JPR INFO
Journal of Pharma Research

Journal of Pharma Research
Available online through
www.jprinfo.com

Research Article
ISSN: 2319-5622

Inhibitory Potential of Aqueous extract of *Ficus bengalensis* on Human Peripheral Blood Mononuclear Cells

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Received on: 15-11-2014; Revised and Accepted on: 29-11-2014

ABSTRACT

Ficus bengalensis are traditionally used to treat a variety of disorders including inflammatory conditions and infections. Nitric oxide (NO) produced from human peripheral blood mononuclear cells (PBMC) plays an important role in both inflammatory and anti-inflammatory processes. This study examined whether Ficus bengalensis inhibit the production of NO, pro-inflammatory cytokines and blood counts using flow cytometry by human PBMC. The results showed that the aqueous extract of leaves of Ficus bengalensis showed NO production and pro-inflammatory cytokines decreased in a dose-dependent manner. Meanwhile, the aqueous extract of Ficus bengalensis showed rapidly decline in the number of monocytes count as compared to control which is confirmed through CD14 surface marker. These studies show that the aqueous extract of Ficus bengalensis can either increase or decrease NO production by human PBMC and that these effects are predominantly mediated through an effect on pro-inflammatory cytokines expression. These data contribute to a better mechanistic understanding of the medicinal properties of Ficus bengalensis.

Key words: *Ficus bengalensis, PBMC, Nitric oxide.*

INTRODUCTION

Medicinal plants have played an important as well as essential role in human beings because many of the modern medicine comes into the market are directly or indirectly from the medicinal plants [1]. Medicinal plants have the capability to synthesize the number of bioactive compounds from different parts of the plant e.g. roots, stem and leaves [2]. Now a day, there is an emerging trend in research to support the immunopharmacological activities of medicinal plants [3, 4]. Many scientific researchers have been reported about the safety, potency, efficacious and chemotherapeutic role of medicinal plants in the treatment of several diseases [5, 6]. Most of the immunomodulatory agents are of plant origin [7-9] and have shown their numerous capability to reduced the raised level of liver enzymes in viral hepatitis [10, 11]. Most of the medicinal plants have shown lot of immense potential as anti-viral, anti-inflammatory, anti-diabetic, anti-tumor and antioxidant properties [12-16]. Some of the proper medicinal uses of some of plants are well known, and many have still to be explored.

Ficus bengalensis (commonly known as a Banyan tree) is an evergreen tree and belongs to the family Moraceae and it is commonly grown in gardens and road sides and is widely distributed in India from sub Himalayan region and in the deciduous forest of Deccan and south India [17, 18]. According to ancient and modern pharmacopoeias of Indian system of medicine contain more valuable information about the immunopharmacological activities of various parts of Ficus benghalensis. During ancient period, the tree is regarded as a symbol of peace and harmony. It has been traditionally claimed that the whole plant of *Ficus bengalensis* showed some medicinal properties e.g. leaves, fruits and bark are used as anti-oxidant, anti-cancer, anti-microbial properties and also used in the treatment of several diseases e.g. skin, vaginal disorders etc [19-21]. In the traditional system of medicine, the plant is generally used in the treatment of several health problems and diseases. With the advancement of medicinal plants research, it was concluded that plants are one of the biosynthetic laboratories for chemical compounds, which are responsible for curative action of plants. Number of researchers involved in isolating the phytochemicals from medicinal plants and many of them are found very active against many diseases [22, 23]. Therefore, this study was designed to investigate the anti-inflammatory activity using nitric oxide, pro-inflammatory cytokines, estimation of lymphocytes, monocytes and granulocytes count and also estimate the CD14 count by using aqueous extract of leaves of *Ficus bengalensis*.

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MATERIALS AND METHODS

Plant material:

Ficus bengalensis was collected in May 2014 from the garden of Vidya Pratishthan's School of Biotechnology (VSBT), Baramati (Pune), Maharashtra.

