# POSTERS

# Evaluation of protective effect of *Enicostemma littorale* extract against $H_2O_2$ induced apoptosis in islets of Langerhans

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## ABSTRACT

Progressive loss of ß-cells is the major area of concern in type-1 and type-2 diabetes. Oxidative stress is one of the pivotal factors for induction of apoptosis in islets, responsible for reduction of islet mass in diabetic condition. $H_2O_2$-induced apoptosis is a well-established method to study the cytoprotective effect of any antioxidant compound. In our study we confirmed apoptosis in islets of Langerhans (in vitro) upon exposure to $H_2O_2$ by FDA/PI staining, Biochemical assessment of antioxidant enzymes like Catalase, Superoxide dismutase, reduced glutathione and glutathione peroxidase, PARP-1 cleavage, Caspase-3 activation and Comet assay. Reduction in ß-cell function was observed by performing Insulin and Glucagon Immunocytochemistry. Cells exposed to $H_2O_2$ also showed high fluorescence with DCF-DA fluorescence dye; indicator of cell's total oxidative stress. Upon $H_2O_2$ treatment cell's antioxidant defence system goes down and unable to prevent apoptotic event. Pre-treatment with Enicostemma littorale (EL) extract decreases fluorescence in the cells with DCF-DA staining, indicating reduced oxidative stress in these cells and also showed improvement in the antioxidant defence system. This improvement in the antioxidant defence system ameliorated the apoptotic events by decreasing DNA damage and Caspase-3 activity. Also, Insulin and Glucagon presence in immunocytochemistry suggested rescue of ß-cells from oxidative damage. Thus, our results clearly demonstrated that EL extract is able to protect islets of Langerhans from oxidative stress induced apoptosis.

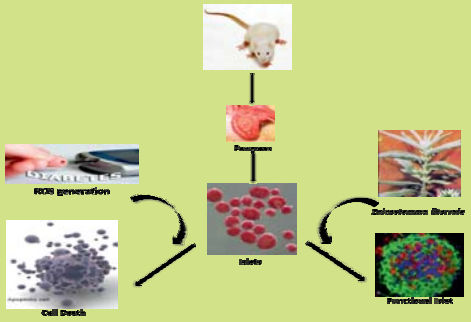
## INTRODUCTION

Generation of ROS can cause cell death either by apoptosis or necrosis. *β*-Cell apoptosis is the defining feature of type-1 and type-2 diabetes and ROS generation during diabetes is a major factor involved.

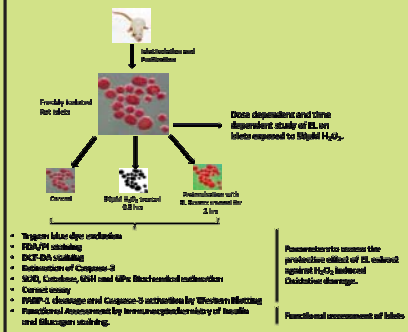
Natural phytochemicals are emerging as potent therapeutic drugs for free radical-mediated diseases. Flavonoids such as butin were investigated for cytoprotective effect against $H_2O_2$-induced cell damage. Isoflavone puerarin was evaluated for its cytoprotective effect against $H_2O_2$ induced rat pancreatic islets damage (Fu-Liang et al., 2006).

*Enicostemma littorale*(EL) Blume is used as a herb for eons by the tribal people of Gujarat. EL contains diversified phytochemicals, some of the important constituents of the plant include betulin, a triterpenoid sapogenin, swertiamarin, a secoirridoid glycoside, monoterpene alkaloids like enicoflavine and gentiocrucine. EL is known to have good antioxidant activity in diabetic rats (Maroo et al., 2003) as well as in newly diagnosed NIDDM patients (Vasu et al., 2003). In this context, the herb *EL* was screened for checking its protection against $H_2O_2$- induced oxidative damage leading to apoptosis on cultured rat pancreatic islets.

## HYPOTHESIS

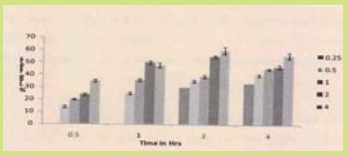


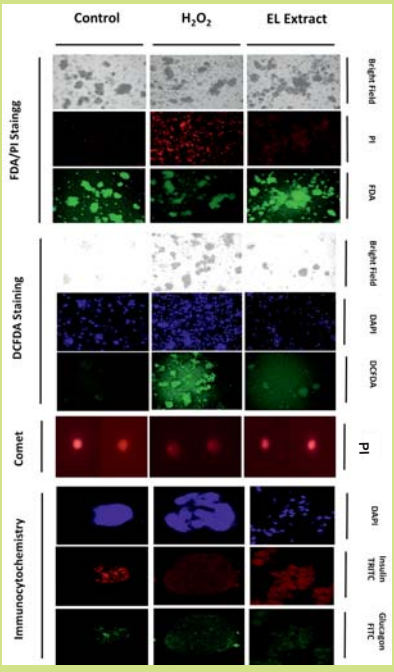

## PLAN OF WORK



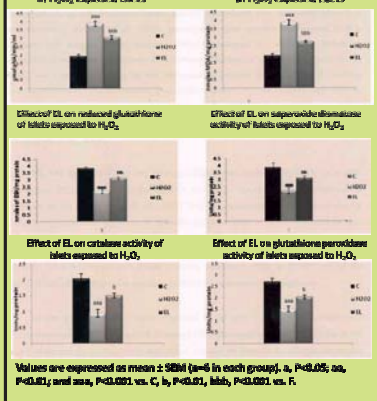

## RESULTS

**Dose dependent and time dependent study of EL extract on islets exposed to 50µM $H_2O_2$ by trypan blue die exclusion.**



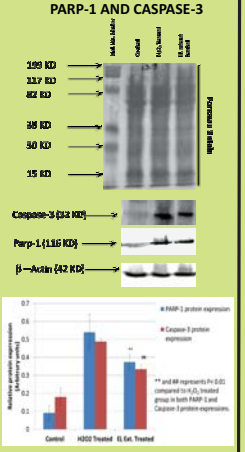




**Biochemical Estimations to assess the protective effect of EL extract against $H_2O_2$ Induced Oxidative Damage**




Values are expressed as mean ± SEM (n=6 in each group). a, P<0.05; aa, P<0.01; and aaa, P<0.001 vs. C, b, P<0.01, bbb, P<0.001 vs. F.

**WESTERN BLOT ANALYSIS OF PARP-1 AND CASPASE-3**




## DISCUSSION

- Diabetes mellitus is characterized by hyperglycemia that promotes ROS generation leading to apoptosis or necrosis of islets.
- EL improves insulin secretion, decreases insulin resistance (Maroo et al., 2002; Vasu et al.,2003) and also decreases oxidative stress by ameliorating antioxidant status in diabetic animals (Maroo et al., 2003). It has also shown hypolipidemic, hepatoprotective, antidematogenic and free radical scavenging properties. (Vasu et al., 2005 and Vaijanathappa et al., 2009).
- In this study, we observed that preincubation with EL (2mg/ml) for 2 hr. caused suppression of ROS generation caused by exposure to 50µM $H_2O_2$ , thus suppressing islet cell death.
- In pancreatic islets, antioxidant enzymes CAT, SOD and GPx genes are expressed at low levels. In present study, significant changes of GSH content, SOD, CAT and GPx activities were found at 0.5 hr afetr $H_2O_2$ addition compared with normal control, whereas with EL preincubation CAT, SOD and GPx acitivity and GSH content improved in islet cells. This suggests that the EL extract strengthened the ability to combat Oxidative stress.
- ROS generation causes DNA damage, which activates Parp-1 and Caspase-3, an apoptotic executor. On treatment with EL the expression levels of these two proteins were significantly lowered indicating protective effect of EL by blocking DNA damage.

## CONCLUSION

El has shown to have hypoglycemic, hypolipidemic and in this study strong antioxidant property, which provides significant evidence for considering EL as a strong herbal therapeutic agent to combat diabetes mellitus.

