International Journal of PharmTech Research
CODEN (USA): IJPRIF, ISSN: 0974-4304
Vol.9, No.1, pp 153-163, 2016

Assessment of immunomodulatory activity of *Ficus benghalensis* Linn. aerial roots

Sneha J Anarthe*, Aleeti Pravalika, Enishetty Malavika and M. Ganga raju

Department of Pharmacology Gokaraju Rangaraju College of Pharmacy, Bachupally, Hyderabad- 500090, India

Abstract: The present study was undertaken to assess *in vitro* antioxidant and immunomodulatory activity for methanolic extract of *Ficus benghalensis* Linn. aerial roots (MEFB). Antioxidant activity of (MEFB)was evaluated by reducing power assay and hydrogen peroxide assay. Immunomodulatory activity was evaluated for specific and non-specific immune response by using *in vitro* assays as plaque forming cell assay and quantitative haemolysis assay and various *in vivo* models as heamagglutination antibody (HA) titer, delayed type hypersensitivity (DTH), T cell population and drug induced myelosuppression. Preliminary phytochemical investigation of (MEFB) showed presence of flavonoids, glycosides, phenols, tannins. (MEFB showed good antioxidant activity by reducing power assay and hydrogen peroxide assay with IC_{50} value 27.5 and 25 µg/ml respectively. (MEFB showed elevated response at a dose of 100 mg/kg bd.wt.for plaque forming cell and quantitave haemolysis assay. Methanolic extract of Ficus bengalensis showed a significant increase in the production of circulating antibody titer in response to sheep red blood cells (SRBC's) at dose of 100 mg/kg bd.wt. Methanolic extract of Ficus bengalensis showed significantly ($p<0.01$) increase in the delayed type hypersensitivity response by facilitating the footpad thickness response, increased the levels of haematological parameters, lymphocytes and rosettes formation, when results were compared with standard as Levamisole. Thus methanolic extract of *Ficus benghalensis* aerial roots has showed significant immunostimulatory activity with specific and non-specific mechanisms which may be due to the presence of prominent amount of flavonoids, phenols and tannins.

Key words: Immunomodulatory, *Ficus benghalensis*, plaque forming cell assay, quantitative haemolysis assay, delayed type hypersensitivity, haemagglutination titer, drug induced myelosuppression

Introduction

The immune system is one of our most complex biological systems in the body. The basic role of the immune system is to distinguish self from non-self[1]. This non-self could be an infectious organism, a transplanted organ or an endogenous cell that can be mistaken as a foreign. The immune responses of the human body against any non-self are of two types: (a) innate (or natural or non-specific) and (b) adaptive (or acquired or specific)[2]. Immune system disorders results in autoimmune diseases, inflammatory diseases, cancer and immunodeficiency[3]. Immunomodulators are the biological or synthetic substances, which can stimulate, suppress or modulate any of the immune system including both adaptive (humoral and cell mediated) and innate arms of the immune response[4]. Immuomodulators with enhanced immune reaction is called as an immunostimulative drug which primarily implies stimulation of non-specific system i.e., granulocytes,

macrophages, complement, certain T-lymphocytes and different effector substances. Immunosuppressant implies mostly to reduce resistance against infections, stress and may occur on account of environmental or chemotherapeutic factors. Many proteins, amino acids, and natural compounds have shown a significant ability to regulate immune responses, including interferon-γ (IFN-γ), steroids[4]. A number of Indian medicinal plants have been claimed to possess immunomodulatory activity[5] and use of plant derived products as immunomodulators is still in a developing stage. A variety of plant-derived compounds such as polysaccharides, lectins, peptides, flavonoids, tannins, sterols and sterolins have been reported to modulate the immune system[6]. Since ancient times, several diseases have been treated by administration of plant extracts based on traditional medicine[7]. The natural immunomodulators act to strengthen weak immune systems, about three quarters of the world population relies on the plants and plant extracts for healthcare. India has an extensive forest cover, enriched with plant diversity.

Ficus benghalensis (family Moraceae) commonly called as banyan tree. It is considered as the sacred tree in hindu mythology as resting place for the god Krishna. It is popular indigenous system of medicine like Ayurveda, Siddha, Unani and Homeopathy. Leaves of *Ficus benghalensis* contain quercetin-3-galactoside and rutin, bark of *Ficus benghalensis* presence of 5,7 Dimethyl ether of leucopelargonidin-3-0-α-L rhamnoside and 5,3 dimethyl ether of leucocynidin 3-0-α-D galactosyl cellobioside, rutin, and quercetin 3- galactoside[8]. In traditional system of medicine various plant parts such as stem bark, root bark aerial roots, vegetative buds, leaves, fruits and latex are used in dysentery, diarrhea, diabetes leucorrhoea, menorrhagia, nervous disorders, tonic and astringent[9].

Materials and Methods

Plant material collection, authentication and extraction

The plant *Ficus benghalensis* was collected from Ecil, of Hyderabad district, Telangana state in the month of February, 2015, identified and authenticated by Dr. Venkatesh. The crude plant material was cleaned, made into small pieces, dried under sun and coarsely powdered. The powdered material was extracted by simple distillation process using methanol as solvent. Filtrate obtained was evaporated to dryness and extract obtained was stored in air tight containers for further use. MEFB was subjected to preliminary phytochemical investigation[11].

