^{th} and 19^{th} day. In immunosuppressed animals, till day 12 not much increase was noticed. However, on day 19, significant increase was obtained. It indicates that both the fractions could produce a significant increase in total WBC count on 19^{th} day in immunocompetent as well as in immunosuppressed animals. The percentage distribution of lymphocytes also showed a significant increase in acetone soluble and acetone insoluble fraction treated immunocompetent animals on 12^{th} and 19^{th} day, where as in immunosuppressed animals, only acetone soluble fraction produced a significant increase on 19^{th} day. A gradual increase in total WBC count and percentage distribution of lymphocytes was noticed in all the groups from day zero to day nineteen.

Total serum protein as well as serum globulin concentration was significantly increased and the albumin-globulin ratio was significantly decreased by acetone soluble and acetone insoluble fractions on 12^{th} and 19^{th} day in immunocompetent animals, while in immunosuppressed animals that was

administered with acetone soluble fraction alone produced a significant increase in total serum protein as well as serum globulin on 19th day. The highest value was observed on day 19 in mice fed with acetone soluble fraction. Both the fractions showed a non-significant increase in both total serum protein and serum globulin in normal as well as in immunosuppressed animals from zero day to nineteenth day.

The superoxide dismutase and catalase levels showed a significant increase in both acetone soluble and acetone insoluble fractions treated immunocompetent animals on 19th day. On 12th day, only acetone soluble fraction showed a significant increase. Even though not significant, an increase in level of these enzymes from 12th to 19th day was observed in immunosuppressed animals also. The acetone soluble fraction treated groups produced a significantly higher superoxide dismutase and catalase level than the acetone insoluble fraction treated groups on 12th and 19th day of the experiment.

The effects on humoral immunity were determined using parameters like antibody titre and the number of antibody producing cells. A significant increase was noticed in antibody titre in both acetone soluble and acetone insoluble fractions treated immunocompetent animals on 12th as well as on 19th day of the experiment. Though not significant, a consistent increase in antibody titre was noticed in all the groups from day zero to day nineteen. The highest antibody titre was noticed in acetone soluble fraction treated immunocompetent animals on 19th day of the experiment.

Numbers of antibody producing cells were significantly higher in the acetone soluble and acetone insoluble fraction treated immunocompetent animals on both 12th and 19th day of the experiment. Even though not significant, acetone soluble and acetone insoluble fraction treated immunosuppressed groups also showed a tendency to increase in the number of plaque forming cells from day twelve to day nineteen. The highest number of plaque forming cells was observed in acetone soluble fraction treated groups on day 19.

The effects of acetone soluble and acetone insoluble fractions on cellular immunity were determined using parameters like bone marrow cellularity, footpad thickness, Macrophage Migration Index and Nitro Blue Tetrazolium dye reduction test (NBT test). Bone marrow cellularity showed a significant increase in acetone soluble and acetone insoluble fractions treated immunocompetent animals on both 12^{th} and 19^{th} day of the experiment. Moreover, immunosuppressed animals also showed an increased cellularity of the bone marrow from day twelve to day nineteen, though not significant. The highest cellularity of the bone marrow was observed in acetone soluble fraction treated immunocompetent group on day 19.

A significant increase in footpad thickness were recorded in acetone soluble and acetone insoluble fraction treated immunocompetent animals on 12^{th} and 19^{th} day, where as in immunosuppressed animals, a significant increase was noted only on 19^{th} day in acetone soluble fraction treated animals. It indicates that even in immunosuppressed animals the delayed hypersensitivity response can be stimulated if continue the administration for 19 days.

In the present study, significantly higher macrophage migration area was recorded in the acetone soluble and acetone insoluble fraction treated immunocompetent animals on 12^{th} and 19^{th} day, where as in immunosuppressed animals significantly higher values were obtained only on 19^{th} day that was administered with acetone soluble fraction. All the groups (both normal and immunosuppressed) showed an increase in migration area from twelfth day to nineteenth day. The maximum macrophage migration area and the macrophage migration index were observed in acetone soluble fraction treated immunocompetent animals on day 19 of the experiment.

Nitro Blue Tetrazolium dye reduction values showed a significant increase in both the acetone soluble and acetone insoluble fraction treated immunocompetent animals on day 12 and 19 of the experiment than the control groups, where as no such significantly higher values were observed in immunosuppressed animals even on day 19. A non-significant increase in NBT reduction values in immunosuppressed animals was observed on day nineteen from day twelve. The

highest value was seen in the mice administered with acetone soluble fraction treated immunocompetent animal on the 19th day of the experiment.

