REPORT

Report on participation of the ICMR International Fellow (ICMR-IF) in Training/Research abroad.

1. **Name and designation of ICMR- IF:** Dr Debabrata Chanda; Principal Scientist, CSIR-CIMAP, Lucknow
2. **Address:** *In-vivo* Testing Facility, Bioprospection and Product Development Division, CSIR-CIMAP, Lucknow – 226015, UP
3. **Frontline area of research in which training/research was carried out:** Molecular Pharmacology and Drug Discovery
4. **Name & address of Professor and host institute:** Professor Vijay Yechoor; Division of Endocrinology and Metabolism, School of Medicine, University of Pittsburgh, W1055 Biomedical Science Tower, 200 Lothrop Street Pittsburgh, PA 15261, USA

5. **Duration of fellowship with exact date:** From 28th March 2023 to 27th June, 2023 (Including travel time)

6. **Highlights of work conducted:**

   i). **Technique/expertise acquired:**

   A. Undertook mandatory eight course works (80% cutoff marks for clearing the exam) to get eligible for bench work for undergoing training and to conduct planned research. The courses are as follows:

   - Basic Introduction to Biosafety – Biosafety-MYIBC (ID 236706)
   - Working with Genetically Modified Mice in Research Setting (ID 238738)
   - Working with Small Animal (ID 238743)
   - Bloodborne Pathogens for Lab Workers (ID 257929)
   - Working with Mice in Research Settings (ID 64774)
   - Conflict of Interest-COI PHS Regulated Course (ID 61825)
   - Responsible Conduct of Research (ID 104590)
   - Use of Controlled Substances in Basic and Animal Research (ID 189469)

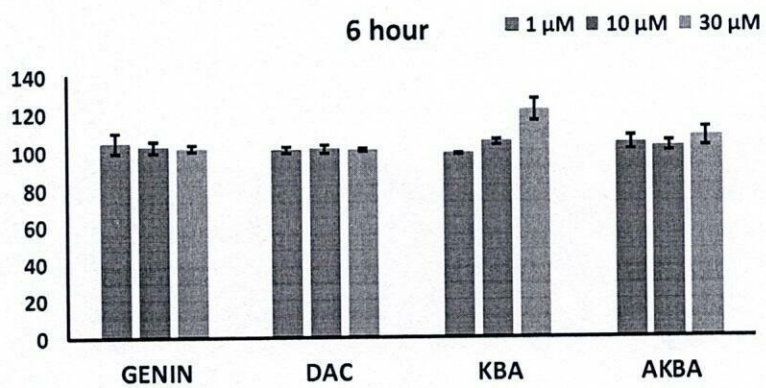
   B. Techniques Learnt: As a short term fellow, priority was given to learn advance techniques and SOP in the area of molecular pharmacology and drug discovery. The techniques learnt with basic exposure are as follows:

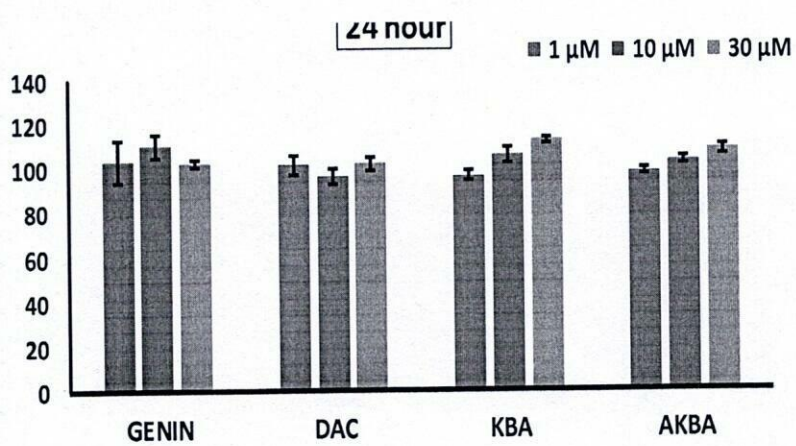
   - Isolation of islets from Pancreas in Mouse
   - Glucose stimulated insulin secretion (GSIS) by β cells of islets
   - GSIS in INS-1 cell lines
   - FACS
   - Distal Pancreatomy (approx. 60%) in mouse
   - Surgical Model of Myocardial infarction in mice: left coronary artery ligation