Animals used

Wistar albino rats (Approx 150 to 180 g) were procured from Albino labs Hyderabad. Present study was carried out in CPCSEA approved animal house of Gokaraju Rangaraju College of Pharmacy, Bachupally, Hyderabad, India (Reg. No.1175/ac/08/CPCSEA).

Antigens

Sheep Red Blood Cells (SRBC) were collected in Alsever's solution from NIN slaughter house Hyderabad, India. SRBC were washed 3-4 times with large quantity of sterile and pyrogen free saline.

Acute toxicity studies

The methanolic extract of *Ficus benghalensis* whole plant was tested for acute toxicity studies as per procedure given in OECD guidelines 425 and limit test method was followed. Mice were starved for 4h and fed orally with methanolic extract of *Ficus benghalensis* at doses 2000 and 5000 mg/kg bd.wt. animals were observed for 14 days for mortality[10].

Estimation of total phenolic content and total flavonoid content

For the estimation of total phenolic content 2 mL of MEFB was prepared, 10 mL of the water and 2 mL of Folin-phenol reagent were added and volume of solution was made up to 25 mL by adding sodium carbonate solution. Resultant solution was kept for 30 minutes for incubation in dark. Then, the absorbance of solutions was measured using UV spectrophotometer at 760 nm[12]. Total flavonoid of MEFB was determined using the method of Liu et al. In brief, MEFB was diluted with 80% aqueous ethanol (0.9 mL). 0.5 mL of extract was added to test tube containing 0.1 mL of 10 % aluminum nitrate, 0.1 mL 1M aqueous potassium

acetate and 4.3 mL of 80 % alcohol. The reaction tubes were set aside for 40 minutes at room temperature. At the end, optical density of each sample was determined at 415 nm using a UV spectrophotometer. Total flavonoids content was calculated by interpolation on a standard curve established with a reference standard, quercetin. Quercetin and Folin-Ciocalteu reagent were obtained from Sigma-Aldrich, Germany[12].

***In vitro* antioxidant assay**

Reducing Power Assay

One mL of MEFB (20 µg/mL) was mixed with phosphate buffer (2.5 mL) and potassium ferricyanide (2.5 mL). The mixture was incubated at 500°C for 20 min. Aliquot of trichloroacetic acid (2.5 mL) was added to the mixture, and centrifuged at 3000 rpm for 10 min. The upper layer of resultant solution (2.5 mL) was mixed with distilled water (2.5 mL) and ferric chloride solution (0.5 mL). The absorbance was measured at 700 nm. Ascorbic acid (20 µg/mL) was used as standard. Increased absorbance of the reaction mixture indicates increase in reducing power[13].

Hydrogen Peroxide Assay

The ability of MEFB to scavenge hydrogen peroxide was determined according to the method given by Ruch *et al*. A solution of hydrogen peroxide (2mmol/L) was prepared in phosphate buffer (pH 7.4). MEFB (1–10 µg/mL) were added to hydrogen peroxide solution (0.6 mL). Absorbance of resultant solution was determined after 10 min at 230 nm against a blank solution, and ascorbic acid was used as reference compound[14].

***In vitro* immunomodulatory assay**

The experiments was done according to the CPCSEA guidelines and approved by the Institutional Animal Ethical Committee. In the present study MEFB was dissolved acacia and in distilled water. Doses selected of MEFB were 100, 200 and 300 mg/kg body weight. Albino rats were divided into groups comprising of six animals each.

Plaque forming cell assay

On 0 day, all groups were sensitized with 0.1 mL of SRBC containing 1×10^8 cells, *i.p.*
Group I served as control and was administered 1% Gum acacia suspension in saline
Group II received 100 mg/ kg bd.wt. of MEFB *p.o.* respectively (1 to 5 days)
Group III received 200 mg/ kg bd.wt. of MEFB *p.o.* respectively (1 to 5 days)
Group IV received 300 mg/kg bd,wt. of MEFB *p.o.* respectively (1 to 5 days)
Group V received standard drug 50 mg/kg bd.wt. Levamisole. *p.o.* respectively (1 to 5 days)
(Immunostimulant)

The PFC assay was performed using the method of Raisuddin *et al*. The animals were humanized on the fifth day of immunization with SRBC. The spleen was removed, cleaned free of extraneous tissues, and a single cell suspension of 10^6 cells/mL was prepared from it in RPMI-1640 medium. For PFC assay, the SRBC were prepared at a density of 5 x 10^8 cells/ mL in PBS. One milliliter of SRBC in medium along with 0.5 mL of diluted rabbit serum complement (1:10 diluted with normal saline) was added to 1 mL of spleen cell suspension. Cuningham chambers were prepared using glass slide, coverslips and double- sided tape (Scotch Brand, St. Paul, MN). The chambers were loaded with a known volume of assay mixture, sealed with petroleum jelly and incubated at 37˚C for 1 h. The plaques were counted under a light microscope (Olympus BX50) and expressed as PFC per 10^6 spleen cells[15].

Quantitative haemolysis of SRBC (QHS) assay

On 0 day, all groups were sensitized with 0.1 mL of SRBC containing 1×10^8 cells, *i.p.*
Group I served as control and was administered 1% Gum acacia suspension in saline
Group II received 100 mg/ kg bd.wt. of MEFB *p.o.* respectively (1 to 5 days)
Group III received 200 mg/kg bd.wt. of MEFB *p.o.* respectively (1 to 5 days)
Group IV received 300 mg/kg bd,wt. of MEFB *p.o.* respectively (1 to 5 days)

Group V received standard drug 50 mg/kg bd.wt. Levamisole. *p.o.* respectively (1 to 5 days) (Immunostimulant)

QHS assay was performed using the methods of Simpson and Gozo and with some modifications. Spleens were removed and a cell suspension of 1 $X10^6$ cells /mL was prepared in PBS. One mL of 0.2% SRBC and 1 mL of 10% rabbit serum were mixed with cell suspension and incubated for 1hr at 37˚C. After centrifugation at 3000 rpm for 3 min, optical density of the supernatant was measured at 413 nm using a spectrophotometer (Shimadzu UV- 1201)[16].

