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# Inhibitory Potential of Aqueous Leaves Extract of *Mesua Ferrea* and *Mimusops Elengi* on Antigen Specific Immune Response using Human Whole Blood

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**ABSTRACT:** The objective of this study is to examine the effect of variable doses of aqueous leaves extract of *Mesua ferrea* and *Mimusops elengi* (0.5 – 10 mg/ml, 50 µl), medicinal plant on human lysed whole blood (cultured for 48 h) in order to determine the antigen (IBD, infectious bursal disease; virus derived from chicken) specific immune response including CD14 monocyte surface marker which is determined through flow cytometry. The results showed that *Mesua ferrea* and *Mimusops elengi* (10 mg/ml, 50 µl) showed dose dependent decline in antigen specific immune response including CD14 monocyte surface marker as compared to IBD virus and control. IBD used as standard for these studies and the results showed that there is significant enhancement in antigen specific immune response and CD14 monocyte surface marker as compared to control. Moreover, the outcomes of the work provide a platform on the way to discover novel immunosuppressive as well as anti-viral agents against IBD virus from plant origin.

**Keywords:** *Mesua ferrea*, *Mimusops elengi*, Immunosuppressive, Infectious Bursal Disease

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## INTRODUCTION

Medicinal plants are composed of primary (proteins, carbohydrates etc.) and secondary (alkaloids, flavonoids, terpenoids, glycosides etc.) metabolites. These medicinal plants are highly efficient because of these metabolites in order to provide protection against various infections (bacterial, fungal, viral and parasitic) and these plant products (leaves, stem, roots etc.) are generally used to cure various ailments since from ancient times (Maghsoudi, 2005; Gupta et al., 2014a). As per the literature survey, more than 70 % of the population resides in villages is totally dependent on various medicinal plant products and also used as raw material for synthesizing or manufacturing various drugs in pharmaceutical companies (Maghsoudi, 2005; Gupta et al., 2014a).

Although some of synthetic drugs and antibiotics derived from various medicinal plant products brought about a revolution in controlling different diseases. Recently, these drugs (synthetic) reach to millions of people those who live in remote places depend on traditional healers. These medicinal plant products showed various medicinal properties i.e. immunomodulatory (Gupta et al., 2014b; Gupta and Chaphalkar, 2015a), anti-diabetic (Gupta et al., 2014d), anti-inflammatory (Gupta and Chaphalkar, 2015b) etc. In addition, these plant products also showed anti-microbial activity because of proteins or peptides (low molecular mass) have been investigated (Gupta and Chaphalkar, 2015c).

Infectious bursal disease (IBD; poultry disease; double stranded RNA virus; incubation period i.e. 2-3 days) are widely exposed to chicken worldwide, which leads to multi-billion dollar losses in the poultry industry (Alviano and Alviano, 2009; Liu et al., 2005). There are number of strategies in order to control IBD disease using various medicinal plant products.

One of the medicinal plant, *Mesua ferrea* (Nagkeshar, family *Guttifere*) and *Mimusops elengi* (bakul, family *Sapotaceae*), were selected for these studies. These medicinal plants are commonly found in India (Maharashtra and parts of Andhra Pradesh), Pakistan and Bangladesh. This medicinal plant showed various medicinal properties or activities i.e. antiasthmatic, anti-inflammatory, immunomodulatory, anti-oxidant etc (Eldaghayes et al., 2006; Gupta et al., 2014c). In order to achieve this objective, our group focused on the aqueous leaves extract of *Mesua ferrea* and *Mimusops elengi* on antigen specific immune response including CD14 monocyte surface marker in human whole blood using infectious bursal disease (IBD) derived from chicken.

## MATERIAL AND METHODS

### Plant material and HPTLC analysis

Fresh plant leaves (*Mesua ferrea* and *Mimusops elengi*) were gathered from the garden of Vidya Pratishthan's School of Biotechnology (VSBT), Baramati (Pune), Maharashtra. Plant leaves powder of these two medicinal plants was grinded in phosphate buffered saline and collect the supernatant after centrifugation (5000 rpm, 10 minutes, 4 °C). Finally, supernatants were filtered using Whatman filter paper and used for various immunological assays including HPTLC analysis. For HPTLC estimation, aqueous extract of *Mesua ferrea* and *Mimusops elengi* revealed the presence of flavonoids, terpenoids (Rf value 0.96 and 0.92), glycosides (5.44 µg and < 1.8 µg) and phenolics (present only in *Mesua ferrea*).