## REFERENCES


- Maroo, J., Ghosh, A., Mathur, R., Vasu, V. T., and Gupta, S. (2003) Antidiabetic efficacy of *Enicostemma littorale* methanol extract in alloxan induced diabetic rats, *Pharm. Biol. 41*, 388-391.
- Maroo, J., Vasu, V. T., Aalinkeel, R., and Gupta, S. (2002) Glucose lowering effect of aqueous extract of *Enicostemma littorale* blume in diabetes: a possible mechanism of action, *J. Ethnopharmacol. 81*, 317-320.
- Vaijanathappa, J., and Badami, S. (2009) Antiedematogenic and free radical scavenging activity of swertiamarin isolated form *Enicostemma axillare*. *P(anta Med. 75*,12-17.
- Vasu, V. T., Ashwinikumar, C., Maroo, J., Gupta, S., and Gupta, S. (2003), Antidiabetic effect of *Enicostemma littorale* blume aqueous extract in newly diagnosed non-insulin-dependent diabetes mellitus patients (NIDDM): a preliminary investigation, *Orient. Pharma. Exper. Med.* 3, 84-89.
- Vasu, V. T., Modi, H. R., Jyoti V. T., and Gupta S. (2005) Hypolipidaemic and antioxidant effect of Enicostemma littorale Blume aqueous extract in cholesterol fed rats, *J. Ethnopharmacol. 101*, 282.
- Xiong Fu-Liang, Sun Xiao-Hui, Gan Lu, Yang Xiang-Liang and Xu Hui-Bi. (2006), Puerarin protects rat pancreatic islets from damage by hydrogen peroxide, *Eur. J. Pharmacol. 529*,1-7.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# Coalescing Stem Cell Therapy with potent Bioactives: Regenerating Islet of Langerhans in Type I Diabetic BALB/c mice

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## Abstract

Mesenchymal stem cells (MSCs) have both regenerative and immunomodulatory properties. For over a decade, researchers have been exploring the potential of MSCs in preclinical and clinical studies that can resolve injuries by enhancing endogenous repair opening a new paradigm in stem cell therapy for the treatment of diabetes.

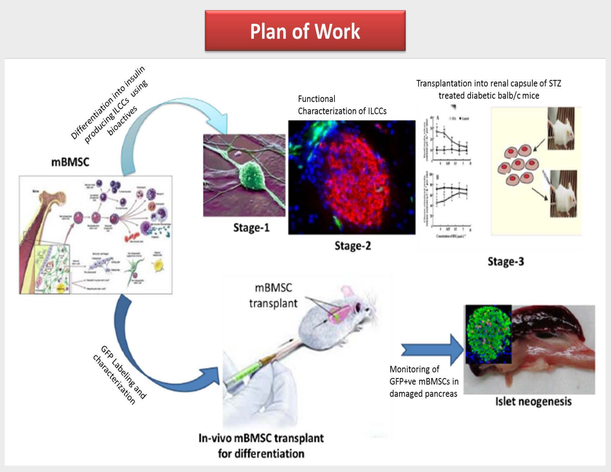
In our study, mouse Bone marrow mesenchymal stem cells (mBMSCs) were isolated, purified and characterized by Flow Cytometry. These were then differentiated using bioactives into insulin producing islet like cell clusters, which were found to be very efficient in diabetes reversal upon transplantation in streptozotocin (STZ) treated diabetic BALB/c mice. Further, we created GFP labelled mBMSCs and performed in-vivo lineage tracing experiment, where we successfully demonstrated endogenous differentiation of GFP+ve mBMSCs into newly generated islets in STZ induced diabetic mice endocrine pancreas. The transplantation in combination with bioactives enhanced the homing of mBMSCs to the damaged pancreas in turn boosting pancreatic regeneration. Also, BMSCs have a wider clinical acceptability as their harvesting protocols are already well established, which serve as a wonderful source of pluripotent/multipotent stem cells that can differentiate into multiple lineages including insulin producing cells. Thus, the potential of mBMSCs along with our bioactive compound can be effectively translated as therapeutic tool for the treatment of type 1 diabetes.

## Introduction

BMSCs offer an excellent source of stem cells and provide clinical option for regeneration of pancreas or islet differentiation and transplantation for curative and definitive treatment of insulin-dependent diabetes (Phadnis *et al.*, 2010). By now, bone marrow mesenchymal stem cells (BMSCs) have been shown to ameliorate diabetes in animal models(Sordi and Piemonti, 2010). The mechanism, however, remains largely unknown. BMSC can be obtained with relative ease from each patient, allowing potential circumvention of allograft rejection. Few earlier studies have shown that mouse BMSCs spontaneously differentiate into endocrine pancreatic cells *in vivo* (Ianus *et al.*, 2003), but still a major unanswered question remains, whether BMSCs are able to differentiate into β-cells *in-vivo* or they are able to mediate recovery and or regeneration of endogenous β-cells by facilitating growth and differentiation of other tissue specific progenitors.

An approach for in vivo endogenous differentiation of any stem cell type is the actual therapeutic regenerative intervention for any diabetic patient in treatment. Drugs or bioactive agents which can be capable of enforcing organ stem cells or circulating stem cells like BMSCs to form functional islets into diabetic individuals endogenously will create a paradigm shift in regenerative stem cell therapy. Targeting this, we attempted mBMSC differentiation into islets both under *in vitro* and *in vivo* condition with swertisin as positive differentiating agent, which has been demonstrated in our earlier published report. (Dadheech N. *et al.*, 2013).

## Results



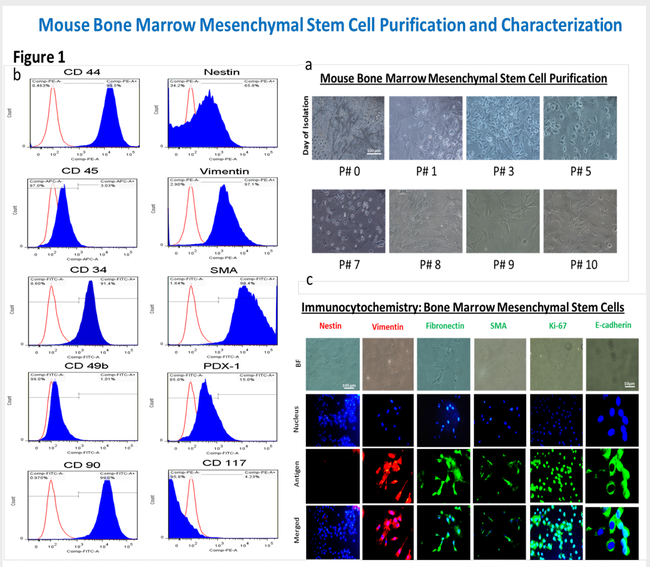




Figure 1: Fig 1a showing phase contrast images demonstrate step-wise purification of mBMSC from P# 0 to P# 10. Fig 1b & 1c demonstrate Characterization of mBMSC by Flow Cytometry and Immunocytochemistry.

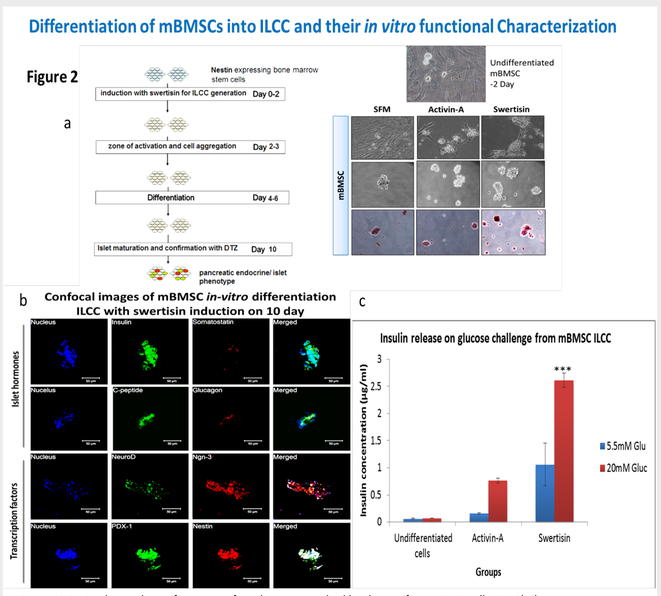



Figure 2: Fig 2a shows Cluster formation of insulin-positive islet-like clusters from mBMS cells. Fig 2b depicts intense positive staining for Insulin (red color), C-peptide (Green color), Glucagon (Red color) and Somatostatin (pink color). Fig 2c represents in-vitro insulin release from undifferentiated mBMSC, activin-A and swertisin induced ILCC on 10th day of differentiation. Results are expressed as mean ±SEM n=3 *** p< 0.001 vs undifferentiated and activin-A.

