



Virucidal potential of *Prosopis spicigera* and *Mangifera indica* on human peripheral blood mononuclear cells

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ABSTRACT

Introduction: The treatment of viral infections with the available antiviral drugs is not free of side effects. Therefore, in the present study, our group focused on antiviral activity against Newcastle disease (NDV) and IBD viruses using medicinal plants especially leaves of *Prosopis spicigera* and *Mangifera indica*.

Methods: Different medicinal plant products especially leaves of *P. spicigera* and *M. indica* were tested in the form of aqueous leaves extracts (0.5- 30 mg/mL; 50 µL) for anti-microbial activities on human peripheral blood mononuclear cells (PBMC) pertaining to determine their proliferation rate (cytotoxicity assay), tumor necrosis factor alpha (TNFα) production and CD14 monocyte surface marker.

Results: Three medicinal plant aqueous extracts showed significant antimicrobial activity against PBMC at higher doses with respect to decline in proliferation assay, TNFα production and CD14 monocyte surface marker as compared to control.

Conclusion: Aqueous leaves extract of *P. spicigera* and *M. indica* showed antimicrobial activities and might be useful for the treatment of various viral diseases.

Implication for health policy/practice/research/medical education:

Prosopis spicigera and *Mangifera indica* (aqueous leaves extract) demonstrated a significant decrease in proliferation, TNFα production including CD14 surface marker against NDV/IBD which may indicate antiviral properties of these components.

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Introduction

Medicinal plant products have a long traditional history with respect to their medicinal properties and uses. The major advantage of these medicinal plants over synthetic drugs is low side effects (1,2). In this regard, there is an increasing trend in the field of immunopharmacology for discovering novel drugs based on various herbal formulations. Plants synthesize a variety of phytochemicals most of which are extractable with various immunopharmacological activities (3,4). So, medicinal plant products proved to be a major resort for the treatment of animal and human diseases (3,4).

Mangifera indica (Mango; family *Anacardiaceae*) and *Prosopis spicigera* (Shami, family *Fabaceae*) showed number of medicinal properties e.g. anti-inflammatory, antioxidant, anti-asthma, etc. In addition, leaves of *M. indica* are used traditionally in India especially in weddings and religious ceremonies (5-7).

Newcastle disease (NDV) remains a constant threat or problem for poultry farm worldwide, strains of NDV (*Paramyxoviridae* family and *Avulavirus*, genus) contain one serotype and are also known as *avian paramyxovirus* serotype-1 (APMV-1). NDV (pleomorphic in shape, single-stranded, non-segmented and negative sense RNA viruses) is reported to infect birds and thus has a wide host range. Low virulent NDV typically produce subclinical disease with some morbidity, whereas virulent isolates can result in rapid and high mortality of birds (8).

One of the highly contagious diseases in chickens is IBD (*birnaviridae*; RNA viruses family) manifested by inflammation and successive atrophy of bursa of Fabricius (birds) including various degrees of nephroso-nephritis and immunosuppression. Clinically, IBD disease is seen only in those chickens that are older than 3 weeks (9). Recently, treatments are not available for NDV and IBD. However, vaccination is an effective method to control this disease

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but it does not provide any protection. Thus there is a need to search for those medicinal plant products which are able to restrict the load of NDV and IBD diseases in poultry animals. In the present study, therefore, we focused on antiviral activity against NDV and IBD using medicinal plant products especially leaves of *Prosopis spicigera* and *Mangifera indica*.

Materials and Methods

Collection and preparation of plant material

Fresh plant leaves (From *P. spicigera* and *M. indica*) were obtained from Nakshatra garden of Vidya Pratishthan's School of Biotechnology in Baramati region, Maharashtra, India. These medicinal plants were washed and air dried (away from direct sunlight). Leaves of these medicinal plants were crushed in mortar and pestle to prepare fine powder.

Estimation of secondary metabolites (qualitative and quantitative)

Secondary metabolites of fresh plant leaves were determined qualitatively in the aqueous leaves extract of *P. spicigera* and *M. indica*. Different assays were performed qualitatively in order to determine the presence of secondary metabolites and showed qualitatively the presence of flavonoids (alkaline reagent test); terpenoids (acetic anhydride test) and phenolic compounds (ferric chloride test).

