



IMMUNOMODULATORY EFFECT OF FLAVONOID FROM MUSA PARADISIACA ON HUMAN WHOLE BLOOD AGAINST SPECIFIC PROTEIN ANTIGEN

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ABSTRACT

Objective- The objective of our study is to investigate its immunomodulatory properties of flavonoid isolated from peels of *Musa paradisiaca*. **Methods-** Immunological activities of flavonoid were evaluated on human whole blood against specific protein (rubella vaccine) antigen using various assays i.e. Elisa, proliferation and total cellular content. **Results-** In this study, flavonoids isolated from peel powder of *Musa paradisiaca* and determined its immunomodulatory potential against rubella vaccine antigen. The results showed that these flavonoids showed dose dependent increased in antibody titre at higher doses as compared to control. In addition, these flavonoids also showed enhancement in proliferation assay and total cellular content at lower doses. **Conclusion-** Overall, the results of the present study indicate that the flavonoid isolated from the peels of *Musa paradisiaca* having potent immunomodulatory activities.

KEYWORDS: *Musa paradisiaca*; flavonoid; antibody; proliferation; cellular.

INTRODUCTION

As per the literature, these polyphenols (> 8000 phenolic structures) are also called as phenolic compounds and these are widely distributed group in plant kingdom.^[1] Structurally, these polyphenolic compounds are totally differ as well as varied from simple molecules (i.e. phenolic acids).^[1, 2] One of the well-known phenolics that are reported in human diet i.e. polyphenols and flavonoids. So, these phenolic compounds includes one aromatic ring along with one or more hydroxyl groups and it may be classified as flavonoids and non-flavonoids.^[1-3] One of the major group of polyphenols i.e. Flavonoids (polyphenolic compounds) that are included under the broad category of secondary metabolites and these are categorized on the basis of its chemical structure (i.e. flavonols, flavones, flavanones, isoflavones, catechins, anthocyanidins and chalcones). As per the literature survey, more than 4,000 flavonoids are reported till now and many of them are reported in fruits, vegetables and beverages.^[1-5] Recently, researchers showed considerable interest on flavonoids because of its medicinal properties with respect to human health and reported as antimicrobial, anti-tumor, antioxidant activity etc.^[6, 7]

One of the well-known fruits i.e. *Musa paradisiaca* (banana; hybrid between *Musa acuminata* and *Musa balbisiana*) are reported in India especially Maharashtra. These fruits especially *Musa paradisiaca* reported as sweet, astringent, anthelmintic, aphrodisiac, antiscorbutic, demulcent and tonic.^[8-10] In this regard, number of anti-inflammatory drugs that are available and is associated with various side effects i.e. gastric irritation, anorexia, diarrhoea, rashes, stomach ulcers, GIT bleeding, kidney damage, liver damage, hypertension etc.^[11] Till now, there is no scientific data related to secondary metabolites i.e. flavonoids extracted from the peels of *Musa paradisiaca* for determining its immunomodulatory activity against specific protein antigen.

MATERIALS AND METHODS

Collection of plant material

Fresh unripe fruit (*Musa paradisiaca*) were collected from Vidya Pratishthan's School of Biotechnology (VSBT), Baramati, Maharashtra, India. Peel of *Musa paradisiaca* (n =10) were taken and then washed firstly with alcohol and then with sterile distilled water pertaining to remove dust particles. Afterwards, peels

were cut into small pieces and dried in a shady area for 1 week. Afterwards, peels were macerated in liquid nitrogen and prepared fine powder for isolation of flavonoids.

Extraction of flavonoids from *Musa paradisiaca*

For flavonoid extraction, 1 g of peel powder of *Musa paradisiaca* were macerated in liquid nitrogen (using mortar and pestle) to prepare fine powder and refluxed with 10 ml of 80% methanol for 2-3 h at 100°C. Thereafter, peel solution containing powder was cooled down and proceed for filtration (using Whatman filter paper). After filtration, add distilled water and ethyl acetate in the ratio of 2:1 in collected filtrate of *Musa paradisiaca*. These samples were incubated for 4-5 at room temperature. After incubation, two different layers were observed i.e. upper layer containing ethyl acetate was removed and then dried peels powder in the form of flavonoids. For confirmation related to flavonoid content using lead acetate solution, yellow precipitation appears. This yellow colour precipitation indicated the presence of flavonoid content.^[5-7]

Phytochemical screening

The phytochemicals screening of *Musa paradisiaca* extract was carried out using standard procedures and revealed the presence of flavonoids, polyphenols, saponins and glycosides.

