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Immunosuppressive and antidiabetic activity of polyphenols from *Musa paradisiaca*

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Abstract
The objective of our study is to examine its immunosuppressive and antidiabetic potential of polyphenols extracted from the peels of *Musa paradisiaca*. In this study, indirect ELISA was performed in order to determine its immunogenicity against rubella vaccine antigen (Serum institute of India Limited, India) using variable doses of polyphenols (0.312 - 5 mg/ml; 100 µl). In addition, studies were also conducted on normal human whole blood samples in order to examine its proliferation against rubella vaccine antigen (1:500 dilution; 10 µl). Similarly, polyphenols of variable concentration were also tested in diabetic human whole blood samples and measured total cellular content. The results showed that polyphenols at higher doses (5 mg/ml; 100 µl) showed decline in antibody production (immunogenicity) and proliferation assay (rubella vaccine) in human whole blood samples. In contrast, polyphenols also showed reduction in total cellular content in diabetic human whole blood sample at a very low concentration (0.312 mg/ml; 100 µl). Overall, the results indicate that polyphenols extracted from the peels of *Musa paradisiaca* showed immunosuppressive and anti-diabetic effect.

Keywords: polyphenols, *musa paradisiaca*, immunosuppressive, diabetic

1. Introduction
One of the types of immunomodulators i.e. Immunosuppressant that are used actually in the form of drugs which is responsible for suppressing the strength of the body's immunity [1]. Most of these immunosuppressive drugs are generally used to make the body less likely to reject a transplanted organ such as a liver, heart or kidney. These drugs are called anti-rejection drugs [2]. Other immunosuppressant drugs are often used to treat autoimmune disorders such as lupus, psoriasis and rheumatoid arthritis. The most familiar example of immunosuppressant drugs are generally used to treat autoimmune diseases [3]. These immunosuppressant drugs are involved in order to weaken the immune system, they suppress this reaction. This helps reduce the impact of the autoimmune disease on the body [4].
Infectious diseases are considered as one of the most common and serious complication related to diabetes mellitus [5]. Now a day, number of cases related to diabetes increasingly day by day. As per the literature, different types of immunosuppressant medications are available e.g. corticosteroids, tacrolimus, cyclosporine, mycophenolate, azathioprine, sirolimus etc. but it showed several side effects [6]. One of the major side effects of corticosteroids i.e. osteoporosis (density of bones is decreased, increasing the risk for fractures) [7]. In this regard, researchers focused on immunosuppressive agents extracted from fruit materials or medicinal plant products.
Musa paradisiaca, commonly known as banana and is reported as a hybrid between *Musa acuminata* and *Musa balbisiana*. This fruit are widely distributed in all over the world including India especially Maharashtra [8-10]. As per the literature, this fruit showed many medicinal properties i.e. anthelmintic, aphrodisiac, antiscorbutic etc. [8-10] In the present study, we focused on secondary metabolites especially polyphenols extracted from the peels of *Musa paradisiaca* and determined its immunosuppressive (against specific protein antigen) and anti-diabetic potential in human whole blood samples.

2. Materials and methods
2.1 Collection of plant material
Fresh unripe fruit (*Musa paradisiaca*) were collected from Vidya Pratishthan's School of Biotechnology (VSBT), Baramati, Maharashtra, India. For these studies, peels of *Musa paradisiaca* (n =10) were collected and washed firstly with alcohol (100 %) and then with sterile distilled water in order 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 polyphenols.

2.2 Extraction of polyphenols
In this study, fresh peels of *Musa paradisiaca* (1 g) were macerated using 20 ml of solvent (n- ethanol) a maceration procedure was employed and the extraction time in this case was 5 h. The extracts were filtered and concentrated until dryness.

2.3 ELISA
In this study, we determined the antibody (IgG) titre of polyphenols isolated from the peels of *Musa paradisiaca* against rubella vaccine antigen. For these studies, coated Elisa plates with Rubella vaccine (1:500 dilution, 100 µl) in high binding 96 well plate. Wash Elisa plate with PBS (pH 7.4) and

add blocking buffer (1 % BSA, 100 µl). Incubate the plate for 1 h at room temperature and then add variable concentration of polyphenols from *Musa paradisiaca* 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 H_2SO_4). The optical density was measured at 450 nm [11].

2.4 Immunological activities for determining immunosuppressive and anti-diabetic properties

Non-infected (control) and infected (Diabetic) human whole blood samples were received from our local pathology lab (Mangal laboratory) in Baramati, District Pune, Maharashtra. In this study, we examined the polyphenol content in normal human whole blood samples in order to examine its immunomodulatory effect against rubella vaccine antigen and also observed its activity in diabetic human whole blood samples. For anti-diabetic studies, huminsulin 50/50 used as standard whereas in case of immunomodulatory effect, rubella vaccine used as standard.