Virus sample collection (IBD and NDV)

Poultry suspected disease samples of NDV and IBD were collected aseptically from suspected birds including Baramati poultry farm and the study was conducted under Biovillage programme scheme, Vidya Pratishthan's School of Biotechnology, Baramati, India.

For NDV collection, dead birds underwent postmortem examinations on them concurrent infections were determined through various laboratory tests. Briefly, pooled tissues (lung, spleen, intestine etc.) were properly minced and dissolved in phosphate buffered saline (PBS) containing penicillin and streptomycin. Then, the samples were cleared by centrifugation at 3000 rpm for 10 minutes and the supernatant was collected and determined haemagglutination (HA) test for detection of NDV. Thereafter, 0.2 ml of supernatant was directly injected into the allantoic cavity route of embryonated (9-11 day old) chicken eggs that were totally pathogenic free (Venkys India Ltd). These chicken eggs (big sized embryos) were selected for inoculation and observed at regular time intervals to examine or determine embryo motility rate. After death, fluid of amnio-allantoic cavity was harvested and identified the presence of virus which was determined through haemagglutination (HA, 128) titre (8).

Similarly, IBDVs were processed by using chicken embryo fibroblast cell culture (9). Briefly, bursal sample was macerated into small pieces using mortar and pestle and finally dissolved in PBS containing penicillin and streptomycin. The samples were incubated for 1 hour and shaken every 5 minutes. After incubation, the supernatant was inocu-

lated into sterile blood agar media for bacteriological sterility and was incubated at 37°C for 24 hours. This sterile suspension (bacteriologically) was used as inoculums for isolation of virus.

Proliferation assay and TNF alpha production

Fresh anti-coagulant (EDTA) human whole blood samples were collected (Mangal Pathology Laboratory, Maharashtra, India) and the human PBMC was separated by means of density gradient centrifugation and then cultured (10^5 cells/well) in 96 well plate for 48 h incubation with variable doses of aqueous leaves extracts (0.5–30 mg/mL, 50 μ L) of *Prosopis spicigera* and *Mangifera indica* along with NDV (1:80 dilution, 10 μ L) or IBD (1:100 dilution, 10 μ L). Thereafter, the plates (96 well) were centrifuged at 2600 rpm for 12 minutes at 4°C and then the supernatant (after centrifuging) was collected for estimation of TNF alpha production and then equal volume of fresh complete medium was added into the 96-well plates. Again, the plates were incubated for another 4 hours along with MTT (5 mg/mL, 10 μ L). Afterwards, the plates were centrifuged and the supernatant was collected and finally dispersed or dissolved in dimethyl sulphoxide (DMSO) solution. The optical density was measured at 570 nm (8,9). In addition, measurement of TNF alpha production from PBMC cell culture supernatant of *P. spicigera* and *M. indica* (exposed to NDV and IBD virus) was carried out using conventional ELISA kits and reagents (BD Biosciences, according to the manufacturer's instructions (10)).

CD14 monocyte surface marker

In another set of experiment, human PBMC were cultured with variable concentrations of aqueous leaves extract of *Prosopis spicigera* and *Mangifera indica* along with or without NDV (1:80 dilution, 10 μ L) or IBD (1:100 dilution, 10 μ L) for 48 hours in 96-well plates. After incubation, treated and non-treated PBMC samples of *Prosopis spicigera* and *Mangifera indica* were collected and stained with CD14 FITC (5 μ L) monoclonal antibody. The samples were then incubated (at room temperature), lysed (red cell lysis buffer containing ammonium chloride, potassium bicarbonate and EDTA) and washed with PBS (pH, 7.2). The resulting stained cell pellet was resuspended in 500 μ L of PBS and run on a FACS Calibur flow cytometer (11).

Statistical analysis

All values were mentioned as mean \pm S.E. Data were analysed by one-way analysis of variance (ANOVA) test (Bonferroni multiple comparison test).