Preparation of aqueous extract:

The leaves of *Ficus bengalensis* were properly washed with tap water and then dried in a shady area at room temperature and finely macerated with liquid nitrogen to form the fine powder. The plant leaves powder was grinded in phosphate buffered saline by continuously stirring. The aqueous extract of plants was centrifuged at 8000 rpm for 10 minutes. The supernatants were filtered through Whatman filter paper and the supernatant was collected and was used for various immunological assays.

High performance thin layer chromatography (HPTLC) profile:

The secondary metabolites of the aqueous extract of *Ficus bengalensis* were determined through HPTLC. The plates used for the determination of secondary metabolites through HPTLC (10 x 10 cm) were purchased from Merck and most of the solvents (used in mobile phase) from Qualigens and detect its wavelength at 366 nm. The stock solution of aqueous extract of *Ficus bengalensis* was prepared for HPTLC studies and dissolved the 3 g of weighed compound in phosphate buffered saline in a final volume of 30 ml. The aqueous extract showed the presence of saponin, terpenoids and flavonoids in the phytochemical profile of *Ficus bengalensis*. The retardation factor *(Rf)* values of terpenoids (0.96) and saponin (0.36, 0.48 and 0.79).

Human blood samples and determined the nitric oxide production and monocytes (CD14 marker) from PBMC:

Before blood collection, informed consent letter was collected from healthy volunteers and does not show any symptoms

of disease or any other illness. Briefly, human peripheral blood mononuclear cells (PBMC, 10^5 cells/ml) were separated by means of Ficoll–Hypaque gradient centrifugation and plated in 96 well plates were pre-incubated for 24 h with or without LPS and then treated with serial dilutions of *Ficus bengalensis* at 37°C for 24 h. The plates were centrifuged at 2500 rpm for 10 minutes and then the supernatant (100 µl) was collected for the estimation of nitric oxide and cytokine profile.

In NO estimation, PBMC cell culture supernatant was mixed with same volume of Griess reagent (1% sulfanilamide and 0.1% naphthylethylenediamine dihydrochloride in 2.5% phosphoric acid) and incubated the flat bottom 96 well plates at room temperature for 10 minutes, and the absorbance at 540 nm was measured in a microplate reader. The fresh culture medium (RPMI containing 10 % fetal bovine serum) was used as a blank. The nitrite quantity was determined from a sodium nitrite standard curve. All experiments were performed in triplicates [24].

In addition, human PBMC cells were cultured in the presence of variable concentration of *Ficus bengalensis* and stained with CD14 FITC surface marker. The samples were centrifuged (300 – 400 × *g*) and the supernatant was aspirated or discarded and washed two to three times with phosphate buffered saline. After centrifugation, pellet dissolved in PBS and observed the cells through flow cytometer [12, 13].

Measurement of pro-inflammatory cytokines (IFN-gamma TNF alpha) production:

The aqueous extract of *Ficus bengalensis* was diluted with cell culture medium containing PBMC and incubates the plate for 24 h. The plates were centrifuged at 5000 rpm for 10 minutes and the supernatant was collected for the estimation of Th1 type of cytokines. The effect of the aqueous extract on pro-inflammatory cytokine (TNF alpha and IFN-gamma) production in PBMC and was determined by ELISA as described in the manufacturer's instructions (BD Optia kits). All experiments were performed in triplicate [25, 26].

Statistical analysis:

All values are mentioned as Mean ± S.E. Data is represented by One way ANOVA test (Boniferroni multiple comparison test).

RESULTS

Estimation of NO production from human PBMC:

The effect of *Ficus bengalensis* was observed in human PBMC as shown in **Fig. 1**. PBMC were cultured in LPS (10 µg/ml) and the contents of the indicated nitric oxide (NO) were measured in the supernatants as a function of macrophage activation. There was a significant decreased in NO production elicited by the aqueous extract. The inhibitory concentration of aqueous extract at 30 mg/ml as compared to control.

CD14 monocyte marker estimation:

The effect of *Ficus bengalensis* in human PBMC on CD14 monocyte marker as shown in **Fig. 2**. The results showed that in human PBMC, there is dose dependent decrease in monocyte marker CD14 as compared to control. The inhibitory effect of aqueous extract in human PBMC on CD14 monocyte marker at higher doses (i.e. 30 mg/ml) as compared to control.