### Infectious bursal disease

Infectious bursal disease viruses (IBDVs) were collected aseptically from Baramati poultry form and stored at -20°C until processed for the isolation of IBD virus using chicken embryo fibroblast cell culture. Each bursal sample was macerated using phosphate buffered saline (PBS) into small pieces using mortar and pestle. The supernatant were collected and treated with various antibiotics (penicillin and streptomycin). Incubate the supernatant samples containing antibiotics at room temperature for 30 minutes and shaked gently every 5 minutes. After incubation, the suspension was inoculated into sterile blood agar media for bacteriological sterility and was incubated at 37 °C for 24 h. This sterile suspension (bacteriologically) was used as inoculums for the isolation of virus.

### Chicken embryo fibroblast cell culture

Chicken embryo fibroblast cell culture were grown in 24 well tissue culture plates and used for the isolation of IBD virus. After 24 h incubation, growth medium was removed and inoculate 50 µl of sterile suspension was added into the 24 well plate. Three wells of 24 well plates (performed in triplicates) treated as infected control and rest three wells treated as uninfected control. The plates were incubated at 37°C for 1h to allow the virus to adsorb. After 1h incubation, one ml of fresh medium was added to each well. Again, the plates were incubated at carbon dioxide incubator and observed daily under inverted fluorescent microscope for the appearance of cytopathic effect. On day 5, cells were collected and frozen at -20 °C irrespective of the appearance of cytopathic effect. When maximum cytopathic effect was manifested, the tissue culture was harvested after three to four cycles of freezing and thawing. The harvested tissue culture fluid was centrifuged for 10 minutes at 4000 rpm. The supernatant (fluid) was collected and stored in 1 ml aliquots in vials and stored at -20 celsius and identified the titre through haemagglutionation test (128 HA).

### Standardization of IBD virus in human and chicken whole blood

Anti-coagulant EDTA human and chicken blood samples were collected from *Mangal Pathology laboratory* and Poultry farm, Baramati region, District Pune, Maharashtra, India. For its IBD standardization with respect to estimation of blood counts in chicken and human whole blood. Whole blood was taken into the eppendorf tube and treat with variable concentration of IBD virus (1:10; 1:20; 1:40; 1:80 and 1:100 dilution). Incubate the samples for 2 h in dark and then lysed and washed two times with phosphate buffered saline. After lysing and washed the samples then proceed for the estimation of monocytes through flow cytometer (Gupta et al., 2014 b,e).

### Determination of antigen (IBD) specific immune response including CD14 monocyte surface marker in lysed human whole blood

Antigen (IBD) specific immune response were evaluated in human lysed whole blood and was cultured for 48 h in presence of variable doses of *Mesua ferrea* and *Mimusops elengi* (0.5 – 10 mg/ml, 50 µl) along with standardized dose of IBD (1:100 dilution) in 96 well flat bottom tissue culture plates.

In first set of experiment, after 48 h incubation, MTT solution (2.5 mg/ml; 10 µl) were added and incubated for 2-3 h in carbon dioxide incubator. Again, the plates were centrifuged (1800 rpm for 5 minutes) and supernatant was eliminated. Add DMSO (100 µl solution) to the formazan crystals and the absorbance was evaluated in an ELISA reader at 570 nm (Gupta and Chaphalkar, 2014b; Gupta et al., 2014e).

In second set of experiment, after 48 h incubation, cells were stained with CD14 monocyte surface marker. Incubate the samples in dark for 30 minutes at room temperature. After lysing and wash the samples with PBS and proceed for flow cytometric estimation.

### Statistical analysis

The difference between the control and treated groups of IBD along with variable doses of *Mesua ferrea* and *Mimusops elengi.* Data is represented by One way ANOVA (Boniferroni multiple comparison) test.

## RESULTS

### Estimation of standardized dose of IBD virus

The effect of variable doses of IBD virus on human and chicken whole blood as shown in **Fig. 1**. The results showed that there is drastic enhancement of monocytes count in case of 1:80 (chicken) and 1: 100 (human) dilution of IBD virus as compared to control.