Results

Proliferation assay

The effect of aqueous leaves extract on PBMC proliferation assay using NDV and IBD is shown in Figure 1. The results showed that aqueous leaves extract at higher doses significantly inhibited the PBMC proliferation assay as compared to control. NDV and IBD used as standard and there was significant increase in proliferation as compared

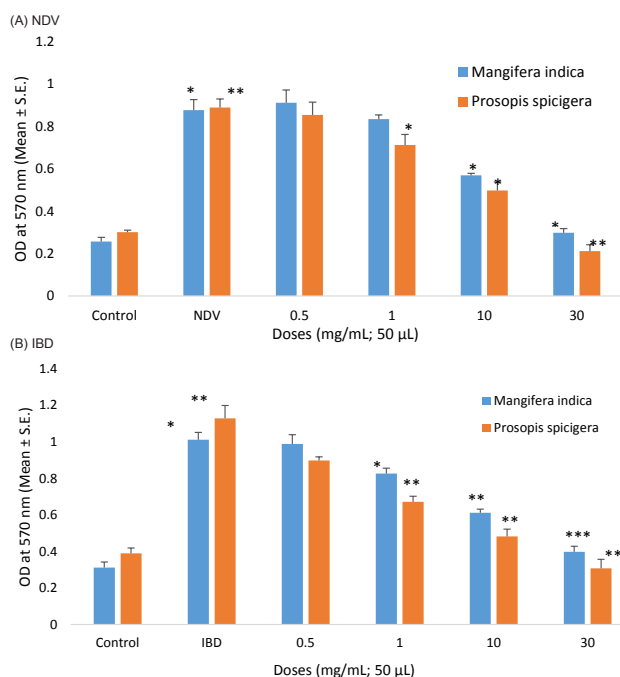


Figure 1. PBMC proliferation assay. Human PBMC (10^5 cells/well) were cultured with NDV or IBD along with variable doses of aqueous leaves extract or NDV/IBD alone. After 48 hours, proliferation was measured by MTT assay. The results are presented as mean \pm SE. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ as compared to control.

to control.

TNF alpha production

The effect of aqueous leaves extract on TNF alpha production from PBMC cell culture supernatant (using NDV and IBD) is shown in Figure 2. The results showed that aqueous leaves extract at higher doses significantly inhibited TNF alpha production as compared to control.

CD14 monocyte surface marker

The effect of aqueous leaves extract on CD14 monocyte surface marker using NDV and IBD is shown in Figure 3. At higher doses, aqueous extract significantly inhibited CD14 monocyte surface marker as compared to control.

Discussion

The objective of our present work was to examine the antiviral activities of medicinal plants especially *P. spicigera* and *M. indica* against NDV and IBD. As per the literature, numerous medicinal plant products were reported and showed antiviral properties against number of viruses. In this study, our group analyzed anti-viral activities of aqueous leaves extract of *P. spicigera* and *M. indica*. In the initial screening of these medicinal plants, our group used aqueous leaves extract and determined their anti-inflammatory activities against hepatitis B surface antigen (12). It was easier to screen many pathogenic samples i.e. NDV and IBD. In this study, treatment of aqueous leaves extract on human PBMC, which is exposed with NDV and/or IBD in two different sets of experiments, showed anti-

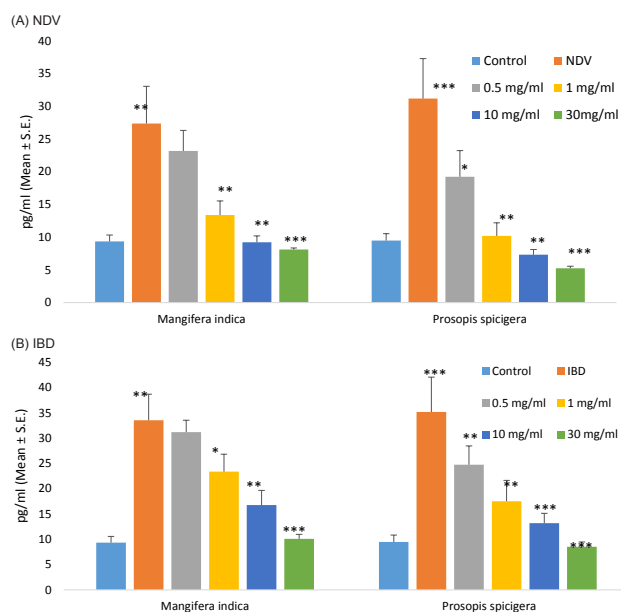


Figure 2. TNF alpha production from human PBMC cell culture supernatant. Human PBMC (10^5 cells/well) were cultured with NDV or IBD along with variable doses of aqueous leaves extract or NDV/IBD alone. After 48 hours, supernatant was collected for the estimation of Th1 cytokine (TNF alpha). Values are expressed as mean \pm SE. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ as compared to control.