In this study, control and diabetic human whole blood (25 µl) samples was taken and incubate with variable concentration of polyphenols extracted from the peels of *Musa paradisiaca* in presence or absence of rubella vaccine for 2 h at 37°C carbon dioxide incubator. This experiment was performed in two different sets i.e. one set for immunomodulatory activity and second for anti-diabetic effect (total cellular content).

In immunomodulatory studies, after incubation with rubella vaccine along with variable concentration of polyphenols, add red cell lysis buffer (1 ml) with gentle mixing followed by incubation for 8-10 min. Theraftter, add MTT solution (5 mg/ml, 5 µl) and then incubate it for another 3-4 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 [12].

In diabetic studies, after incubation with variable concentration of polyphenols, add red cell lysis buffer (1 ml) with gentle mixing followed by incubation for 8-10 min. finally, supernatant was aspirated after centrifuging and washed two times with PBS, pH 7.4. Finally, pellet dissolved in PBS (pH 7.4) and examined its optical density (OD) at 570 nm using UV-spectrophotometer [13].

2.5 Statistical analysis

The difference between control and treated groups of polyphenols extracted from the peels of *Musa paradisiaca* is determined through Bonferroni multiple comparison test (One way ANOVA test).

3. Results

3.1 ELISA

The results showed that polyphenols showed no enhancement in antibody titre at higher doses. Overall, the results showed that polyphenols from *Musa paradisiaca* showed immunosuppressive effect against rubella vaccine antigen (Fig.1).

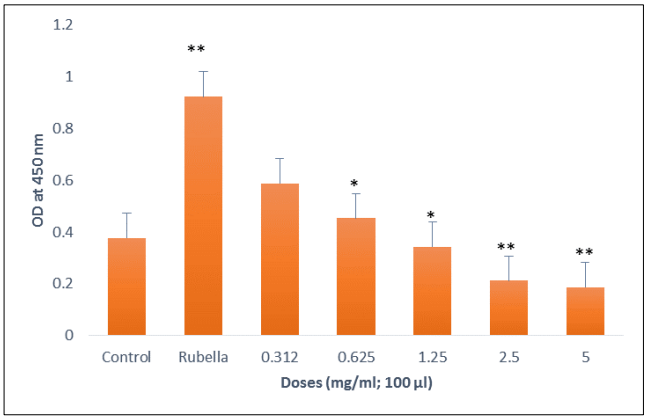

Fig 1: ELISA assay. Indirect ELISA was assayed using rubella vaccine as coating antigen using variable doses of polyphenols for determining antibody titre. Horse anti-serum used as secondary antibody. The difference between control and variable doses of polyphenols is determined through one way ANOVA test (Bonferroni multiple comparison test). *P < 0.05; **P < 0.01 and ***P < 0.001

3.2 Proliferation assay for determining immunomodulatory activity

The results of these studies showed that polyphenols at higher doses showed declined in proliferation rate against rubella vaccine antigen. From these studies it is confirmed that polyphenols showed immunosuppressive effect (Fig.2).

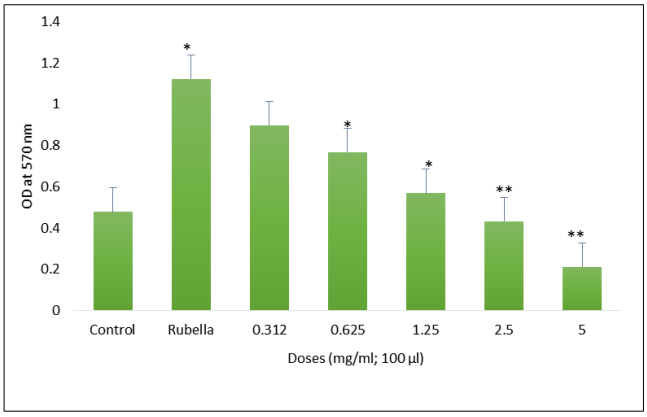

Fig 2: Proliferation assay. Lysed human whole blood (non-infected) were cultured with variable concentration of polyphenols extracted from the peels of *Musa paradisiaca* in presence of rubella vaccine. After incubation, centrifuge the samples and add MTT solution (5 mg/ml, 10 µl). Fresh formazan crystals were appeared and settled at the bottom and then finally dissolved in dimethyl sulphoxide (DMSO) in a final volume of 0.2 ml. The optical density (OD) was measured at 570 nm. The difference between control and variable doses of protein is determined through one way ANOVA test (Bonferroni multiple comparison test).
*P < 0.05; **P < 0.01 and ***P < 0.001

3.3 Antidiabetic activity

Human diabetic blood samples were collected in order to analysing its antidiabetic effect using polyphenols extracted from the peels of *Musa paradisiaca*. The results showed that polyphenols at lower doses showed antidiabetic effect as compared to control. Huminsulin 50/50 used as standard and showed drastic declined in total cellular content as compared to control (Fig.3).

