Form 3

Report for Joint/Usage Research Program for Endocrine/Metabolism (Fiscal Year 2025)

Date:(2026)/(4)/(27)

To Director of Institute for Molecular and Cellular Regulation, Gunma University

Principal Applicant	
Institution	College of Molecular Medicine, Ziauddin University.
Position	Associate Professor
Name	Abdul Hameed

We report on the results of joint research in fiscal year 2025 as below.

（Program No. 24006）

1． Research Title	**Roles of Hispidulin as Potent Insulin Secretagogue and its Mechanism(s)**
2． Purpose and Significance of the research project	**Purpose**: The present research project aims to identify natural, safer, efficacious insulin secretagogues with novel drug targets for the regulation of glucose-dependent insulinotropic effects. Hispidulin was identified as a new natural insulin secretagogue that stimulates insulin release in hyperglycemic condition, and appears to be a better drug candidate than synthetic marketed drugs. In this project, we explored the insulinotropic mechanism(s) of hispidulin in rat INS1-832-13 cells, BH1 human beta cells, mouse islets, and Human islets. **Significance of the research project**: Insulin secretory impairments resulting from β-cell dysfunction are reported to be predominantly involved in Asian type 2 diabetic subjects. Therefore, preserving and improving β-cell mass and insulin secretory function could be the key to prevent and treat type2 diabetes. Unfortunately, limited marketed drugs are available to directly target β-cells, such as glinide and sulfonylurea, that potentiate insulin release irrespective of glucose, hence posing continuous stress on β-cells, worsening the situation further by enhancing β-cells functional impairments, hypoglycemia, and other adverse effects. Therefore, identification of new, safer insulin secretagogues that selectively act under high glucose conditions could reduce the side effects of already marketed drugs. This collaborative project aims to identify safer natural insulin secretagogues that exclusively act on novel molecular drug targets in the presence of high glucose, thereby avoiding overstress on β-cells and offering a promising therapeutic alternative for a large number of diabetic patients. This research project revealed several potential findings that show the promise of addressing the challenges with the current anti-diabetic drug. Importantly, the project has established a valuable scientific link between Japan, a leader in biomedical research, and Pakistan, where scientific capabilities are still under development. Overall, this initiative would play a crucial role in the development of potential anti-diabetic drugs and lay the foundation for future advancements. This research would empower young researchers in Pakistan to pursue further exploration and enhance their research capabilities to develop novel anti-diabetic therapies.

3．Period of The Program	April 1, 2025～ March 31, 2026

4．Project Members

Name	Age	Sex	Affiliation	Position	Role
(Principal Applicant) Abdul Hameed	43	M	Ziauddin University, College of Molecular Medicine	Associate Professor	PI of the project supervised and monitor aspects of the project.
(Research Collaborators) Muhammad, Moazzam Tau-heed	34	M	Ziauddin University, College of Molecular Medicine	Graduate student	Performed experiments under the supervision.
Zikra Khan	25	F	Ziauddin University, College of Molecular Medicine	Graduate student	Performed experiments under supervision.

※If additional space is required, please attach a separate sheet.

5．Collaborating Researcher of IMCR	Name of Laboratory	Diabetes and Metabolic Disorders	Name	Kohichi Matsunaga

6． Research Plans

Hispidulin was tested for its insulinotropic mechanism(s) in INS1-832/13 cells, mouse pancreatic islets, under stimulatory glucose conditions, using agonists and antagonists targeting key insulin signaling pathways. The involvement of AKAP-9 was examined through siRNA-mediated knockdown and protein interaction analysis. Intracellular cAMP and PKAα levels were assessed using ELISA and Western blotting. The impact of AKAP-9 knockdown on hispidulin-induced glucose-stimulated insulin secretion was also evaluated. For the translational value, the effect of hispidulin was also evaluated in BH1 human beta cells and human pancreatic islets.

Research Plans for the Proposed Project:

1. Cell culture and transfection validation
2. Effect of AKAP-9 knockdown and hispidulin treatment on insulin secretion and intracellular PKA activation
3. Role of hispidulin in insulin secretion kinetics using perifusion experiments
4. Role of hispidulin in insulin secretory events using TIRF microscopy
5. Roles of hispidulin on Ca^{2+} kinetics using Confocal microscopy.
6. Effect of hispidulin on insulin secretion in the BH1 human beta-cell line and human islets.
7. Data analysis, result compilation, and report preparation.

Research plans 1, 2, and 6 have been completed. Research plans 3, 4, and 5 will be conducted soon by Kohichi Matsunaga at the Institute for Molecular and Cellular Regulation (IMCR), Gunma University, Japan.

7 . Research results:

Please describe the details of the contribution of the joint research with IMCR in obtaining the results.