The results of the present study indicates that both the acetone soluble and acetone insoluble fraction of ethanolic extract of dried *Emblica officinalis* fruit pulp stimulate both humoral and cellular immunity in immunocompetent as well as in immunosuppressed animals.

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.

A number of active ingredients from plants have been identified as a promising source of immunomodulators that will have a great role in enhancing the immune function. In the current era, the use of the individual active ingredients are much promising, greatly because of its specific activity and less toxicity. Considering these aspects, the use of acetone soluble and acetone insoluble fractions of ethanolic extract of dried *Emblica officinalis* fruit pulp can be recommended as an immunomodulator in normal as well as immunosuppressed animals. Acetone soluble fraction is found to have a better immunostimulating capability than the acetone insoluble fraction.

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IMMUNOMODULATORY EFFECT OF FRACTIONS OF ETHANOLIC EXTRACT FROM *Emblica officinalis* (AMLA) FRUIT PULP IN MICE

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ABSTRACT

The immunomodulatory activity of acetone soluble and acetone insoluble fractions of ethanolic extract of *Emblica officinalis* was investigated in the present study. The extracts were also qualitatively tested for the presence of various active principles. One hundred and forty four male swiss albino mice were used to assess humoral and cellular immunity by feeding the extracts at dose level of 200 mg per kg body weight for 19 days. Few animals were also administered with dexamethasone at the dose rate of 0.75 mg per kg body weight intra peritoneally for seven days before the start of the experiment to suppress the immune system and thereby to study the effect of extracts on immunosuppressed animals also. The control group received vehicle alone (five percent gum acacia).

Various physiological, haematological, biochemical, enzymatic and immunological parameters like body weight, relative organ weight, total leukocyte count, differential leukocyte count, total serum, protein, serum globulin, albumin-globulin ratio, quantification of superoxide dismutase and catalase, haemagglutination test, Jerene's plaque forming assay to assess the humoral immune response and tests like delayed type of hypersensitivity, macrophage migration index, nitroblue tetrazolium reduction test to assess the cellular immune response were performed.

Both the fractions increased the body weight, spleen weight and liver weight in normal as well as in immunosuppressed animals. A significantly increased total WBC counts on days 5, 12 and 19 and the distribution of lymphocytes on days 12 and 19 were seen in normal animals. In immunosuppressed animals, non-significant increase in both total leukocyte counts and distribution of lymphocytes from day zero to nineteen was seen.

Total serum protein as well as serum globulin concentration was significantly increased and albumin globulin ratio was significantly decreased in immunocompetent animals treated with both the fractions on 12^{th} and 19^{th} day.

However, both the fractions showed only a non-significant increase in total serum protein and serum globulin in normal as well as in immunosuppressed animals, from zero day to 19th day. Significant increase was noticed in superoxide dismutase and catalase level in immunocompetent animals treated with both the fractions on 19th day.

A significant stimulation of humoral immune response as indicated by an increase in antibody titre and number of antibody producing cells on both 12th and 19th day of acetone soluble and acetone insoluble fraction treated immunocompetent animals was noted. Immunosuppressed animals also showed a non-significant increase in both antibody titre and number of antibody producing cells through out the experiment.

The bone marrow cellularity and foot pad swelling reaction showed a significant stimulation in immunocompetent animals on 12th and 19th day. In immunosuppressed animals also, foot pad swelling response showed a significant increase on 19th day but bone marrow cellularity was not significant.

In immunocompetent animals the acetone soluble and acetone insoluble fractions showed a significant increase in macrophage migration index (MMI) and Nitro blue Tetrazolium (NBT) dye reduction test value on 12th and 19th day. However, in immunosuppressed animals both the fractions showed only a non-significant increase.

The phytochemical study on both the fractions of ethanolic extract of dried *Emblica officinalis* fruit pulp revealed that diterpenes and triterpenes were present in acetone soluble fraction and tannins, phenolic compounds, flavonoids, glycosides, and saponins were present in acetone insoluble fraction. Thus the present study showed the higher immunostimulant activity for acetone soluble fractions in immunocompetent as well as immunosuppressed animals.