***In vivo* immunomodulatory activities**

Antibody (HA) titre response to SRBC

On 0 day, all groups were sensitized with 0.1 mL of SRBC containing 1×10^8 cells, *i.p.*
Group I served as control and was administered 1% Gum acacia suspension in saline
Group II received 100 mg/kg bd.wt. of MEFB *p.o.* respectively (1 to 7 days)
Group III received 200 mg/kg bd.wt. of MEFB *p.o.* respectively (1 to 7 days)
Group IV received 300 mg/kg bd.wt of MEFB *p.o.* respectively (1 to 7 days)
Group V received 50 mg/kg bd.wt. of standard, Levamisole, *p.o.* respectively (1 to 7 days)

On 7th day before challenge, blood was withdrawn from retro-orbital plexus of each animal. Blood was centrifuged, and serum was separated. Serial two fold dilutions were made i.e. 50 µL of serum was added to 1st well of 96-well micro titer plate containing 50 µL normal saline. To this 1% SRBC (50 µL) dissolved in normal saline was mixed. From 1st well 50 µl of diluted serum was added to 2nd well containing 50 µl normal saline and 50µl 1% SRBC. Such dilutions were done till 24th well. Plates were incubated at 37ºC for 1h highest dilution that has shown visible agglutination was considered as haemagglutination antibody[17].

Delayed type hypersensitivity

On 0 day, all groups were sensitized with 0.1 mL of SRBC containing 1×10^8 cells, *i.p.*
Group I - Control, 1% Gum acacia suspension in saline
Group II received 100 mg/kg bd.wt. of MEFB *p.o.* respectively (1 to 7 days)
Group III received 200 mg/kg bd.wt of MEFB *p.o.* respectively (1 to 7 days)
Group IV received 300 mg/kg bd.wt of MEFB *p.o.* respectively (1 to 7 days)
Group V received 50 mg/kg bd.wt. of standard, Levamisole, *p.o.* respectively (1 to 7 days)

On 7th day prior to injection, right hind footpad thickness was measured with digital vernier callipers (Mitutoyo digimatic). Then animals were challenged by injecting 1% SRBC (20 µL) into the right hind footpad. On 8th and 9th day footpad thickness was again measured. Difference between prior and post challenge footpad thickness was reported as DTH response[17].

T-cell population

On 0 day, all groups were sensitized with 0.1 mL of SRBC containing 1×10^8 cells, *i.p.*
Group I - Control, 1% Gum acacia suspension in saline
Group II- received 100 mg/kg bd.wt. of MEFB *p.o.* respectively (1 to 10 days)
Group III- received 200 mg/kg bd.wt. of MEFB *p.o.* respectively (1 to 10 days)
Group IV- received 300 mg/kg bd.wt. of MEFB *p.o.* respectively (1 to 10 days)
Group V- received 50 mg/kg bd.wt. of standard, Levamisole, *p.o.* respectively (1 to 10 days).

On 11th day, blood was collected from the retro-orbital plexus and anticoagulated with Alsever's solution in separate test tubes. Test tubes containing blood were kept in sloping position (45˚) at 37˚C for 1 h. RBCs were allowed to settle at bottom and supernatant was collected from each test tube by using micropipette which contains lymphocytes. An amount of 50 µl of this lymphocyte suspension & 50 µl SRBC were mixed in test tube and incubated. Resultant suspension was centrifuged at 200 rpm for 5 min and kept in a refrigerator at 40˚C for 2 h. The supernatant fluid was removed and one drop of cell suspension was placed on a glass slide. Total lymphocytes were counted and a lymphocyte binding with three or more erythrocytes was considered as rosette[18].

Drug induced myelosuppression

On 0 day, all groups were sensitized with 0.1 mL of SRBC containing 1×10^8 cells, *i.p.*
Group I – (Control) received, 1% gum acacia suspension in saline
Group II - Negative control, received, 2 mg/kg bd.wt Azathioprine. *p.o*. respectively (on 11th, 12th and 13th day).
Group III- received 100 mg/kg bd.wt of MEFB *p.o*. respectively (1 to 13 days)
Group IV- received 200 mg/kg bd.wt. of MEFB *p.o.* respectively (1 to 13 days)
Group V- received 300 mg/kg bd.wt. of MEFB *p.o.* respectively (1 to 13 days)
Group VI received 50 mg/kg bd.wt. of standard, Levamisole, *p.o.* respectively (1 to 13 days)

On 0 day, blood was withdrawn from retro-orbital plexus of animals of each group and subjected to haematological parameter determination. MEFB was administered to Group-III, IV, V and VI from 1 to 13 days. Azathioprine (2 mg/kg, bd.wt) is given to all animals on 11th, 12th and 13th day, 1h after MEFB administration except control and standard group. On day 14th blood was withdrawn from retro-orbital plexus and hematological parameters were estimated[19].