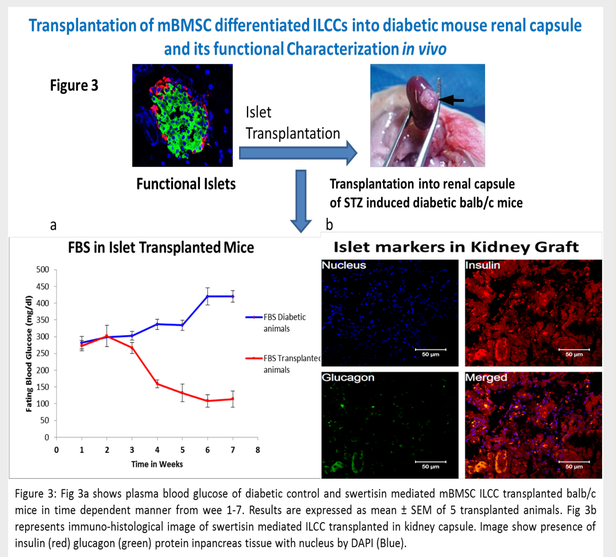



Figure 3: Fig 3a shows plasma blood glucose of diabetic control and swertisin mediated mBMSC ILCC transplanted balb/c mice in time dependent manner from wee 1-7. Results are expressed as mean ± SEM of 5 transplanted animals. Fig 3b represents immuno-histological image of swertisin mediated ILCC transplanted in kidney capsule. Image show presence of insulin (red) glucagon (green) protein inpancreas tissue with nucleus by DAPI (Blue).

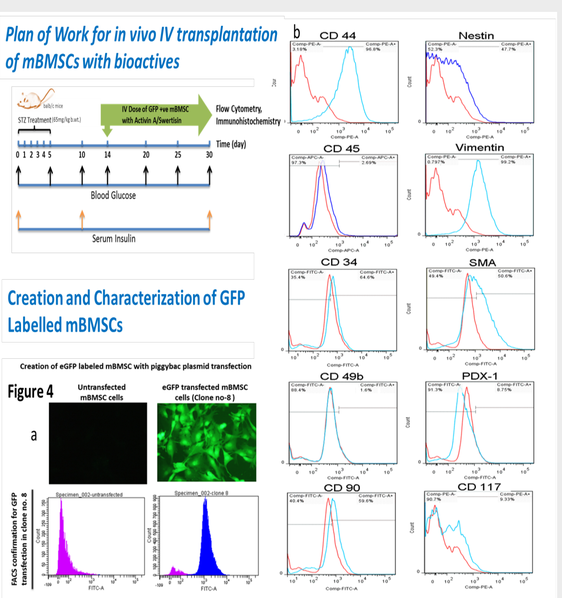



Figure 4: Fig4a representing creation of eGFP labeled mBMSC cells using piggybac vector transfection. Clone no-8 shows GFP (green) protein in fluorescent image and purity of transfected clone in FACS profile (blue peak). Fig4b is characterization of mBMSCs using Flow Cytometry.

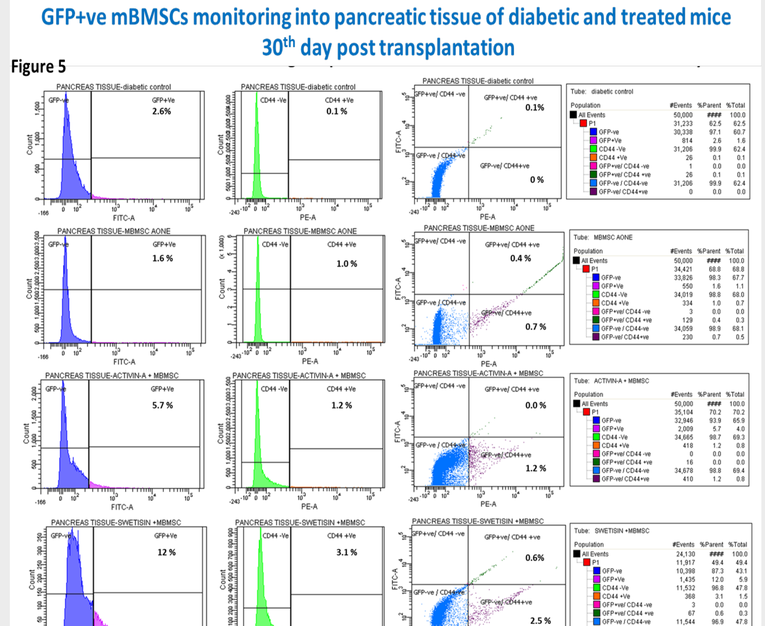



Fig 5 show flowcytometry data of GFP labeled mBMSC cells in pancreas tissues from mBMSC alone, Activin-A and swertisin treated animals. Histograms with blue and green peak show non GFP and CD44-ve cells in pancreas, While pink and orange peek denotes GFP+ with CD44+ve mBMSC cells. Dot plots represents identification of GFP and CD44 duel population.

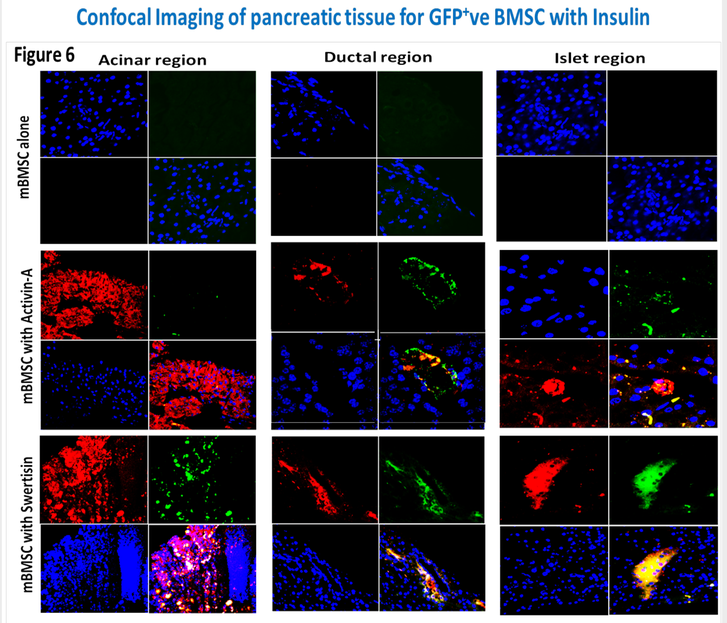



Fig 6 shows confocal images of pancreatic tissue sections of mBMSC-GFP cells transplanted alone and in combination with activin-A and swertisin for differentiating mBMSC endogenously. Three different region of each pancreatic tissue was observed in order to monitor GFP and insulin co-labeled cells. Insulin is showing in red while GFP in green and nucleus was stained with DAPI in blue.

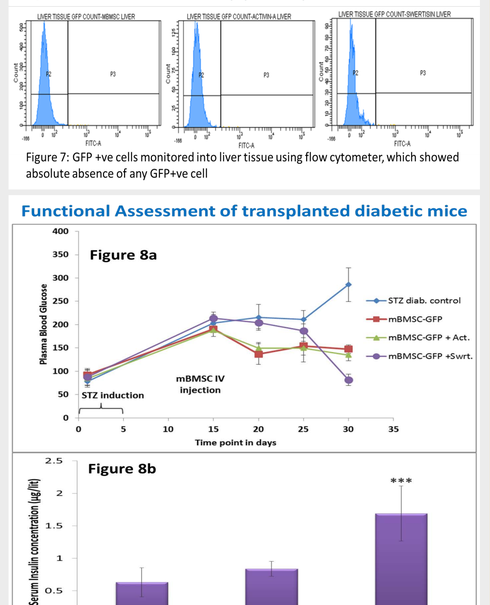



Figure 7: GFP +ve cells monitored into liver tissue using flow cytometer, which showed absolute absence of any GFP+ve cell

Fig 8a and b: Blood glucose and insulin profile of mBMSC-GFP transplanted animals. Fig a shows plasma blood glucose in mBMSC-GFP transplanted animals in time dependent manner. Fig b represents serum insulin levels in these transplanted animals on 30th day of the study. Data represents mean ±SEM. N=3 animals in each group, ***p< 0.001.