ELISA

In this study, we determined the antibody (IgG) titre of flavonoids isolated from the peels of *Musa paradisiaca* against Rubella vaccine (Serum institute of India Limited, India) antigen. For these studies, coated Elisa plates (Himedia, India) with Rubella vaccine (1:500 dilution, 100 µl) in high binding 96 well plate (Himedia, 96 well plate, India). Wash Elisa plate with PBS and add blocking buffer (1 % BSA, 100 µl). Incubate the plate for 1 h at room temperature and then add variable concentration of flavonoids after washing with PBS and then incubate. After 4h incubation, add secondary antibody (i.e. horse anti-serum; 1:1000 dilution; 100 µl) and incubate it for 1 h at carbon dioxide incubator. After incubation, substrate solution (trimethyl benizidine, TMB, 100 µl) was added and keep it in dark for 15 minutes and then add stop solution (1N H₂SO₄). The optical density was measured at 450 nm.^[12]

Estimation of total cellular content and proliferation assay using rubella vaccine

Immunopharmacological studies of flavonoids isolated from the peels of *Musa paradisiaca* were determined in non-infected (normal) human whole blood samples (n=5; 100 µl) against specific protein (rubella vaccine) antigen. In this study, normal human blood samples were exposed to variable concentration of flavonoids along with rubella vaccine (1:1000 dilution, 10 µl) for determined its immunomodulatory activity. Rubella vaccine used as standard. Incubate these samples in 96 well plate for 24 h

incubation in carbon dioxide incubator. This experiment was performed in duplicates.

In one set, after incubation centrifuge the samples at 10,000 rpm and estimated its total cellular content using Nanodrop method. In second set, MTT solution (5 mg/ml, 10 µl) was added after incubation period and then incubate it for another 4 h at carbon dioxide incubator. Finally, centrifuging the samples after incubation, fresh formazan crystals were settled at the bottom and these crystals were dissolved in dimethyl sulphoxide (DMSO) in a final volume of 0.2 ml. The optical density (OD) was measured at 570 nm.^[13, 14]

STATISTICAL ANALYSIS

The difference between control and variable doses of flavonoids isolated from the peels of *Musa paradisiaca* is determined through one way ANOVA test (Bonferroni multiple comparison test).

RESULTS

ELISA

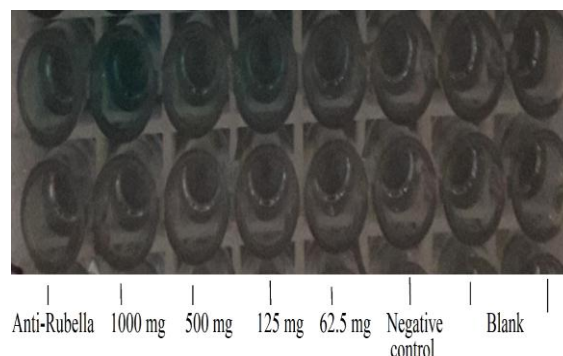
The results of these immunological studies revealed that flavonoids showed dose dependent increased in antibody titre at higher doses (Fig.1).

Total cellular content

Immunological studies were conducted on human whole blood against rubella vaccine as shown in Fig.2. The results showed that these flavonoids at lower doses showed enhancement in total cellular content as compared to control. Rubella vaccine used as standard for these studies and also showed enhancement in total cellular content.

Proliferation assay

These studies were conducted in order to determine its proliferation rate against rubella vaccine antigen. The results of these studies showed that flavonoids at lower doses showed enhancement in proliferation rate as compared to control.



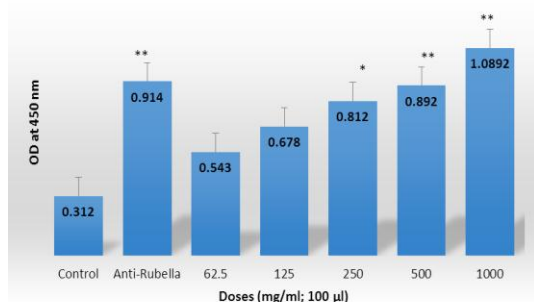


Fig.1. ELISA assay. Indirect Elisa was performed using Rubella vaccine (1:500 dilution) as coating antigen. Variable concentration of flavonoids were added and determined its Anti-rubella antibody titre. Horse anti-serum used as secondary antibody and absorbance in the form of optical density measured at 450 nm.

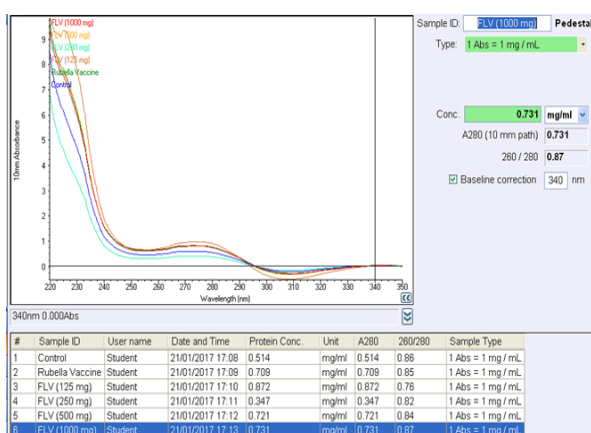


Fig.2. Effect of variable doses of flavonoid on total cellular content. Human whole blood were cultured with variable doses of flavonoids. Total cellular content was measured after high speed centrifugation and collect supernatant for estimation of total cellular content.