Th1 type of cytokines (IFN-gamma and TNF alpha) estimation:

The effect of *Ficus bengalensis* was observed in Th1 type of cytokines from the cell culture supernatant of human PBMC as shown in **Fig. 3**. PBMC were cultured in LPS (10 µg/ml) and the contents of the cytokines (IFN-gamma and TNF alpha) were measured in the supernatant. There was a significant decreased in Th1 type of cytokine production as compared to control.

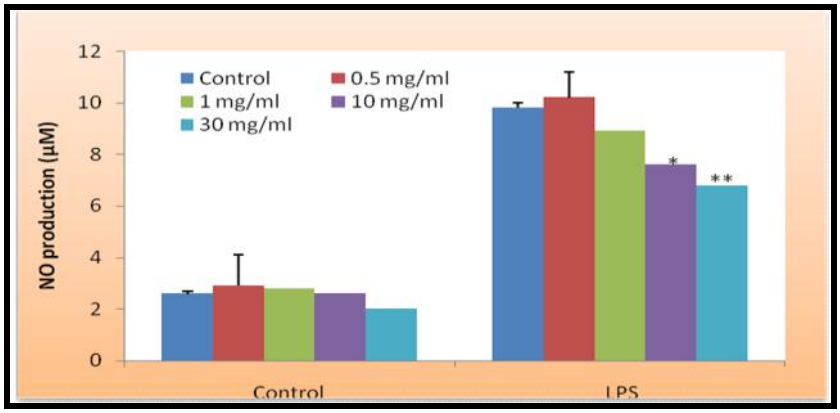

Fig. 1: Production of nitric oxide (NO) by human peripheral blood mononuclear cells (PBMC). The supernatant nitrite concentration was determined by Griess reagent after the 24 h culture of cells in presence of aqueous extract of *Ficus bengalensis*. Values are expressed as Means ± S.E. and The difference between the control and treated groups is determined by One way ANOVA test (Bonferroni multiple comparison test). **P < 0.05; **P < 0.01; ***P < 0.001*

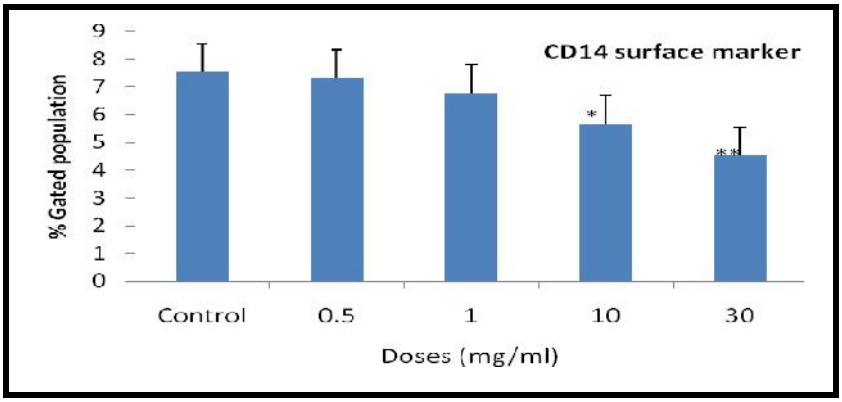

Fig. 2: Estimation of CD14 surface marker on human peripheral blood mononuclear cells (PBMC) using flow cytometry. Cells were stained with CD14 FITC surface marker and then lysed and wash the cells in PBS and then analyzed in a flow cytometer (FACS Calibur). Values are expressed as Mean ± S.E. The difference between the control and treated groups is determined by One way ANOVA test (Bonferroni multiple comparison test). **P < 0.05; **P < 0.01; ***P < 0.001*