## Discussion

- BMSCs reside in bone marrow and are multipotent, and can differentiate into lineages of mesenchymal tissues, such as bone, cartilage, fat, tendon, muscle, adipocytes, chondrocytes, osteocytes (Florio *et al.*, 2000). Similarly, Islet like cell clusters (ILCC) obtained from differentiating BMSCs were able to release insulin in response to glucose stimulation.
- It is important to note that in this study, swertisin mediated mBMSC ILCCs showed timely regulated insulin release upon glucose induction and showed improved blood glucose parameters with presence of insulin and glucagon hormones in ILCC grafts retrieved from renal capsule post transplantation in STZ treated diabetic Balb/c mice.
- Hess *et al*. reported a lowering of blood glucose levels within a week after intravenous infusion of green fluorescence protein(GFP)-tagged allogeneic bone marrow into STZ-induced diabetic mice (Hess *et al.*,2003). With quantitation of GFP insulin cells it was postulated that only 0.5-2%cells migrated to pancreas and differentiated to insulin producing cells. Later in another report no significant transdifferentiation of BMSCs was observed into insulin producing cells *in vivo* (Lechner A. *et al.*, 2004). However, present study supports Hess *et al's.*, report but emphasizes on using effective differentiating bioactives along with BMSCs for augmenting their potential in homing and repair of damaged endogenous tissues, which resulted in 12% GFP+ve mBMSCs of the total pancreatic cell population migrating into the pancreatic tissue.

## Conclusion

In conclusion, the results indicate that

- BMSCs alone fails to produce any significant endogenous islet differentiation while the same was achieved in combination with Activin-A and swertisin treatment.
- Taken together, the data presented in this study indicated that BMSCs proves to be the best adult stem cell source for its advantages such as large potential donor pool, rapid availability, absence of discomfort to the donor with low risk of rejection.
- BMSCs could be programmed to differentiate into ILCCs both *in vitro*, and endogenously *in vivo* with potential differentiating agent. Swertisin proved to be an excellent inducing agent to be used as potential therapy along with BMSCs for diabetes therapeutics.

## Acknowledgement

We would like to thank DBT, Gov. of India for funding this research, the DBT-MSUB-ILSPARE Instrumentation facility and Sanger Institute for providing us with piggybac vectors which were used for GFP tagging in this study.

## References

- Dadheech N., Soni S., Srivastava A. et al., 2013. A Small Molecule Swertisin from Enicostemma littoraleDifferentiates NIH3T3 Cells into Islet-Like Clusters and Restores Normoglycemia upon Transplantation in Diabetic Balb/c Mice. Evidence-Based Complementary and Alternative Medicine 2013:20 pages; 2013
- Florio, P., S. Luisi, P. Marchetti, R. et al., 2000. Activin A stimulates insulin secretion in cultured human pancreatic islets. J Endocrinol Invest. 23:231-4.
- Hess, D., L. Li, M. Martin, et al., 2003. Bone marrow-derived stem cells initiate pancreatic regeneration. Nat Biotechnol. 21:763-70.
- Ianus, A., G.G. Holz, N.D. Theise, and M.A. Hussain. 2003. In vivo derivation of glucose-competent pancreatic endocrine cells from bone marrow without evidence of cell fusion. J Clin Invest. 111:843-50.
- Lechner A, Yang YG, Blacken RA, et al., 2004. No evidence for significant transdifferentiation of bone marrow into pancreatic beta cells in vivo. Diabetes. 53:616-23.
- Phadnis, S.M., M.V. Joglekar, M.P. Dalvi, et al., 2010. Human bone marrow-derived mesenchymal cells differentiate and mature into endocrine pancreatic lineage in vivo. Cytotherapy. 13:279-93.
- Sordi and Piemonti, 2010. Mesenchymal Stem Cells as Feeder Cells for Pancreatic Islet Transplants. The Review of Diabetic Studies. 7(2): 132–143

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**Presented at, National Symposium on 'Emerging Trends in Biochemical Sciences' at the Department of Biochemistry, The Maharaja Sayajirao University of Baroda, Vadodara; 29th-30st Dec., 2014**

# PARP-1 protein regulates Islet differentiation in mouse intra-islet progenitors through Smad signaling


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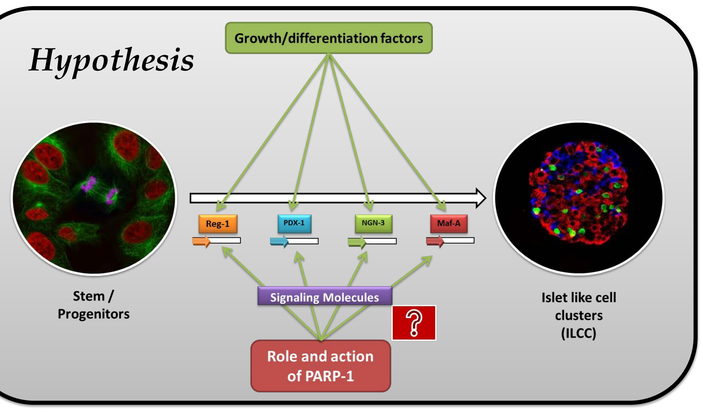
## Abstract

Pancreatic progenitors are the major source for the replenishment of lost beta cells. Numerous reports provide evidence for existence of intra islet precursors differentiating into new islet cells. Beta cell death is a characteristic hallmark of diabetes and is associated with a DNA repair enzyme, Poly (ADP ribose) Polymerase-1 (PARP-1). PARPs are now known to be involved not only in cell death and DNA repair but also in cell differentiation, proliferation and gene expression control. In this in-vitro study, PARP-1 gene was silenced in mouse intra islet progenitor cells (mIPCs). PARP-1 knockdown affected the morphology of mIPCs and clonogenic assay & growth kinetics demonstrated significant increase in doubling time of PARP-1 deplete mIPCs. In a comparative molecular characterization for their pancreatic markers, down regulation of Neurog3 and Pdx1, the key pancreatic transcription factors were significantly reduced post knockdown. However, the mesenchymal stem cell surface markers mostly remained unaffected. Further, the failure of PARP-1 knockdown mIPCs to differentiate into adipocytes, osteoblasts and chondrocytes during trilineage differentiation established that PARP-1 is vital for differentiation. To understand the role of PARP-1 in islet development we further focused on islet differentiation from mIPCs with PARP-1, PARP-1 knockdown and PARP-1/2 inhibitor (ABT888) treated cells. Here, we observed that the islet cluster formation capability of PARP-1 knock down cells was greatly hampered but ABT888 treated cells retained their islet differentiation potential. The islet functionality was measured by Diathizone staining, c-peptide release assay and Immunocytochemistry of c-peptide and glucagon. This suggested that PARP-1 enzymatic activity is not a prerequisite but PARP-1 protein is essential for islet differentiation. In this study, Activin A and Swertisin were used as islet differentiating agents, which follow activin mediated Smad pathway as previously reported. Time dependent gene and protein expression studies provided with evidence that PARP-1 might be controlling expression of key transcription factors via Smad pathway. Protein interaction studies with PARP-1 confirmed a direct interaction of PARP-1 with the Smad proteins which would regulate the expression of key transcriptional regulators during islet differentiation. In conclusion, PARP-1 is essential to regulate islet differentiation via Smad signaling.