Histopathology of spleen and thymus

For histopathology the rats were sacrificed by cervical dislocation and their spleen and thymus gland were dissected out. Tissues (spleen and thymus) obtained from all the experimental groups were washed immediately with saline and then fixed in 10% buffered neutral formalin solution. After fixation, the tissues were processed by embedding in paraffin. The tissues were sectioned and stained with haematoxylin and examined under high power microscope (200 & 400X) and photo micrographs were taken.

Statistical analysis:

Graph Pad prism 3 software and MS excel was used for statistical analysis of data. All the results were expressed as mean±standard error of mean (SEM), analyzed for ANOVA and Dunnet's t-test (Multiple). Differences between groups were considered significant at $p<0.05$, $p<0.01$ levels.

Results and discussion

Acute toxicity testing:

The acute toxicity testing was performed in female animals. The animals treated with different doses of *Ficus benghalensis* aerial roots showed no signs of toxicity. No animal was found to be in moribund state and no animal died even after 14 days. So, it was confirmed that the *Ficus benghalensis* aerial roots was safe up to 5000 mg/kg bd.wt.

The preliminary phytochemical investigation for MEFB showed the presence of carbohydrates, alkaloids, terpenes, glycosides, flavonoids, phenolics, steroids and tannins. Saponins are present in minor quantities. The total phenolic and flavonoid content of MEFB was found to be 2.95 µg/mg of gallic acid and 2.4 µg/mg of quercetin equivalent.

MEFB showed good antioxidant activity by reducing power assay and hydrogen peroxide assay with IC_{50} value 27.5 and 25 µg/mL respectively. Ascorbic acid was used as reference standard showed the IC_{50} value 24 and 19.5 µg/mL with reducing power assay and hydrogen peroxide assay respectively (Table. 1).

Table 1: Effect of methanolic extract of *Ficus benghalensis* for *in vitro* antioxidant assays

Test drug	Reducing power assay IC_{50} (µg/mL)	Hydrogen peroxide scavenging assay IC_{50} (µg/mL)
MEFB	27.5	25
Ascorbic acid(Standard)	24	19.5

Values are expressed as mean ± SEM

Table 2: Effect of methanolic extract of *Ficus benghalensis* for *in vitro* immunomodulatory assays

Groups	Treatment	PFC×10^6 mean±SEM	OD×10×10^6 mean±SEM
I	Control	572.5±6.18	0.671±0.0025
II	MEFB 100 mg/kg	665.0±4.9^{**,a}	0.772±0.0039^{**,a}
III	MEFB 200 mg/kg	620.8±3.7^{**,a}	0.748±0.0042^{**,a}
IV	MEFB 300 mg/kg	563.0±1.3^{**,a}	0.701±0.0110^{**,a}
V	Standard	678.8±1.70^{a}	0.788±0.0026^{a}

Values are expressed as mean± SEM, (n=6). All the groups were compared with control group and standard group (Dunnett's t- test). Significant values are expressed as control (**=p<0.01) and standard (a=p<0.01).

MEFB was evaluated for *in vitro* immunomodulatory assay where MEFB showed good immunomodulatory activity with plaque forming cell (PFC) and QHS assay (Table. 2).

Number of antibody secreting cells from spleen was determined using plaque forming cell assay. The PFC assay is considered to be one of the most highly predictive single assays for detection of the immunomodulatory/ immunotoxic potential of several substances and drugs. It was used to assess potential modulation of the humoral immune response, which quantifies the number of B cells producing SRBC-specific IgM. It is considered that substances and preparations inducing PFC increases are immunostimulators. The increased number of PFCs in animals treated with MEFB exhibited significant values which suggest MEFB showed presence of immunomodulatory activity.

The humoral mediated immunity involves the interaction of B cells with the antigen and their subsequent proliferation and differentiation into antibody secreting plasma cells. Antibody functions as the effector of the humoral immune response by binding to antigen and neutralizing it or facilitating its elimination by cross-linking to form clusters that are more readily ingested by phagocytic cells. The effect of methanolic extract of *Ficus benghalensis* have indicated that immunostimulation achieved through humoral immunity.

MEFB was evaluated for *in vivo* immunomodulatory acitivity with antigen-antibody titer response i.e. haemagglutination titer. MEFB showed significant antibody titer, when compared to control (p<0.01) and standard (p<0.01) (Table. 3).

***In vivo* immunomodulatory models**

Table 3: Haemagglutination titer test for methanolic extract of *Ficus benghalensis* aerial roots.

Groups	Treatment	Antibody titer Mean ± SEM (n=6)
I	Control	1.33±0.21
II	MEFB 100 mg/kg	6.5±0.428^{**,a}
III	MEFB 200 mg/kg	5.5±0.428^{**,a}
IV	MEFB 300 mg/kg	4.16±0.3^{**,a}
V	Standard	8.16±0.52^{a}

Values are expressed as mean± SEM, (n=6). All the groups were compared with control group and standard group. (Dunnett's t- test). Significant values are expressed as control (**=p<0.01) and standard (a=p<0.01).

When particulate antigen combines with its antibody in the presence of normal saline at a suitable pH and temperature the antigen-antibody complexes forms. The complex is either large clumps or aggregates or agglutinate that are visible and can be seen with unaided eye. Such reactions are called agglutination reactions. If the antigen is an erythrocyte, the term haemagglutination is used. Antibody molecules which are secreted by plasma cells mediate the humoral immune response. This augmentation of the humoral response to SRBC indicated an enhanced responsiveness of the macrophages and T and B lymphocyte subsets involved in antibody synthesis. The maximum serum dilution that shows visible agglutination is considered as antibody titer.