### Evaluation report on antigen (IBD) specific immune response

The effect of variable doses of *Mesua ferrea* and *Mimusops elengi* on antigen specific immune response (IBD induced proliferation including CD14 monocyte surface marker) as shown in **Fig. 2.** The results showed that *Mesua ferrea* and *Mimusops elengi* showed drastic reduction at higher doses in antigen specific immune response as compared to standard IBD and control. In addition, similar response was observed in case of CD14 monocyte surface marker. The results showed that there is dose dependent decline in CD14 monocyte surface marker treated with IBD virus along with *Mesua ferrea* and *Mimusops elengi* as compared to control **(Fig. 2).**

## DISCUSSION

Infectious bursal disease (IBD, double stranded RNA virus) is an acute and contagious poultry disease. Outbreak of this disease could result in 10 –75 % mortality of birds; hence it has gained socio-economic importance worldwide. In order to control the burden of IBD disease, medicinal plants have shown number of immunopharmacological activities including anti-viral against RNA and DNA viruses. There are number of medicinal plants which showed anti-viral activity against different kind of viruses (Liu et al., 2005; Eldaghayes et al., 2006). To further explore the knowledge of these medicinal plants in order to determine the cytotoxic and anti-viral activities of aqueous extract of *Mesua ferrea* and *Mimusops elengi* on human whole blood using flow cytometry.

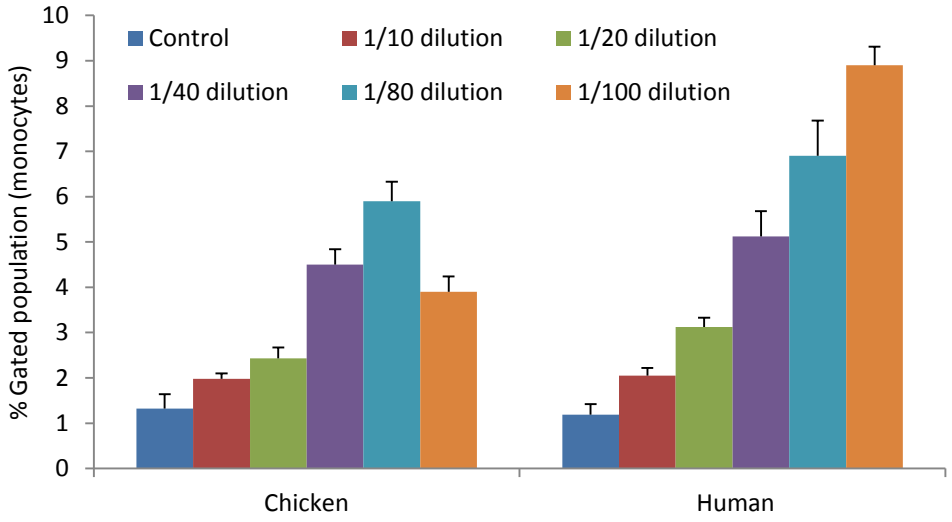


**Fig.1**. **Standardization of IBD virus in chicken and human whole blood.** Values are expressed as Means ± S.E. The difference between the control and treated groups is determined by One way ANOVA test (Bonferroni multiple comparison test). **P < 0.05; **P < 0.01; ***P < 0.001*

In order to achieve the objective of this study with respect to the involvement of aqueous leaves extract showed maximum inhibition of antigen specific immune response has been demonstrated (**Fig. 2**). The inhibition of antigen (IBD) specific immune response is due to decline in production of T lymphocytes in human lysed whole blood. T lymphocytes play a central role in the antigen-specific immune response against various pathogens (Gupta et al., 2014e). However, the importance of T cells in IBD virus disease, have shown that cell-mediated immunity may be responsible for IBD viral infections. Our data show that aqueous leaves extract of *Mesua ferrea* and *Mimusops elengi* decline in the proliferation of T cells in human lysed whole blood with respect to IBD stimulation. Overall, the data reveals that *Mesua ferrea* and *Mimusops elengi* decrease the IBD proliferation rate at higher doses.

In another study, CD14 cell surface marker was first identified on monocytes (blood) and macrophages (tissues). The results obtained from this study and showed that aqueous leaves extract of *Mesua ferrea* and *Mimusops elengi* showed decline in CD14 monocyte marker (exposure with IBD virus) at higher doses in human lysed whole blood with a dosage-dependent relationship. As seen in this experiment, the ability of this aqueous leaves extract of *Mesua ferrea* and *Mimusops elengi* to suppress antigen specific immune response when it is applied after the onset of viral infection is likely to be due to the genuine anti-viral activity.