## Introduction

Intra-islet progenitors are a major source for the replenishment of lost beta cells. Islet neogenesis is inevitably controlled by sequential and timely expression of few key transcription factors which imparts endocrine fate (insulin positivity) to progenitor cells. It has been considered that the regulation or expression of these transcription factors are controlled by certain factors and PARP-1 is considered to be one of them apart from its regular DNA repair and cell death. Transcription control, chromatin remodelling in differentiation and epigenetic control are the few properties of PARP-1 which are now highlighted by many groups. Several earlier studies have implicated PARP-metabolism in different stages of diabetes from development of the disease to its complications. However, none have addressed the possible role of PARP-1 in islet neogenesis from progenitors/stem cells. Hence, a comprehensive understanding of the role of PARP in formation of islet cells from its precursors will allow us not only to improve the yield of islets for transplantation in diabetic patients, but also provide a more fundamental understanding of the mechanism of action of islet neogenesis, which can be targeted for future therapies.

## Hypothesis




## Discussion

- Research on PARP-1 has revealed its involvement in differentiation and diabetes but its role in Islet Neogenesis is a complete grey area. PARP-1 knock down abates islet differentiation and is restored with recovery of PARP-1 which makes it essential for the same.
- Its been well established now that PARP-1 has transcriptional control activity. It has been proposed that PARP-1 protein upregulates transcription of regenerating gene Reg-1 in RINm5F insulinoma-derived cells, which helps in proliferation of these cells in the presence of PARP-inhibitor that can prevent catalytic activation of PARP-1 but not its binding to DNA [1]. This also promotes earlier data where treatment of rats after partial pancreatectomy with weak PARP-inhibitors resulted in enlarged islets with β-cell mass [2]. In one such previous report the role of PARP-1 was examined in hyperglycemia mediated downregulation of insulin biosynthesis by cells by suppressed activity of MafA gene, because weak PARP-inhibitors could increase insulin biosynthesis under these circumstances in INS-1 β-cells [3]. Here, we observed that PARP-1 protein was essential for islet neogenesis and not its activity as the inhibitor treated cells also differentiated into mature islets.
- Nuclear Smad function is regulated by PARP-1 in TGFβ signaling in human HaCaT Keretinocytes and Vascular smooth muscle cells[4,5,6].
- In the present study we observed a direct interaction of PARP-1 with Smads on stimulation with both Activin A and Swertisin that follow TGFβ Smad pathway; interaction with Smads 2/3 decreased and increased with its phosphorylated form as the differentiation progressed. This study suggests the necessity of PARP-1 for Smads for executing their regulatory role during islet neogenesis.

## Conclusion

In Conclusion, PARP-1 protein is essential for Islet differentiation. PARP-1 protein interacts with Smad signaling molecules during islet differentiation which can regulate many key transcriptional factors involved in islet neogenesis.

## Future Prospects

Understanding the role of PARP-1 and its DNA-Protein interaction with key transcription factors like PDX-1, Ngn-3, MafA and Reg-1 in PARP null Human/ Mice in primary cultured pancreatic precursor cell will highlight PARP's significance in Islet neogenesis.


## Acknowledgement

We would like to thank DBT, Gov. of India for funding this research, the DBT-MSUB-ILSPARE Instrumentation facility and Dr. Girish Shah, Laval University, Quebec, Canada for providing us with valuable suggestions in experimental design & PARP-1 shRNA and recovery vectors.


## References


1. Akiyama *et al.*, 2001, Proc Natl Acad Sci USA 98, 48-53.
2. Yonemura *et al.*, 1984, Diabetes 33, 401-404.
3. Ye *et al.*, 2006, Diabetes 55, 742-750.
4. Lonn *et al.*, 2010, Molecular Cell 40, 521-532.
5. Dahl *et al.*, 2014, Plos One 9(8), e103651.
6. Huang *et al.*, 2011, Plos One 6(10), e27123.
7. Levetan *et al.*, 2010 Journal of Diabetes 2, 76–84


## Results

**Figure 1: PARP-1 Gene knockdown in mIPCs**

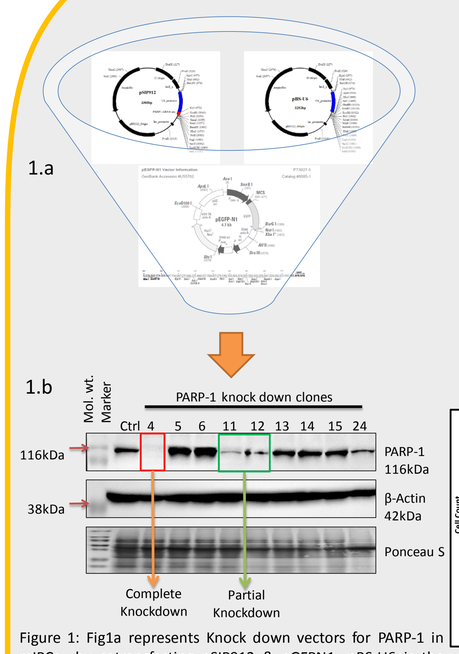



Figure 1: Fig1a represents Knock down vectors for PARP-1 in mIPCs by cotransfecting pSIP912 & pGFPN1, pBS-U6 is the empty vector which was used as control with pGFPN1. Fig1b shows confirmation of PARP-1 knockdown by immunoblotting.

**Figure 2: Comparative characterization of mIPC (PARP-1) and mIPC PARP-1 KD**

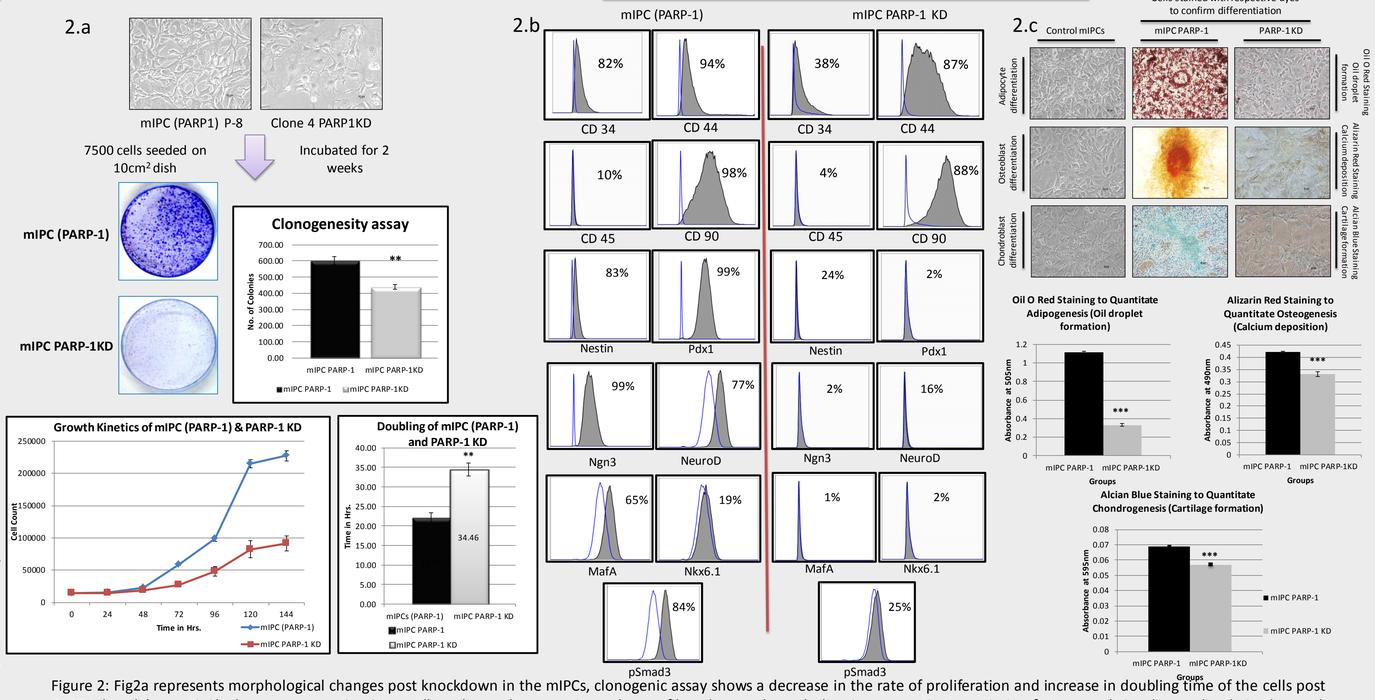



Figure 2: Fig2a represents morphological changes post knockdown in the mIPCs, clonogenic assay shows a decrease in the rate of proliferation and increase in doubling time of the cells post PARP-1 knockdown. Fig2b show a comparative Stem cell marker and pancreatic marker profile where a sharp decline in pancreatic transcription factors and signaling molecule is observed. Fig2c represents the trilineage differentiation where differentiation is abolished on PARP-1 knock down in mIPCs.