MEFB showed significant inhibition of hypersensitivity reaction at the dose of 100 mg/kg bd.wt. compared with control ($p<0.01$) and standard ($p<0.01$) (Table. 4).

Table 4: Delayed type hypersensitivity of methanolic extract of *Ficus benghalensis* aerial roots.

Groups	Treatment	DTH Response (mm) 24 h mean±SEM	DTH Response (mm) 48 h mean±SEM
I	Control	0.25±0.007	0.25±0.007
II	MEFB 100 mg/kg	1.68±0.071^{**,a}	1.345±0.082^{**,a}
III	MEFB 200 mg/kg	0.78±0.03^{**,a}	0.63±0.029^{**,a}
IV	MEFB 300 mg/kg	0.5±0.02^{**,a}	0.47±0.024^{**,a}
V	Standard	1.47±0.07^{a}	1.12±0.078^{a}

Values are expressed as mean± SEM, (n=6). All the groups were compared with control group (Dunnett's t- test). Significant values are expressed as control (**=$p<0.01$) and standard (a=$p<0.01$).

DTH requires the specific recognition of a given antigen by activated T lymphocytes, which subsequently proliferate and release cytokines. It is a Type IV hypersensitivity reaction that develops when antigen activates sensitized T_{DTH} cells.. Activation of T_{DTH} cells by antigen (SRBC) presented through appropriate antigen presenting cells results in the secretion of various cytokines including Interleukin-2, Interferon, macrophage migration inhibition factor and tumor necrosis factor. The overall effects of these cytokines are to recruits macrophages into the area and activate them, increasing concentration of lytic enzymes ie., by increasing vascular permeability, induce vasodilation, macrophage accumulation and activation, promoting increased phagocytic activity for more effective killing . Several lines of evidence suggest that DTH reaction is important in host defense against parasites and bacteria that can live and proliferate intracellularly. Treatment of methanolic extract of F. *benghalensis* enhanced DTH reaction, which is reflected from the increased footpad thickness compared to control group suggesting heightened infiltration of macrophages to the inflammatory site. This study may be supporting a possible role of methanolic extract of *F. benghalensis* in assisting cell-mediated immune response. The interaction of sensitized T-cells with presented antigen is known to be associated with the release of mediators, such as histamine. Products of arachidonic acid metabolism and interferon-gamma eventually lead to DTH. Therefore, the inhibitory action could be due to an influence of fraction on the biological mediators. Significant inhibition of hypersensitivity reaction was observed at a dose of100 mg/kg when compared to standard value. It was found that as the dose increases the inhibition activity occurred in minute levels. This states that 100 mg/kg is the optimal dose to reduce maximum inflammation.

MEFB showed a significant increase in lymphocytes and rosettes when compared with control ($p<0.01$) and standard ($p<0.01$). 100 mg/kg bd. wt. was found to be potent dose amongst other doses as 200 and 300 mg/kg bd.wt. (Table. 5).

Table 5: Lymphocytes and rosettes count for methanolic extract of *Ficus benghalensis* aerial roots

Groups	Treatment	Lymphocytes count mean±SEM	Rosettes count mean±SEM
I	Control	131.6±1.8	9.83±0.3
II	MEFB 100 mg/kg	171±1.7^{**,a}	23±0.96^{**,a}
III	MEFB 200 mg/kg	161.3±1.7^{**,a}	20.6±0.71^{**,a}
IV	MEFB 300 mg/kg	148±1.2^{**,a}	14.8±0.47^{*,a}
V	Standard	180±1.8^{a}	25.6±1.02^{a}

Values are expressed as mean± SEM, (n=6). All the groups were compared with control group (Dunnett's t- test). Significant values are expressed as control (**=$p<0.01$), (*=$p<0.05$) and standard (a=$p<0.01$).

Increase in rosette formation and lymphocyte formation in T cell population indicate effect of methanolic extract of *Ficus benghalensis* aerial roots on cell mediated immunity. Methanolic extract of *Ficus benghalensis* may activate the CD4 and CD8 cells which influence T-cell mechanism results increase in T-cell

immune response significantly. In present study increasing doses has showed significant increase in lymphocytes and rosettes when results were compared with control and standard.

MEFB showed a significant effect in restoration of haematological parameters when compared with control ($p<0.01$), and standard ($p<0.01$, $p<0.05$) (Table. 6).

Myelosuppression is a decrease in the production of blood cells. Azathioprine immunosuppressive agents which act at various levels on cells involved in defense mechanism against various invaders by inhibiting both cell mediated and humoral immunity. Azathioprine significantly decreases the Hb, RBC, and WBC counts. Azathioprine treatment for the period of 3 days showed significant reduction in WBC count, RBC count and Hb count and thereby exerted immunosuppressant effect when compared to control animals.

Histopathology of spleen was confirmed the immunostimulant activity of MEFB (Fig. 1 a,b,c,d,e). The standard levamisole showed prominent stimulant activity evident by large amount of RBC's in the cortex region and very mild atrophy and lymphoid depletion in the medullary region. Similarly MEFB exhibited significant immunostimulant activity indicated by normal cortex with more RBC's and medullary area with varying size of spleenic corpuscles with lymphocytes. MEFB showed potent immunomodulatory actiity at the dose of 100 mg/kg bd. wt. amongst other doses. This might be due to maximum/ceiling effect of MEFB.