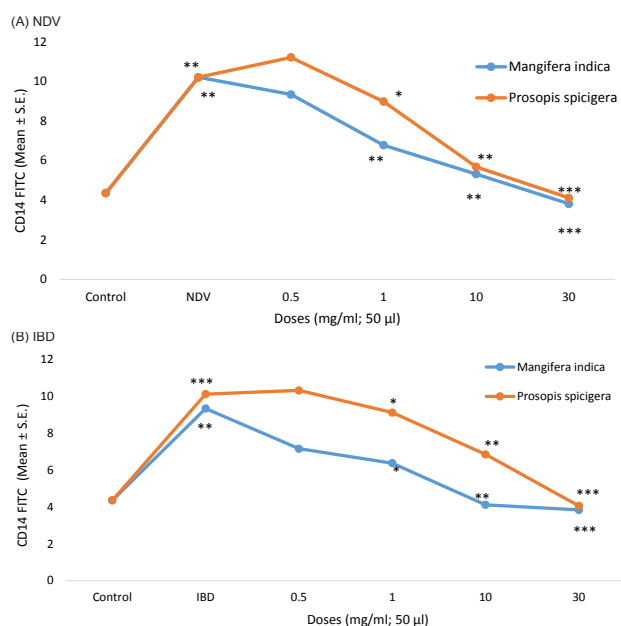


Figure 3. CD14 monocyte surface marker. Flow cytometric analysis of aqueous leaves extract (0.5–30 mg/ml; 50 µL) extracted from *Mangifera indica* and *Prosopis spicigera* for determine its effect (CD14, monocyte surface marker) in human PBMC against NDV and IBD. Data acquisition of 10000 events and fraction or separation of cell populations representing different phenotypes analysed using cell quest software. Values are expressed as mean \pm SE. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

viral properties because of rapid decline in proliferation rate at higher doses. In addition, NDV and IBD viruses were not able to mutate with the medicinal plants because of the presence of secondary metabolites (terpenoids, fla-

vonoids, saponin and phenolics). There is only one major difference on the basis of phytochemical investigation, glycosides are absent in *P. spicigera* and alkaloids in *M. indica*.

For these studies, basic parameters were selected i.e. proliferation assay, TNF alpha production and CD14 monocyte surface marker, that are totally interconnected with our immune system and provide general information about aqueous leaves extract of *P. spicigera* and *M. indica*. In this regard, NDV and IBD are two important viruses related to poultry farm and showed enhancement in the number of monocytes count (CD14 surface marker), proliferation rate and TNF alpha production. The results indicate that aqueous leaves extracts of *P. spicigera* and *M. indica* have a dosage-dependent effect and could significantly reduce the CD14 count in human PBMC exposed to NDV and IBD viruses. The results of our immunopharmacological studies on human PBMC after exposing with NDV and IBD suggested that the aqueous leaves extracts had antiviral effects on human PBMC.

Currently, TNF alpha inhibitors from natural origins are generally used for the treatment of various inflammatory disorders. Enhancement of TNF alpha is totally associated with diabetes, arthritis etc (13). Currently, only protein based drugs are available for the clinical inhibition of TNF alpha activity. So, overall results indicated that aqueous leaves extract of *Prosopis spicigera* and *Mangifera indica* had anti-viral activities against NDV and IBD.

Conclusion

These studies suggest that aqueous leaves extract of *Prosopis spicigera* and *Mangifera indica* significantly inhibits proliferation rate, TNF alpha production and CD14 monocyte surface marker. Further investigations of the aqueous leaves extract should be done through in vivo assessment for immunopharmacological (anti-viral) studies in mice models with identification of the major active candidates responsible for anti-microbial activities.

Authors' contributions

Dr Gupta designed the study, wrote the protocol and interpreted the data. Dr Gupta anchored the field of the study, gathered the initial data and performed preliminary data analysis. Dr Gupta and Dr Chaphalkar managed the literature searches and produced the initial draft. Both the authors read and approved the final manuscript.

Conflict of interests

The authors declared no competing interests.

Ethical considerations

Ethical issues (including plagiarism, misconduct, data fabrication, falsification, double publication or submission and redundancy) were completely observed by authors.

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