**Figure 3: Comparative Islet Differentiation profile**

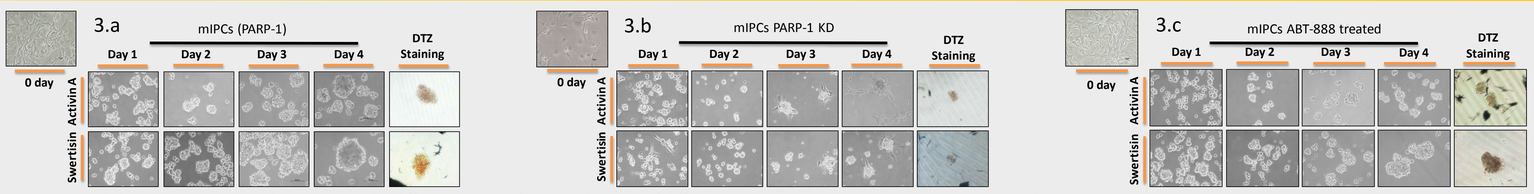



Figure 3: Fig. 3a,b &c represents temporal microscopic Islet differentiation profile through 4 days of differentiation in mIPCs, mIPCs PARP-1 knock down and mIPCs treated with ABT-888 (PARP-1 specific inhibitor) respectively. It is observed that islet cluster formation is abolished in PARP-1 knock down cells but not in the ABT-888 treated cells

**Figure 4: Transcriptional Pathway for Islet formation**

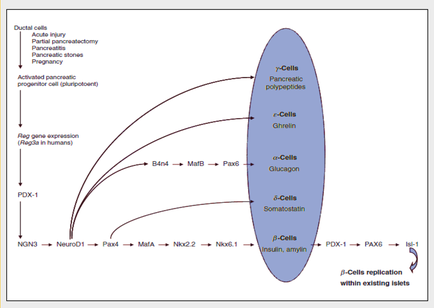



Figure 4: Exhibits the key transcription factors involved in islet differentiation and replication[7].

**Figure 5: Comparative temporal gene and protein expression profile**

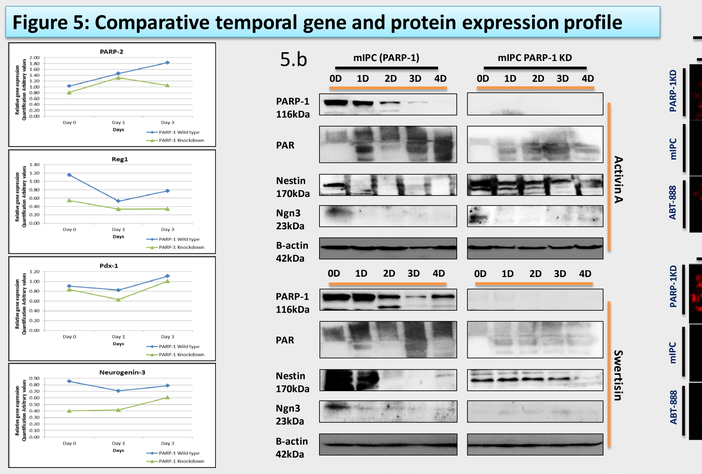



Figure 5a&b: Demonstrates the key pancreatic gene downregulation post PARP-1 knock down during islet differentiation

**Figure 6: Comparative Functional Characterization of differentiated Islets**

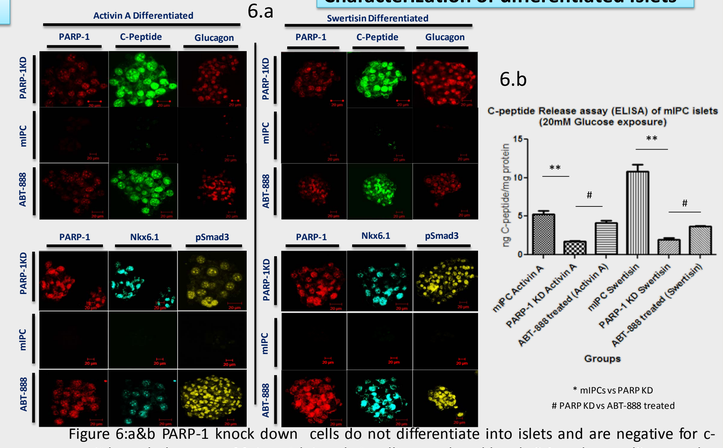



Figure 6:a&b PARP-1 knock down cells do not differentiate into islets and are negative for c-peptide and glucagon . Mature islet and signalling markers like nkx6.1 and psmad3 were also downregulated.

**Figure 7: PARP-1 Recovery**

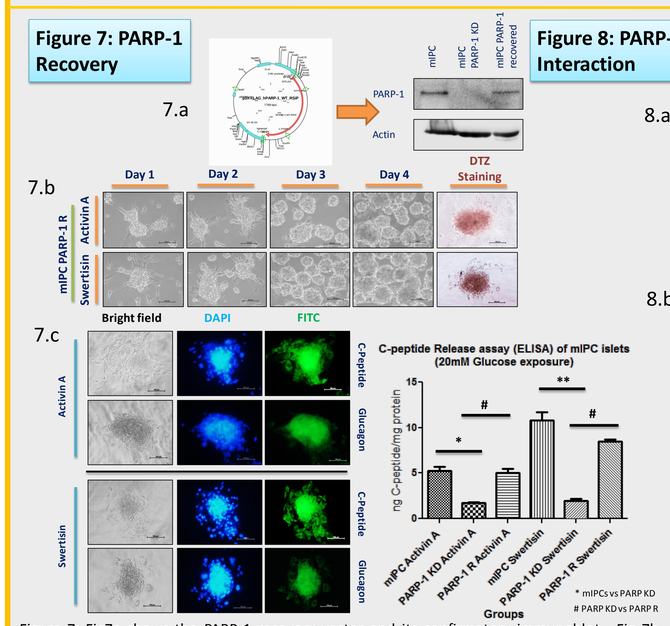



Figure 7: Fig7a shows the PARP-1 recovery vector and its confirmatory immunoblot. Fig 7b shows successful islet differentiation post PARP-1 recovery. Fig 7c gives the functional characterization and confirms mature islet formation post PARP-1 recovery.

**Figure 8: PARP-1:Smad Interaction**

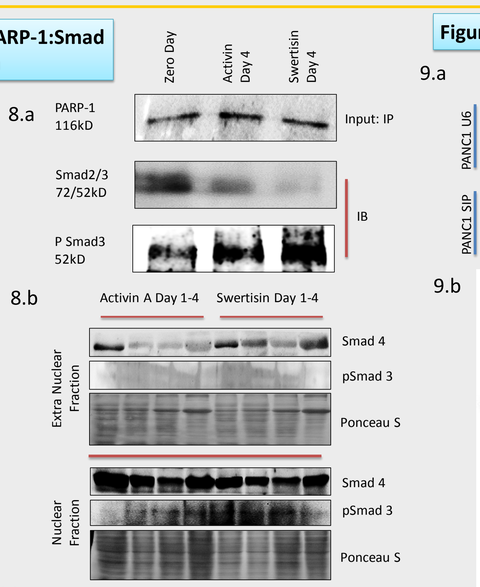



Figure 8: Fig8a shows IP of PARP-1 at 4th day of differentiation Where PARP-1:Smad interaction is observed. Fig8b is a fractionation blot which shows localization of Smad complex in The nucleus during islet differentiation.