Table 6: Haematological parameters for methanolic extract of *Ficus benghalensis* aerial roots (Drug induced myelosuppression)

Groups	Treatment	WBC count ($\times10^3$/mm^3) mean±SEM		RBC count ($\times10^6$/mm^3) mean±SEM		Hb count (g/dL) mean±SEM	
		0 day	14th day	0 day	14th day	0 day	14th day
I	Control	10.71±0.25	10.74±0.19	12.58±0.14	12.3±0.16	10.15±0.20	10.3±0.19
II	Negative control	9.5±0.341	4.0±0.11^{**,a}	10.51±0.29	4.9±0.1$^{**\,a}$	10.01±0.16	5±0.13^{**,a}
III	MEFB 100 mg/kg	10.56±0.29	9.8±0.17^{**,a}	11.53±0.140	10.±0.2$^{**\,a}$	11.06±0.21	9.2±0.19^{**,a}
IV	MEFB 200 mg/kg	9.30±0.34	8.0±0.15^{**,a}	10.94±0.12	7.8±0.2$^{**\,a}$	10.18±0.23	8.5±0.14^{**,a}
V	MEFB 300 mg/kg	9.28±0.29	7.0±0.21^{**,a}	10.29±0.16	7.0±0.1$^{**\,a}$	9.83±0.44	7.4±0.19^{**,a}
VI	Standard	11.15±0.17	11.61±0.24^{b}	12.96±0.13	13.1±0.22^{b}	11.23±0.17	11.3±0.1^{a}

Values are expressed as mean± SEM, (n=6). All the groups were compared with control group (Dunnett's t-test). Significant values are expressed as control (**=$p<0.01$) and standard (a=$p<0.01$), (b= $p<0.05$),

Histopathological studies of rat spleen for methanolic extract of *Ficus benghalensis* aerial roots