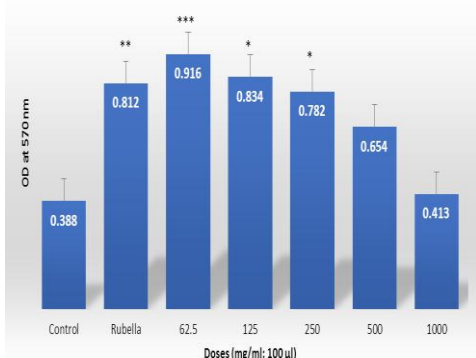


Fig.3. Proliferation assay. Normal human blood samples were exposed to variable concentration of flavonoids along with rubella vaccine (1:1000 dilution, 10 µl). Incubate these samples in 96 well plate for 24 h incubation in carbon dioxide incubator and determined its proliferation assay using MTT. Values are expressed as Mean $\pm$ S.E. The difference between control and variable doses of aqueous extract is controlled by one

way ANOVA test (Bonferroni multiple comparison test). * $p < 0.05$; ** $p < 0.01$ and *** $p < 0.001$

DISCUSSION

Immunomodulatory agents extracted from various medicinal plant products that are reported and it's totally free from side effects and which can be administered for long duration to obtain a continuous immune activation are highly desirable for the prevention of animal and human diseases.^[15] Lot of chemically derived compound that are reported and showed immunomodulatory activity i.e. levamisole, glucan, IL-2, interferon's, etc. which are used in combination with cisplatin, adiramycin, 5-fluorourcil, etc. against many types of carcinomas. But most of them have side effects namely fever, myalgias fatigue, etc.^[16] In this regard, researchers still focused on medicinal plant products which is totally free of side effects and tested against specific and non-specific antigen.

Musa species, antique fruit crop of the world and is commonly known as apple of the paradise. It is one of the cheapest fruit in all over the world and is extensively consumed by the people. Basically, this plant can live or survive for more than 2 years and its shoot can flower only once and ultimately will die after production of fruit.^[8-10] As per the literature, India is the largest producer of banana in the world and major banana growing states are in the northeastern and southern parts of the country. In our country i.e. India, Tamil Nadu (state) has the largest area followed by Maharashtra and Karnataka. Tamil Nadu also ranks first in production, followed by Maharashtra, but the highest productivity is recorded in Maharashtra, followed by Tamil Nadu and Madhya Pradesh.^[8-10] In this regard, we focused on its immunopharmacological activities especially peels and determined its immunomodulatory potential against specific protein antigen.

This study was carried out in order to find out the possible immunomodulatory activity of crude flavonoid isolated from the peels of *Musa paradisiaca*. For these studies, antigen (rubella vaccine)-specific antibody production is the major component for determining humoral immunity. In this study, we evaluated the effects of flavonoid using variable doses on humoral immunity through ELISA test against rubella vaccine antigen. For experimental purpose, various methods are applied for evaluating humoral immunity i.e. antibody titer against rubella vaccine. In other words, antibody titer is the direct way to evaluate humoral immunity. So, Elisa technique is one of the most preferable method for determining antibody production against rubella vaccine antigen. The results showed clear cut evidence that flavonoids at higher doses showed enhancement in antibody production against rubella vaccine antigen. In other words, these metabolites especially flavonoids from *Musa paradisiaca* are used as raw material for extraction that were used as adjuvant or immunostimulator for vaccine antigen. Similarly,

flavonoid at various concentrations on normal human lymphocytes and exposed to rubella vaccine for determined its proliferation using MTT assay and also determined total cellular content. From these studies, data revealed that flavonoid isolated from *Musa paradisiaca* was found to enhance the proliferation rate of lymphocytes and total cellular content at lower doses. The results of this experiment showed that the flavonoid isolated from *Musa paradisiaca* exhibited higher growth stimulations even at low concentrations, indicating its potential as an effective immunomodulatory compound.

Further investigations are still under progress in order to find out the effect of crude flavonoid on other immune parameters such as macrophage activity and natural killer (NK) cell activity and to characterize and elucidate the structure of the active principle behind the activity.

CONCLUSION

One of the most commonly plant i.e. *Musa paradisiaca*, used as food and medicine. Several immunopharmacological studies were conducted and proved that this medicinal plant is very effective in curing various animal and human diseases through its medicinally active secondary metabolites especially flavonoids. This study is totally confirmed its immunomodulatory activity of flavonoid isolated from peel of *Musa paradisiaca* against specific protein antigen.

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This work was carried out in collaboration between four authors. AG designed the study, wrote the protocol and interpreted the data where AP anchored the field study, gathered the initial data related to his M.Sc. Microbiology project work under AG guidance and performed preliminary data analysis. AG, AP, SK and BS managed the literature searches whereas AG and AP produced the initial draft. The final manuscript has been read and approved by all authors.

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