**Figure 9: PANC-1 cells PARP-1:Smad Interaction**

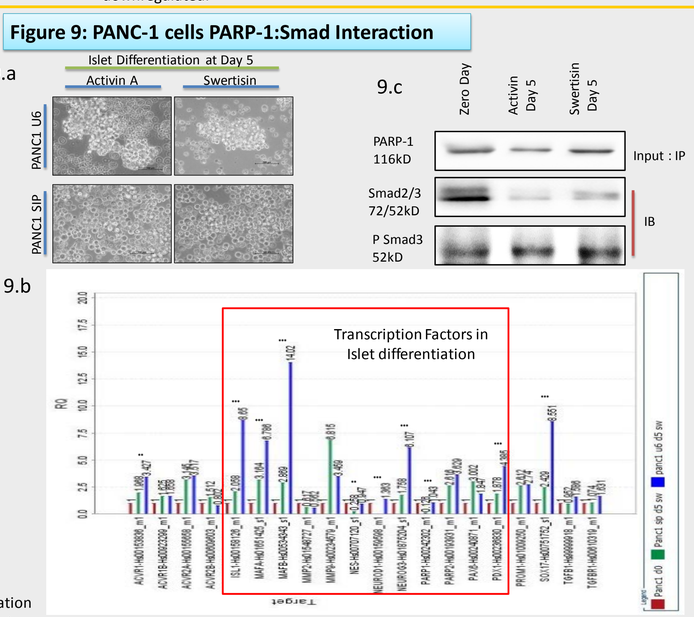



Figure 9: Fig9a shows abatement of islet differentiation at day 5 in panc-1 cells post PARP-1 knockdown. Fig9b is a gene array which shows down regulation of all the important pancreatic genes post PARP-1 knockdown. Fig9c shows PARP-1:Smad Interaction at day 5 of differentiation.

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**Presented at Cold Spring Harbor Laboratories, New York, USA, 3rd Stem Cell Biology Meeting, 7th to 11th October, 2015**

# Novel Bioactive in potentiating Islet Neogenesis from mouse Intra-Islet Mesenchymal Stem Cells


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## Abstract

In the area of regenerative medicine researchers have been exploring the potential of Mesenchymal Stem Cells (MSCs) in preclinical and clinical studies to explore improved therapies that can resolve injuries by enhancing endogenous repair opening a new paradigm in cell therapy. MSCs provide clinically and ethically accepted option over embryonic or induced pluripotent stem cells for their use as autologous source for cell transplant in the treatment of diabetes. Hence, our study has focused on increasing islet mass using a novel bioactive compound, Swertisin isolated from *Enicostemma littorale* demonstrating potent islet neogenic property when evaluated with cells like NIH3T3 and PANC-1. Intra islet precursors were isolated, purified and characterized as MSCs from islets of Langerhans of young BALB/c mice. Further, Intra-Islet MSCs were successfully differentiated into insulin producing islet like cell clusters (ILCCs). These *in vitro* differentiated islets were assessed at morphological, molecular, immunological and functional level. These ILCCs were differentiated into mature functional islets in a span of four days only, which is the fastest reported differentiation period for any isolated MSCs till date. We further confirmed that these ILCCs could sense glucose and secrete both c-peptide and glucagon which implies maintenance of glucose homeostasis. Thus, the significance of this study is in islet replacement therapy that can be exploited by using and stimulating these inherent MSCs using bioactive compounds to achieve the best potential cure for diabetes mellitus. These neo-islets can ameliorate insulin deficiency by preventing exogenous insulin administration in diabetes patients.


## Introduction

Islet development within the pancreas appears to initiate from a pool of undifferentiated precursor cells associated with the ductal epithelium. As a result, the mature islets of Langerhans appear to be derived from stem cells, and these stem cells possess the capacity to differentiate into the four distinct islet-associated endocrine cell populations: glucagon producing alpha cells, insulin-producing beta cells, somatostatin-producing gamma cells, and pancreatic polypeptide-producing delta cells4. The loss of beta cells being the hallmark of diabetes mellitus. Both Type 1 and Type 2 diabetes are directly or indirectly resulted due to insulin producing beta cell death and malfunctioning. Generation of new beta cell in a diabetic pancreas is an event of rare occurrence which is basically attributed to islet neogenesis from pancreatic progenitors (Peck et al., 2002). So far pancreatic ductal progenitors have been very well defined in their role in islet neogenesis whereas, islet neogenesis from intra-islet progenitors have been a debatable and far less explored paradigm. In this present study we have tried to emphasize that a novel small molecule Swertisin along with mouse intra-islet progenitors (mIPCs) with their rapid differentiation capability can provide a potent therapeutic intervention in the treatment of diabetes mellitus.

## Objectives

- To test efficacy of Swertisin in islet differentiation on PANC-1 & NIH3T3 cells.
- To isolate, purify and characterize mouse intra-islet progenitors.
- To differentiate mouse intra-islet progenitors using Swertisin.

## Discussion

- The goal of this study was to target intra-islet progenitors with novel small molecule Swertisin to potentiate their rapid and efficient maturation into Islet of Langerhans.
- The mIPCs that were isolated were found to be Nestin, Pdx-1 and Ngn-3 positive, whereas negative for CK-19, c-peptide and glucagon. Similar results were observed by Gu et al., (2002), where lineage tracing experiments confirmed such cells to be intra-islet progenitors.
- There have been previous reports where pancreatic progenitors were reported to have markers similar to mesenchymal stem cells (Zhang et al., 2005) but these were reports for the cells of the ductal origin. This study confirms that intra-islet progenitors have mesenchymal stem cell characteristics as they have similar cell surface markers and could undergo trilineage differentiation.
- So far for islet differentiation time taken by embryonic stem cells or iPSCs is more than a month. Also, other adult stem cells take approximately ten days to differentiate and produce mature ILCCs (Sui et al., 2013). In this study, we have observed and confirmed neo islet formation from mIPCs in four days, which is the shortest and most rapid islet differentiation reported till date. The mature islets were both C-PEPTIDE and GLUCAGON positive and could release C-PETIDE on sensing glucose.
- The only way to treat chronic diabetes is replacement or replenishment of lost beta cells. We strongly believe that these intra-islet progenitors along with compatible novel small molecules that can specifically stimulate them to differentiate could be an answer for a successful diabetes therapy in the near future.

## Conclusion

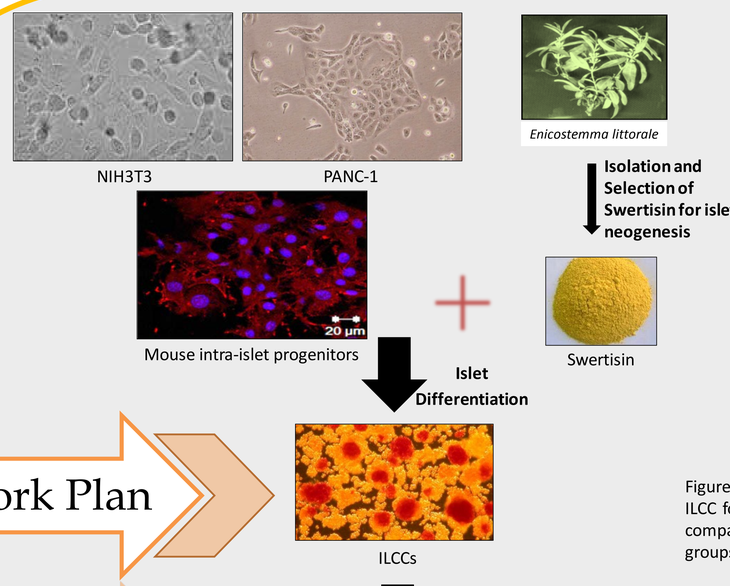
Hence, this study can be a beacon of hope for diabetic patients where their resident intra-islet progenitors can be appropriately stimulated by novel small molecules like Swertisin to replenish their lost insulin producing cells within their impaired Islets of Langerhans in the pancreatic tissue.


## Acknowledgement

We would like to thank DBT, Gov. of India and UGC-NET-Fellowship Grant: BT/PR3564/BRB/10/975/2011 for funding this research and the DBT-MSUB-ILSPARE Instrumentation facility.