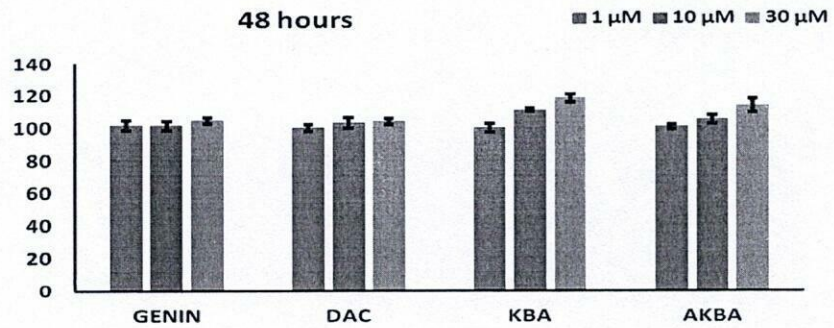
- Differentiation of placenta derived inducible pleuripotent stem cells (iPSCs) into cardiomyocytes with protocols from Stem Cell Technologies
- Pressure volume measurement in through terminal open heart surgery in aortic constriction-induced model of heart failure in mice
- Induction of pulmonary hypertension in mice through surgical banding (partial clamping) of main pulmonary artery

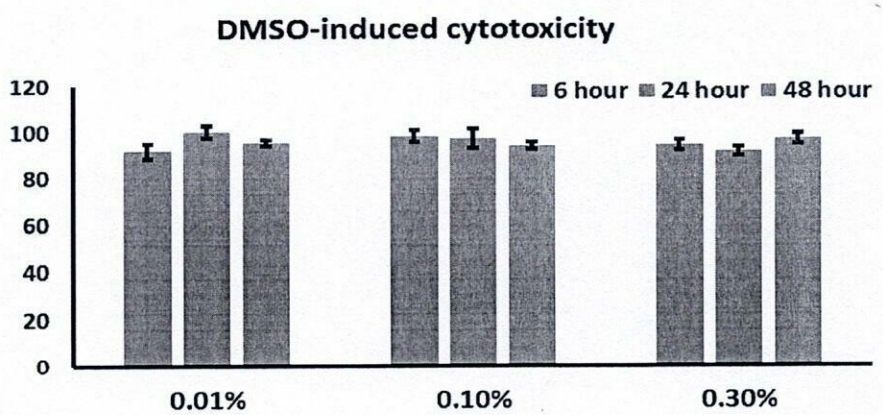
ii) **Research results, including any papers, prepared/submitted for publication:**
In addition to exposure and training with different advanced techniques as stated above, a short study was undertaken in the line of planned grant application with biomarkers from *Gymnema sylvestrae* in INS Cells. The study was planned to evaluate the molecules for cytotoxicity, protection against stress-induced apoptosis/proliferation and expression profile of genes associated with insulin biosynthesis and signaling.

Cytotoxicity of bioactive phytocompounds in INS-2 cells in-vitro after exposure with different concentration and time period:



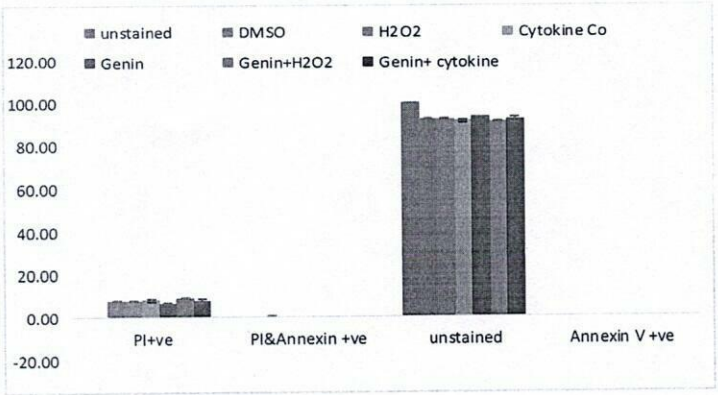


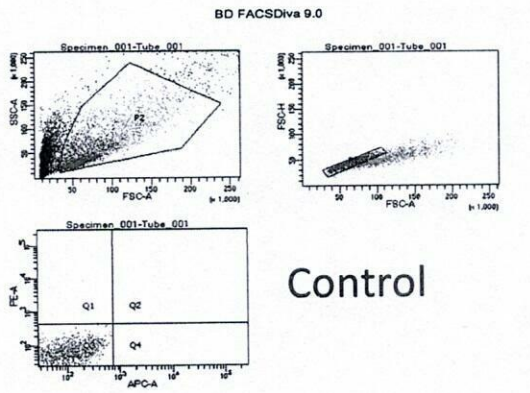


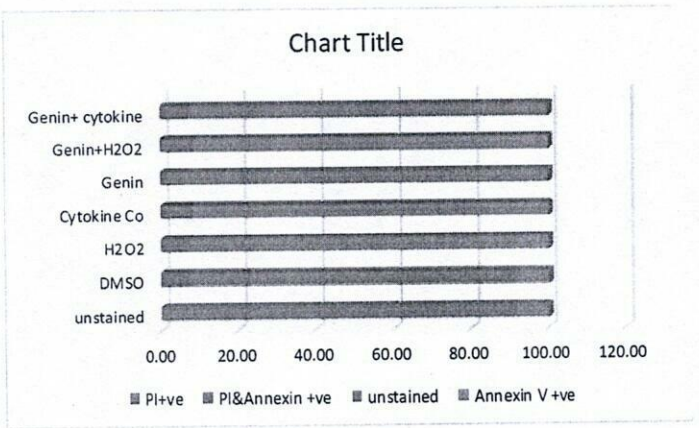


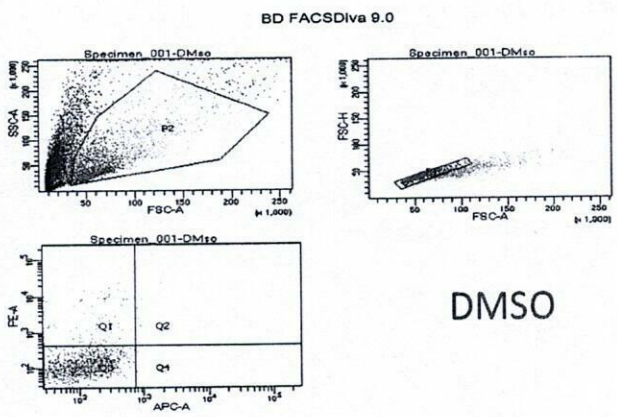
Cells without any treatment was considered control and MTT values were considered 100% (n=3). Non significant changes in all the figure panels. The MTT assay showed that the molecules were nontoxic to cells upto 30µM till 48 hours. Based on our previous studies for vasoreactivity, we found gymnemagenin to be the most potent vasorelaxant and hence was considered for further studies in INS – II cells for exploring its potential in anti-diabetic activity. We have studied its role for protection against cytokine cocktail (IL-1 beta 10ng/ml; IFN gamma 100 ng/ml; TNF alpha 25 ng/ml) and H2O2-idnuced stress in INS-II Cells against apoptosis and cell death using Annexin V and PI staining through FACS analysis and pro or anti proliferative potential using BrdU assay through FACS. The results are presented below.

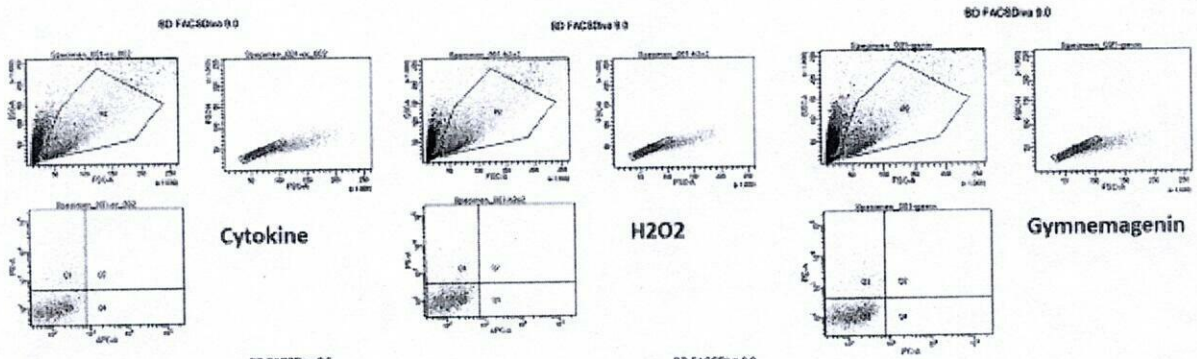
Annexin V and PI assay using FACS:

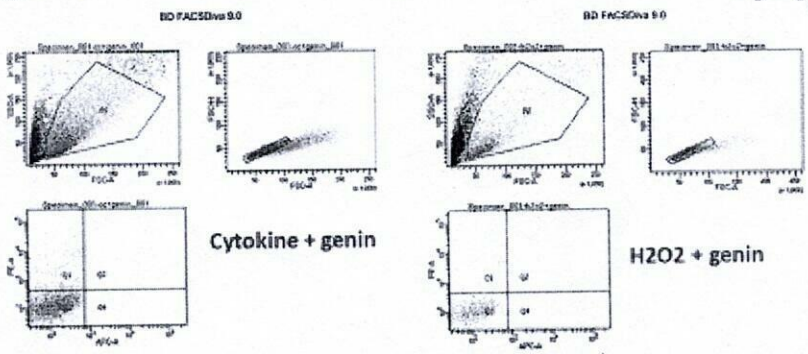




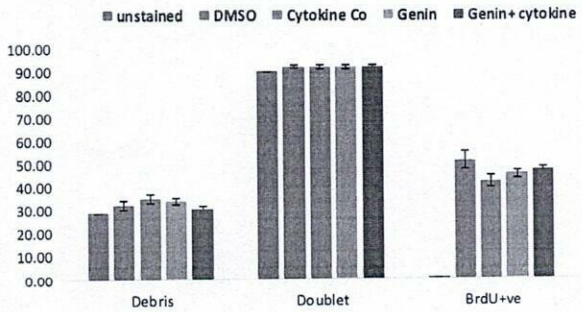


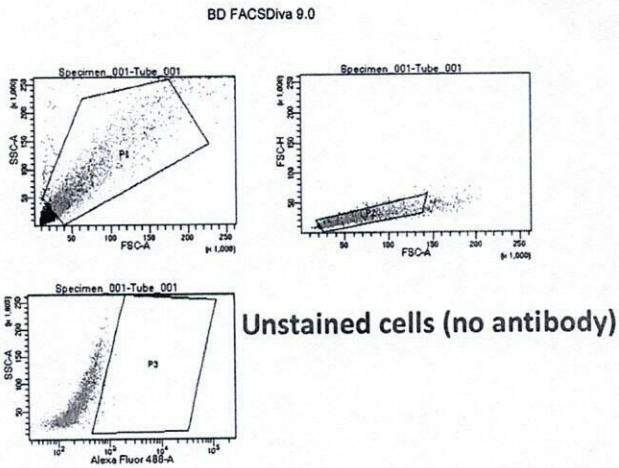




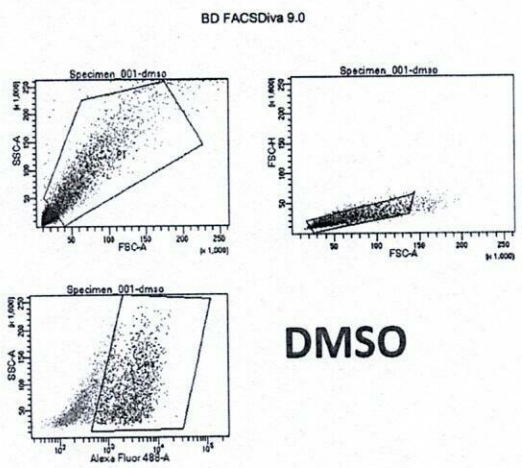


BrdU Assay using FACS:

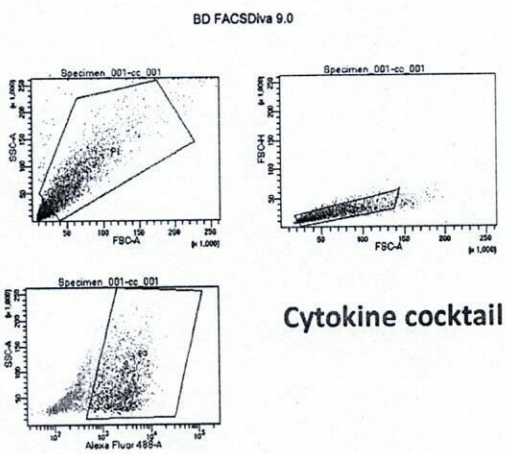




Unstained cells (no antibody)