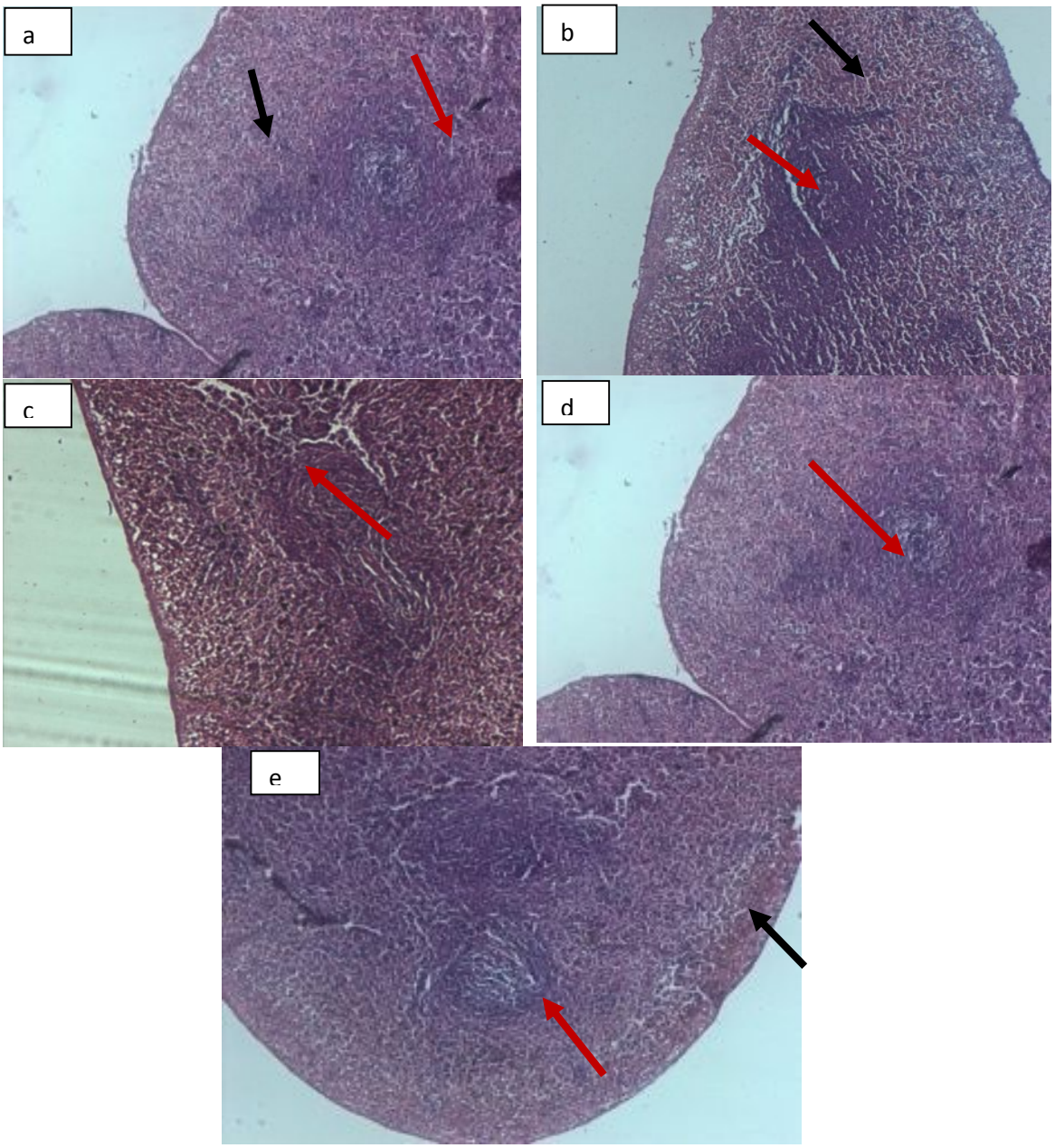

Figure 1. (a-e) rat spleen a) Control- Splenic cortex appeared normal – black arrow, Lymphatic follicles located in the Medullary region appeared normal – red arrow. b) MEFB 100 mg/kg-Spleenic cortex area appeared normal and it contain mostly RBCs [Red bulp] – black arrow. Medullary area containing varying size of spleenic corpuscles or lymphatic follicles containing lymphocytes [white pulp] – Red arrow. c)MEFB 200mg/kg- Lymphatic follicles containing central arterioles appeared normal – arrow, No lymphoid depletion noticed. d) MEFB300mg/kg- Follicular atrophy [fibrosis] noticed in few Lymphatic follicles - red arrow. e) Standard- Spleenic cortex appeared normal and it contain large amount of RBC's Medullary region containing lymphatic follicles showed mild atrophy and lymphoid depletion - arrow.

Histopathological studies of rat thymus for methanolic extract of *Ficus benghalensis* aerial roots

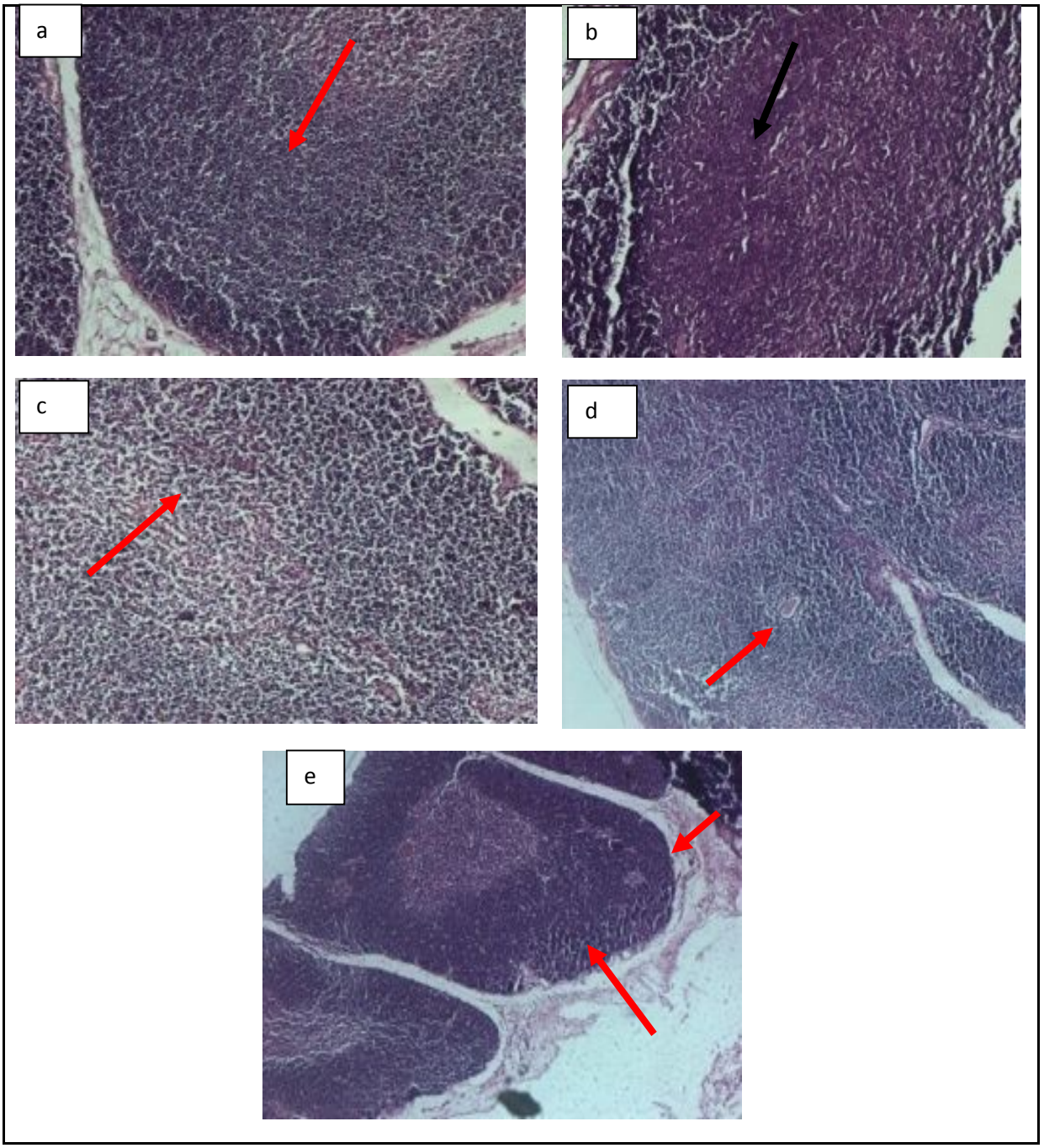

Figure (a-e) rat thymus a) Control - Cortex region of thymus appeared normal [red arrow] it contain pockets/ spread of lymphocytes appeared normal. b) MEFB 100 mg/kg- Medullary region appeared normal – arrow, NO atrophy /fibrosis or lymphoid depletion noticed. c) MEFB 200 mg/kg Moderate to severe lymphoid depletion noticed in the medullar region of thymus arrow. d) MEFB 300 mg/kg Cystic degeneration and mild follicular atrophy noticed in the Medullary region of thymus – red arrow. e) Standard Cortex region appeared normal it contain large amount of lymphocytes appeared as follicles, - red arrow