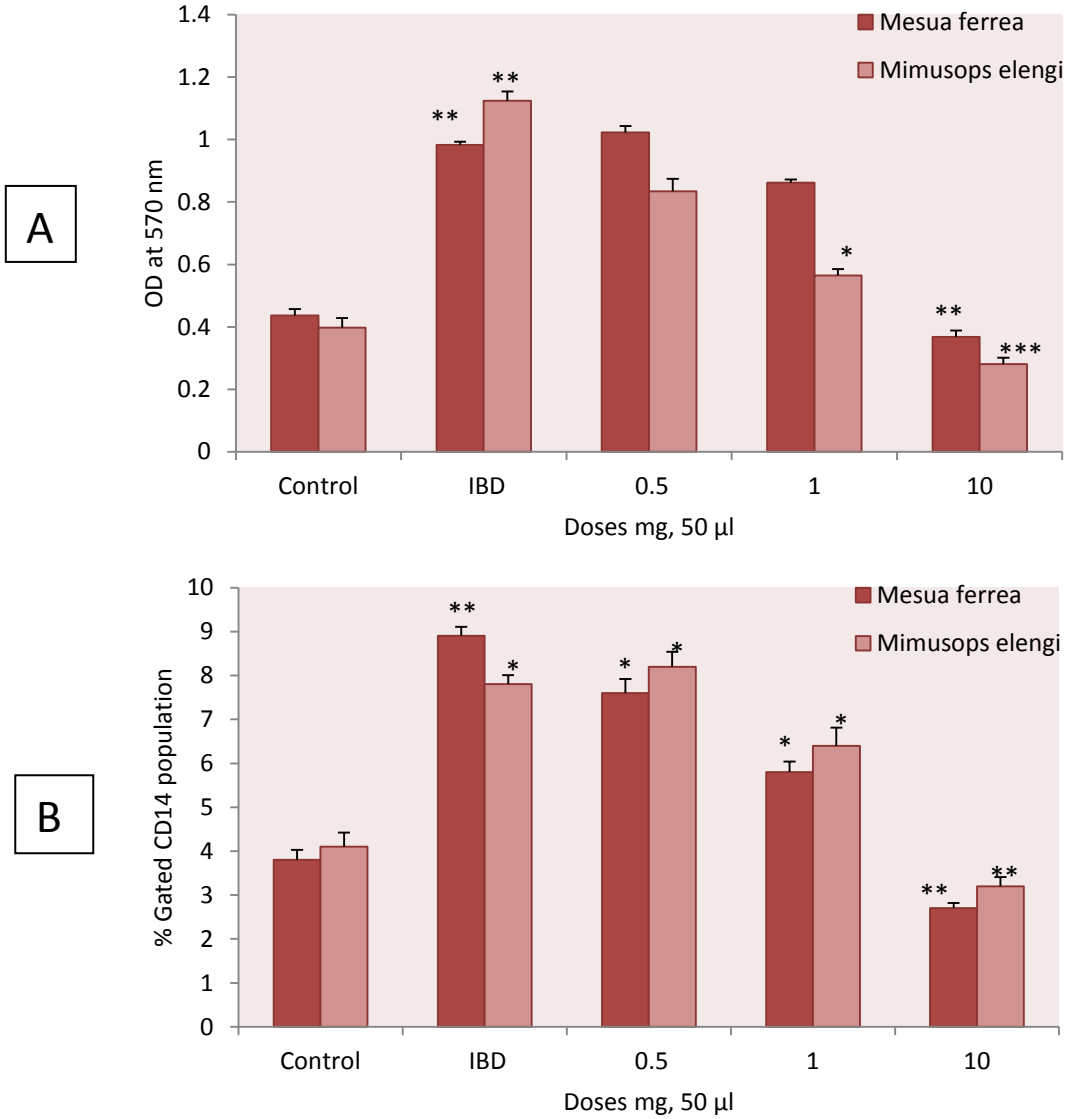


**Fig.2. Effect of aqueous leaves extract of *Mesua ferrea* and *Mimusops elengi* on A) IBD induced proliferation B) CD14 monocyte surface marker on human whole blood.** Values are expressed as Means ± S.E. The difference between the control and treated groups is determined by One way ANOVA test (Bonferroni multiple comparison test). **P < 0.05; **P < 0.01; ***P < 0.001*

## CONCLUSION

The results showed that aqueous leaves extract of *Mesua ferrea* and *Mimusops elengi* showed anti-viral activity i.e. decline in antigen specific immune response including CD14 monocyte surface marker using IBD virus. Since the chicken's immune system is almost similar to that of mammals, chickens provide an attractive model system to study the aqueous extract of *Mesua ferrea* and *Mimusops elengi* in controlling diseases in livestock and

human beings. Overall, the conclusion of this study is that *Mesua ferrea* and *Mimusops elengi* aqueous leaves extract has antiviral activity against IBD. It is expected that using medicinal plant products as therapeutic agents and is generally use of these plant as traditional medicine for treating viral infections.

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