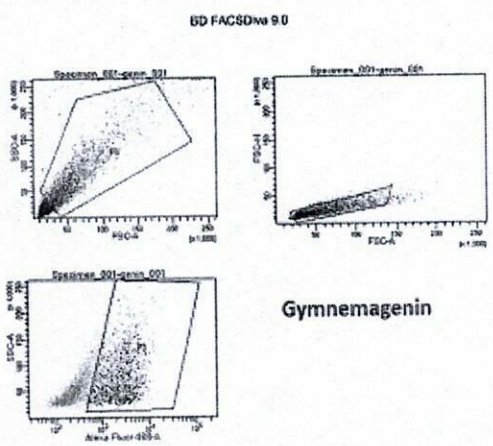


DMSO

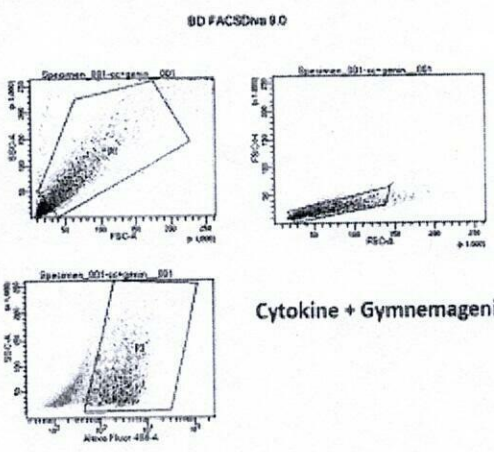


Cytokine cocktail

Evaluation of gymnemagenin against cytokine cocktail-induced cell proliferation in INS-II cells using BrdU assay