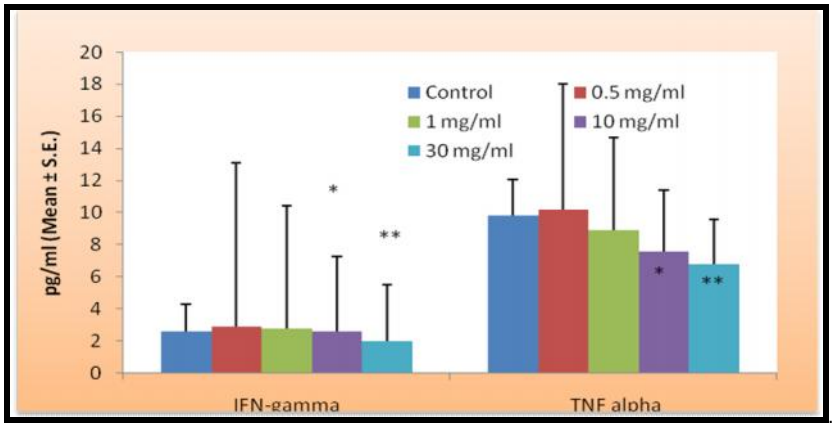

Fig. 3: Effect of aqueous extract on Th1 type of cytokines on human peripheral blood mononuclear cells (PBMC). Concentration of cytokines was determined by ELISA in supernatants of cells cultured for 48 h. Values are expressed as Means ± S.E. The difference between the control and treated groups is determined by One way ANOVA test (Bonferroni multiple comparison test). **P < 0.05; **P < 0.01; ***P < 0.001.*

DISCUSSION

In this study, the aqueous extract of *Ficus bengalensis* were used to assess the effect on various parameters of immune system especially NO production, monocyte marker i.e. CD14 population and estimation of Th1 type of cytokines from cell culture supernatant, in order to validate as well as estimate the potential use of NO assay from PBMC as a immunopharmacological tool in preliminary screening for immunomodulatory effects of compounds or test samples on the immune system. The main advantage as well as criteria for the preliminary screening is its high sensitivity, general feasibility, low cost value and possibility of large scale performance. Since production of NO and cytokines from human PBMC are much more sensitive with respect to the activation signal of LPS, the immunopharmacological screening tests should always be accompanied by careful examination of the test samples for possible contamination with LPS. It has been suggested that production of NO from macrophages, dendritic cells and PBMC may depend on the cell types and their species origin [27, 28], different cells of our immune system having obviously different requirements for signal transduction pathways [29]. In NO production, there is phenomenon that there is an extensive knowledge showing that the increase in the NO production from different types of cells is determined by a number of Th1 and Th2 type of cytokines. There is direct correlation between the NO production and cytokines; direct NO-stimulatory or inhibitory function is dependent on IFN-gamma as well as TNF alpha that triggers or suppress NO production on its own [27-29]. In this study, the results showed that there is dose dependent decrease in NO production as compared to control and also decline in Th1 (IFN-gamma and TNF alpha) type of cytokines. Cytokines such as TNF alpha and IFN-gamma are pro-inflammatory both *in vitro* and *in vivo*. In particular, IFN-gamma is a major pro-inflammatory cytokine that is mainly released by macrophages and is believed to play a considerable role in the pathophysiology of hormonal immune system. In addition, few reports as well as in research papers have already shown about the role of cytokine production regulated by NO; NO production activates directly or indirectly release of cytokines as well as its receptors and also adhesion molecules [30-32]. The effect of NO on expression of TNF-alpha is reported to be likely via activation of a TNF-alpha - converting enzyme (TACE), which is responsible for membrane-bound TNF and thereby activating release of this cytokine [31, 32]. IFN-gamma and TNF-alpha production caused by *Ficus bengalensis* was significantly decreased at higher concentration.

CONCLUSION

In the present study, we found that the *Ficus bengalensis* significantly inhibited the production of the NO production, monocyte surface marker CD14 and pro-inflammatory cytokines (IFN-gamma and TNF alpha). Further investigations will focus on the *in vivo* assessment of the biological activity of these aqueous extracts and on the chemical identification of the major active components responsible for the anti-inflammatory activity in the efficacious extracts.

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How to cite this article:

Amit Gupta et Al.,: Inhibitory Potential of Aqueous extract of *Ficus bengalensis* on Human Peripheral Blood Mononuclear Cells. J. Pharm. Res., 2014; 3(11): 250-253.

Conflict of interest: The authors have declared that no conflict of interest exists.

Source of support: Nil