The results of the histopathology of thymus (Fig. 2 a,b,c,d,e) also correlates with spleen. The standard levamisole, showed marked immunostimulant activity indicated by large amount of lymphocytes in the cortex region of thymus gland. MEFB exhibited significant immunostimulant activity compared to standard levamisole. MEFB at the dose of 100 mg/kg bd.wt. showed better effect evident by normal medullary region with no atrophy or lymphoid depletion than 200 and 300 mg/kg doses in which there was mild to moderate

atrophy and lymphoid degeneration in the medullary region of the thymus gland. This might be due to optimal/ceiling effect of MEFB at 100 mg/kg dose.

Conclusion:

Extensive literature survey revealed the presence of flavonoids, glycosides, steroids and alkaloids in *Ficus benghalensis*, are well established for their antioxidant, anti-inflammatory, analgesic properties. Methanol extract of *Ficus benghalensis* also showed potential effect on haemopoetic system. Immunomodulatory potential of *F. benghalensis* could be attributed for the presence of flavonoids, polyphenols and terpenoids which may modulate and potentiate humoral as well as cellular immunity. This emphasizes the future scope of this study.

References

1. Patil US, Jaydeokar AV, Bandawane DD. Immunomodulators: A Pharmacological Review. Int. J Pharma Pharma Sci., 2012; 4(1): 30-36.
2. Ukpo G, Ogbonnia S, Ehianeta T, Bashir S, 2013. Immunostimulatory and biochemical effects of ethanolic extract of *Mangifera indica* stem bark on dexamethasone-induced immunosuppressed male rats. Int J Pharm Pharm Sci, 5(2): 569-572**.**
3. Benny, K.H., J. Vanitha, Immunomodulatory and antimicrobial effects of some traditional chinese medicinal Herbs: A Review. Curr Med Chem, 2004, 11(11): 1423-1430.
4. Chakraborthy G.S., P. Vikas, N. Kaushik Kailash,. Evaluation of immunomodulatory activity of *Aesculus indica*. J Pharm Res, 2009, 2(3): 385-387.
5. Kuttan, G. Immunomodulatory effect of come naturally occurring sulphur-containing compounds. J Ethnopharmacol, 2000, 72(1-2): 93-99.
6. Bodhankar S., N. Makare, V. Rangari. Immunomodulatory activity of alcoholic extract of *Mangifera indica* L. in mice. J Ethnopharmacol, 2001, 78(2): 133-137.
7. Pezzuto, J.M.,. Plant-derived anticancer agents. Biochem Pharmacol, 1997, 53: 121–133.
8. Gopukumar ST, PK Praseetha. *Ficus benghalensis* Linn. The Sacred Indian Medicinal Tree with Potent Pharmacological Remedies. Int J Pharm Sci Rev Res, 2015, 32(1): 223-227.
9. Murti K, U Kumar, L Vijay, G Vipul. Ethnopharmacological Properties of *Ficus benghalensis* Linn: A Review. Pharmacologyonline, 2010, 2(4):717-726.
10. OECD guideline for testing of chemicals, Acute Oral Toxicity – Up-and-Down Procedure., 425: 2001.
11. Subhashini N, A Thangathirupathi, N Lavanya. Antioxidant activity of *Trigonella foenum graecum* using various *in vitro* and *ex vivo* models. Int J Pharm Pharm Sci 2011, 3(2): 975-1491.
12. Singhal M, A Paul, H Pratap Singh, SK Dubey and G Kalpesh. Evaluation of reducing power assay of Chalcone- semicarbazones. J Chem Pharm Res, 2011, 3(3): 639-645.
13. Nagulendran K, S Velavan, R Mahesh, VH Begum. *In vitro* Antioxidant activity and total poly phenolic content of *Cyperus rotandus* rhizomes. Exp J chem, 2007, 4(3): 440-449.
14. Papiya Mitra Mazumder, S Pattnayak, H Parvani, D Sasmal, P Rathinavelusamy Evaluation of immunomodulatory activity of Glycyrhiza glabra L roots in combination with zinc. Asian Pac J Trop Biomed, 2012, S15-S 20.
15. Simpson MA, JJ Gozzo. Spectrophotometric determination of lymphocyte mediated sheep red blood cell haemolysis *in vitro*. J Immunol Meth, 1978, 21 (1-2): 159–165.
16. Agrawal SS, CK Saurabh, S Gokul. Studies on Immunomodulatory Activity of *Capparis zeylanica* Leaf Extracts. Int J Pharm Sci Nanotech, 2010, 3(1):887-892.
17. S. Verma, K A Shaban, R Purohit, LM Chimata, G Rai and O Verma. Immunomodulatory activity of *Withania somnifera* (L.). J Chem Pharm Res, 2012, 4(1) : 559-561.
18. Gokhale AB, A S Damre and MN Saraf. Investigations into the immunomodulatory activity of *Argyreia speciosa*. J Ethnopharmacol, 2003, 84(1):109-114.
19. S Singh and AK Yadav. Evaluation of immunomodulatory activity of *Grewia asiatica* in laboratory animals. J Chem Pharm Res, 2014, 6(7): 2820-2826.

*****