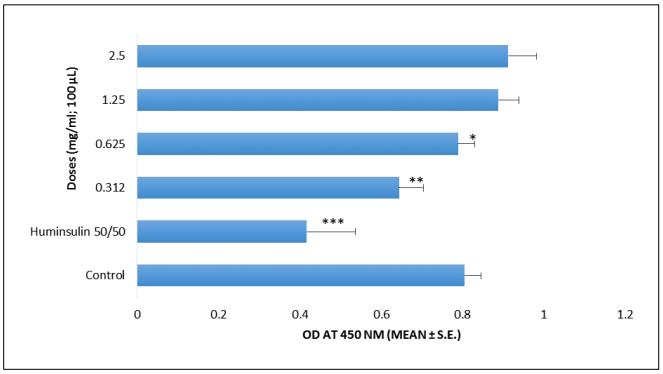

Fig 3: Effect of variable doses of polyphenols on total cellular content in human diabetic blood samples. Lysed diabetic human whole blood were cultured with variable doses of polyphenols extracted from the peels of *Musa paradisiaca*. Total cellular content was measured after high speed centrifugation and collect supernatant for estimation of total cellular content. Values are expressed as Mean ± S.E. The difference between control and variable doses of medicinal plant products is controlled by one way ANOVA test (Bonferroni multiple comparison test). *P<0.05; **P<0.01 and ***P<0.001

4. Discussion

As per the literature, medicinal plant products especially in the form of flavonoids, terpenoids, alkaloids etc. have been reported in order to modulate immune system whether it showed immunostimulatory or immunosuppressive effect [1]. In this regard, we examined the effects of these polyphenols extracted from *Musa paradisiaca* on T cell proliferation against rubella vaccine. From these studies, we found that polyphenols exerted an immunosuppressive effect on T cell proliferation. Similarly, Elisa experiments were performed in order to analysed its antibody production against rubella vaccine antigen and showed that polyphenols at higher doses showed decline in antibody production.

For the last two decades, researchers focused on newer immunosuppressive drugs that directly targeting the co-stimulatory pathways or molecules and avoiding toxicities which is associated with steroids and calcineurin inhibitors i.e. cyclosporine and tacrolimus [14]. Actually, main differences in all these immunosuppressive compounds (naturally or synthetically) were reported on the basis of its mechanism of action e.g. cyclosporine and betamethasone which inhibits T cell proliferation. In this regard, we found that polyphenols not only inhibited T cell population in lysed human whole blood containing rubella vaccine and also showed decline in antibody titre at higher doses *in vitro* (against rubella vaccine) as compared to control. Overall the data indicates its immunosuppressive effect in polyphenols extracted from the peels of *Musa paradisiaca*.

Diabetes, metabolic disorder characterized through chronic hyperglycemic condition resulting from defects in insulin secretion, insulin action or both. Diabetes is of three types i.e. Type 1 diabetes (autoimmune reaction to proteins of the islets cells of the pancreas); Type 2 diabetes (combination of genetic factors related to impaired insulin secretion, insulin resistance and environmental factors such as obesity, overeating, lack of exercise and stress, as well as aging) and gestational diabetes [5, 15]. Lot of antidiabetic drugs that are already available in the market in the form of capsules (Type 2) and formulation (Type 1). As per the literature, number of medicinal plant products showed its potential against cardiovascular disease i.e. diabetes (Type 1 and 2 diabetes; silent killer). Lot of research work is already done related to diabetes and number of medicinal plants are reported and showed anti-diabetic activity e.g. *Syzygium cumini, Emblica officinalis* etc. [5, 15] In continuation of these studies, polyphenols extracted from the peels of *Musa paradisiaca* on human diabetic whole blood samples and the results showed that these polyphenols at a very lower concentration reduced the total cellular content as compared to Huminsulin 50/50 and control. In other words, polyphenols from *Musa paradisiaca* are the major source for drug development. In an effort to reduce the burden of cardiovascular disease i.e. diabetes with Huminsulin 50/50 for Type 1 diabetes which is already available in the market but we need plant based formulation which is effective agent for next generation and considered as potent antidiabetic agent.

5. Conclusion

In this study, we showed significant immunosuppressive and antidiabetic effect of these polyphenols extracted from the peels of *Musa paradisiaca*. Further experimental and clinical trial based studies were followed in order to investigate its immunosuppressive and antidiabetic properties.

6. Authors contribution

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 dissertation work under AG guidance and performed preliminary data analysis. AG, AP, SK and BS managed the literature searches whereas AG produced the initial draft. The final manuscript has been read and approved by all authors.

7. References

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