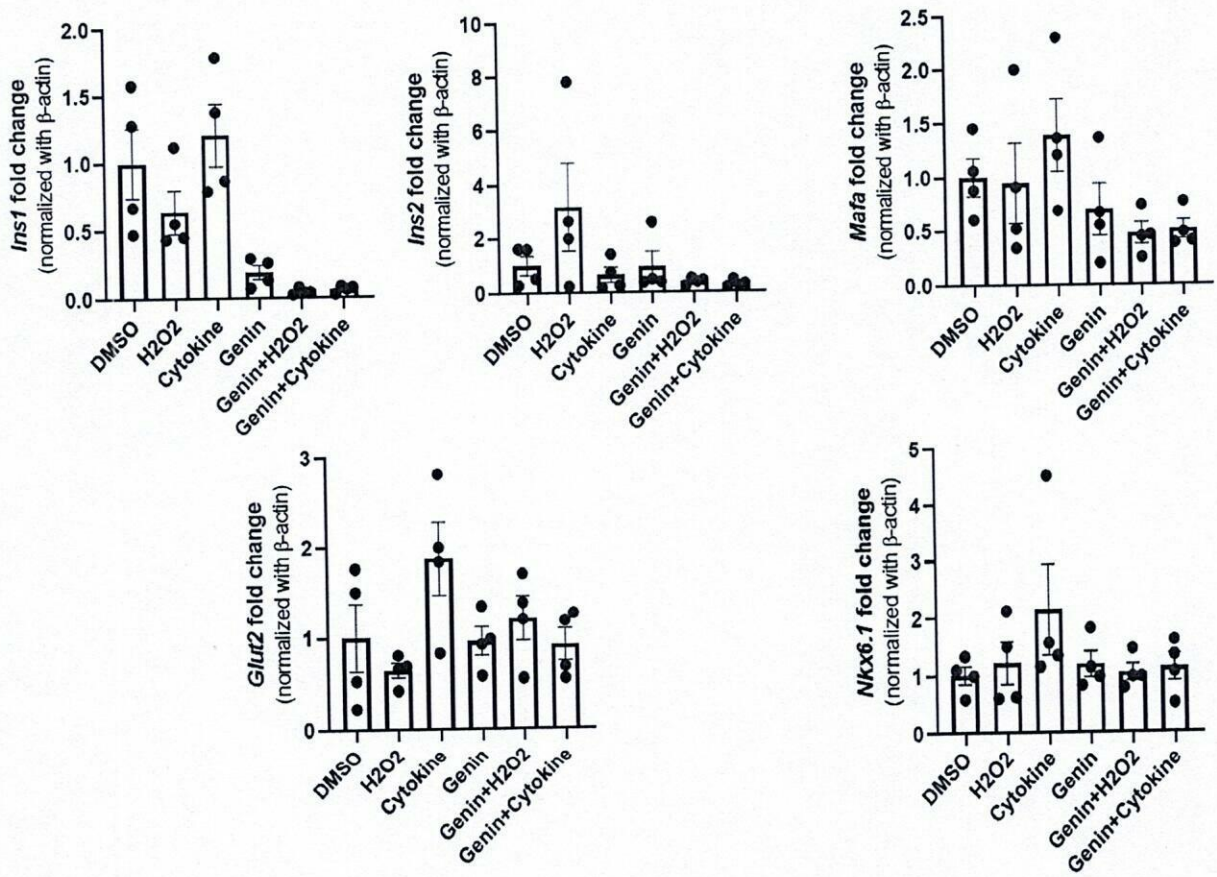
Gymnemagenin



Cytokine + Gymnemagenin

Further expression profile of important target genes like Ins1, Ins2, Mafa, Glu2, Nkx6.1 associated with insulin biosynthesis and signaling were studied in INS-II cells after treatment with gymnemagenin with or without stress induced by cytokine cocktail and or H2O2.

Effect of gymnemagenin against H2O2/cytokine mediated stress induced expression profile of genes associated with insulin signaling/biosynthesis in INS-II Cells:



It was observed from the study that the molecules is nontoxic to the cells and have no apoptotic or proliferative potential in INS-II cells. The molecule has some modulating effects in gene expression. However, it was also observed that neither the cytokine cocktail nor H2O2 could produce any stress or cytotoxicity or proliferation in the INS-II cells. Hence, an optimization study was proposed so that an observable level of stress can be produced with either cytokine cocktail or H2)2 in the cells studied and then preventive potential of the phytomolecule can be studied with 24 hour preincubation with the proposed molecule followed by induction of stress with cytokine cocktail for another 48 hours.

iii) Proposed utilization of the experience in India:

Grant application will be made based on the exposure to different advanced techniques and novel concepts in the area of drug discovery and development.

A. Evaluation of bioactives of medicinal plants with a rich traditional use for rejuvenating potential for efficacy in activations and differentiation of inducible Pluripotent Stem Cells (iPSC):

iPSC are the latest cell based means to treat irreversible organ damage for regenerative treatment. Traditional medicinal plants like aswaganha is well known in Indian System of Medicine for efficacy in irreversible organ damage in brain and heart. The investigator want to address if bioactive molecules from aswagandha can help in inducing differentiation of resting stem cells in vital organs like heart which can help in recovering irreversible damage of heart function in condition like myocardial ischemia.

To simulate the hypothesis in *in-vitro* condition, study will be taken up in iPSC cells for its differentiation into cardiomyocytes where BK channels and wnt pathways plays important role. A host of biomolecules from medicinal plants were found to modulate potassium channel function and wnt signaling and hence is being considered to explore in the differentiation protocol of iPSC cells. Further, the effects of the molecules on the differentiated cardiomyocytes will also be studied for protection against isoproterenol/doxorubicin induced toxicity in cardiomyocytes. The best leads will be taken up for evaluation in isoproterenol/doxorubicin induced toxicity and or against surgically induced MI in rats.

B. Evaluation of biomolecules/standardized extract from Gymnema sylvestrae against cardiometabolic diseases in rodents: Hypertension with diabetes and unitary functional kidney:

- Molecules/extract well known for efficacies against diabetes but have never been studied against hypertension and vasoreactivity. We found decent vasorelaxation and anti-hypertensive activity.
- Diabetes will be induced in SHR Rats/L-NAME-treated hypertensive rats using STZ injection or partial pancreatomy (distal) through surgical procedure
- One kidney will be made non functional by banding (clamping) renal artery
- Pretreatment/post-treatment with extracts will be evaluated for efficacy against hypertension, endothelial function, blood sugar and basic kidney function. Expression profile of pro and anti-inflammatory cytokines, oxidative stress markers, important target genes of cardiometabolic diseases with diabetes will also be studied at mRNA and protein level.

6/7/2023

Signature of ICMR-IF