In the present study, hispidulin, a natural flavone, was evaluated to explore its insulin secretory mechanism(s). Hispidulin showed insulin secretory potential both in INS1832-13 cells and pancreatic mice islets exclusively at stimulatory glucose concentration. The insulin secretory mechanism(s) of hispidulin was investigated by blocking the key signaling pathways using antagonists. Hispidulin was found to have no considerable effect on intracellular cAMP levels, suggesting that it neither affects cAMP production nor inhibits cAMP hydrolysis. Validating these results, hispidulin was found to have an additive effect in both IBMX and forskolin-induced insulin secretion, suggesting hispidulin's effect is on an alternative signaling target. Furthermore, the PKA-dependent and/or epac2-dependent insulinotropic effect of hispidulin was evaluated using H-89, a PKA inhibitor, and ESI-05, an epac2 inhibitor. ESI-05 has no considerable effect on hispidulin-induced insulin secretion. However, complete inhibition of insulin release was observed by H89, suggesting hispidulin's action in a PKA-dependent manner. Exploring the exclusive PKA-dependent effect of hispidulin was done through the affinity pull-down assays to see the affinity binding with the target proteins. Interestingly, hispidulin specifically associates with AKAP9 in pancreatic β-cells, suggesting a potential role of AKAP9-mediated signaling complexes in the cellular actions of hispidulin, especially in the glucose-stimulated insulin secretion. Surprisingly, AKAP9 knockdown does not inhibit hispidulin-induced insulin secretion, but rather amplifies insulin secretion, highlighting that inhibition of AKAP-9 activity, both by knocking down or inhibiting with hispidulin, is responsible for the amplification of glucose-stimulated insulin secretion, suggesting AKAP9 involvement in the cellular and physiological response of hispidulin. Furthermore, we explored whether the binding of hispidulin with AKAP9 is associated with the PKA-related signaling. We found the differential expression of phosphorylation patterns in multiple proteins specially PKA-responsive substrates, in hispidulin-treated cells compared with untreated cells. To confirm whether all these changes in the substrates are accompanied by the catalytic subunit distribution of PKA, immunoblot analysis of PKA catalytic subunit (PKAcs) was performed. Interestingly, Relative differences in PKAcs were observed in the treatment groups compared to the control. These experimental evidences indicate the association of hispidulin treatment with the AKAP9, hence leading to the altered patterns of PKA substrate phosphorylation and changes in the distribution of PKA catalytic subunits in the β cells. As AKAP-9 is a negative regulator of PKA signalosome, further studies are needed to see the expression of AKAP-9 in diabetic pancreatic islets and how hispidulin interferes with and modulates the AKAP-9 expression to improve the diabetic condition. Most importantly, hispidulin also stimulated insulin secretion exclusively at hyperglycemic conditions in the BH1 human beta-cell line, hence showing promise as a drug candidate for translational use in patients in future. Overall, the results showed that hispidulin modulated the AKAP9-PKA signaling cascade for the stimulation of glucose-induced insulin secretion. This data highlights that hispidulin is a potential anti-diabetic drug candidate, with promising translational value. However, to authenticate the conclusive targets is still needed to investigate. Therefore, we extended this joint research project to perform further advanced experiments, such as insulin secretion kinetics using perifusion experiments, insulin secretory events using TIRF microscopy, intracellular Ca^{2+} measurements, and, most importantly, its evaluation and validation in BH1 human beta-cell line and human islets.

８．Present status of academic conference presentations and research papers associated with the results of the joint research, and exchange of information on the joint research with the collaborating researcher at IMCR.
(As much as possible, please state papers that include the names of the collaborating researcher at IMCR or papers stating that the research was supported by the Joint Research Program with IMCR.
Regarding papers, please send a PDF file together with the report to the email address of the general affairs section of the Institute.) Office of General Affairs: kk-msomu4@jimu.gunma-u.ac.jp

① Please list the publications that include the name of the collaborating researcher from IMCR and send a reprint of each publication to IMCR.
Publication is in progress

② Please list the publications that include a description that the research was supported by the Joint Research Program with IMCR, and send a reprint of each publication to IMCR.
Publication is in progress

③ List up to 3 conferences (name of conference, date of conference, and title of the presentation).

④ **Abdul Hameed*, Kohichi Matsunaga**. Hispidulin is an insulin secretagogue targeting the AKAP9-mediated PKA signaling pathway. 9th International Symposium-Cum-Training Course on Molecular Medicine and Drug Research (MMDR-9), November 24-27, 2025 in Karachi, Pakistan.

⑤ **Abdul Hameed*, Kohichi Matsunaga**. Hispidulin is an insulin secretagogue targeting the AKAP9-mediated PKA signaling pathway. 1st International Conference of Molecular Medicine in Healthcare January 7-8, 2026 in Karachi, Pakistan.

⑥ **Abdul Hameed*, Kohichi Matsunaga.** Hispidulin is an insulin secretagogue targeting the AKAP9-mediated PKA signaling pathway. Medical Conference on Clinical Research (MCCR), during April 2-3, 2026, Karachi, Pakistan (**Won the best Oral presenter award**).

⑦ Exchange of information exchange with collaborating researcher from IMCR (please list main points of communication).