## References

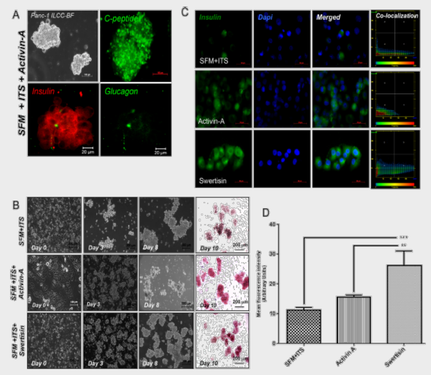

- Peck et al., *J HBP Surgery*, 2002.
- Gu et al., *Dev.*, 2002.
- Dadheech et al., *eCAM.*, 2013.
- Zhang et al., *World J Gastroenterol.*, 2005.
- Sui et al., *J Regen Med.*, 2013.
- Dadheech et al., *PlOS One., 2015.*


Work Plan

## Results

### 1. *In Vitro* Differentiation of PANC-1 cells




### 2. *In Vitro* Differentiation of NIH3T3 cells

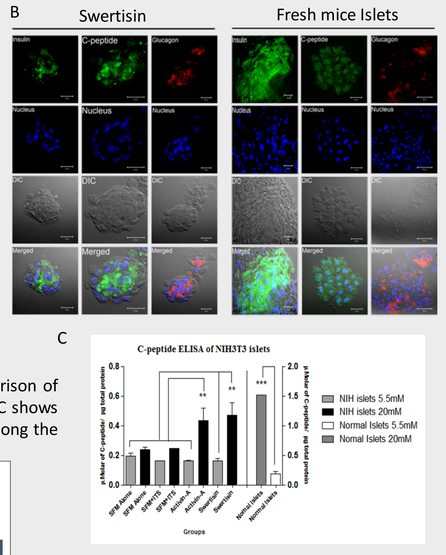



Figure 1: A shows confirmation of differentiation using Activin A. B Comparison of ILCC formation between Serum free media(SFM), Activin A and Swertisin. C shows comparative and qualitative insulin immunofluorescence signals of ILCCs among the groups and D is their quantification.

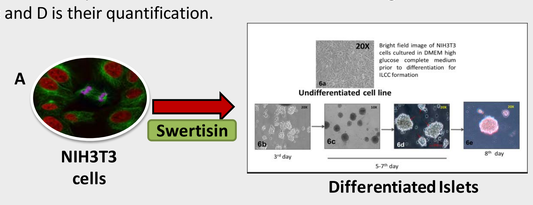



Figure 2: A shows differentiation of NIH3T3 into mature ILCCs, B and C shows functional characterization of these ILCCs using Immunocytochemistry and ELISA respectively.

### 3. Isolation and Characterization of mouse intra-islet progenitors

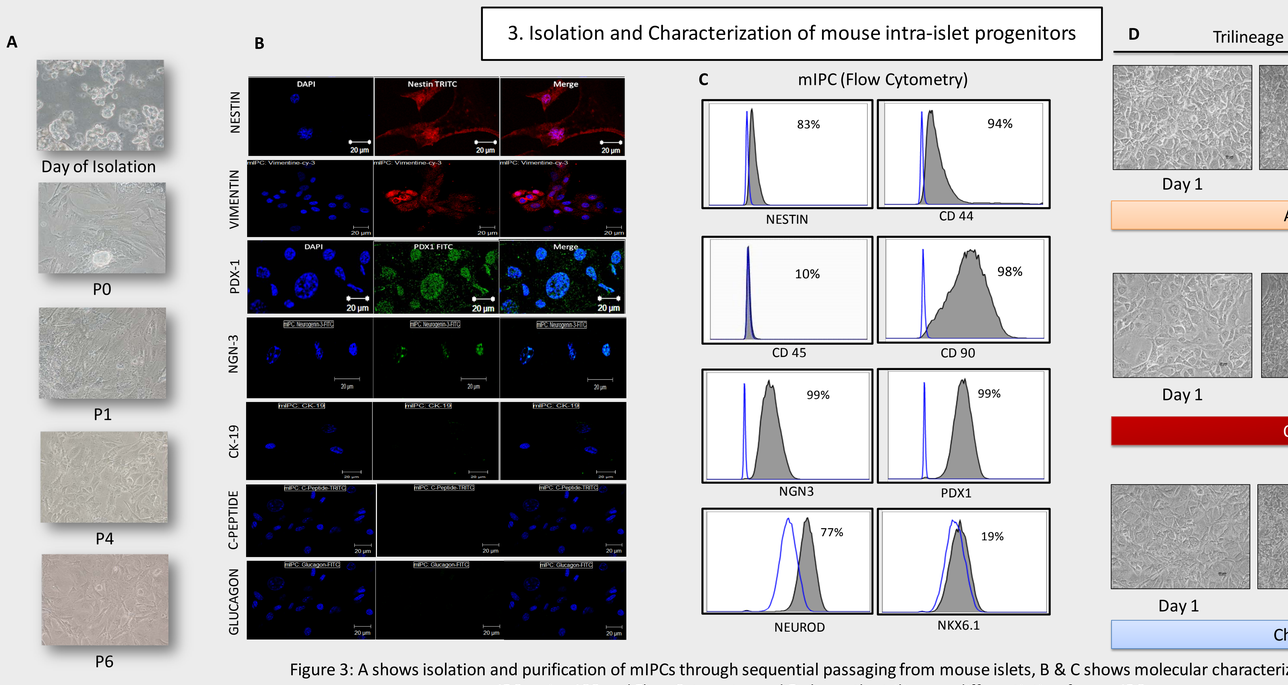



Figure 3: A shows isolation and purification of mIPCs through sequential passaging from mouse islets, B & C shows molecular characterization of mIPCs using ICC and Flow Cytometry and D shows the trilineage differentiation form mIPCs.

### 4. Differentiation of mIPCs into ILCCs

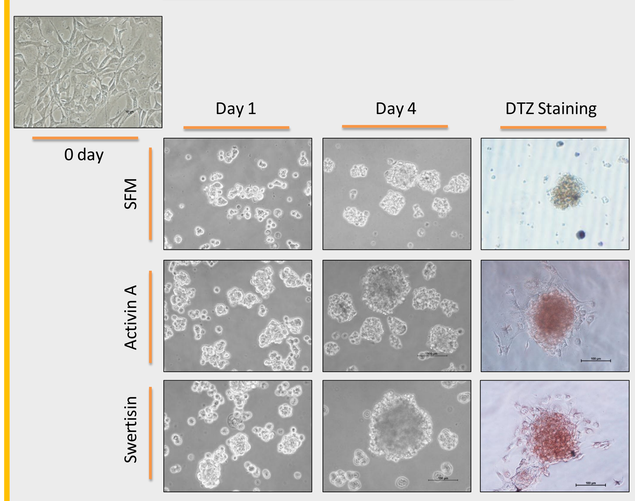



Figure 4: Demonstrate differentiation of mIPCs in serum free media, Activin A and Swertisin into mature ILCCs and Diathiazone staining.

### 5. Temporal gene and Protein Profile

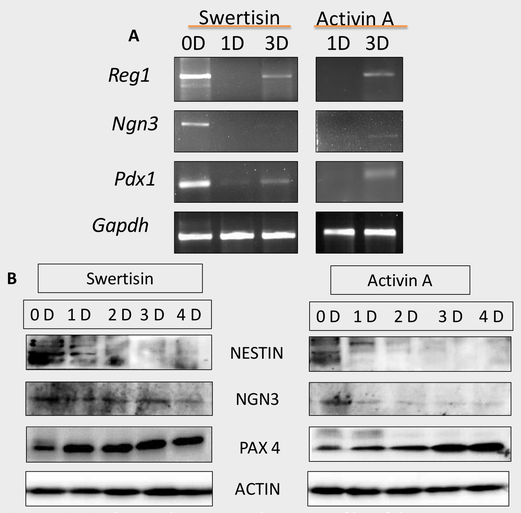



Figure 5: A shows the temporal gene profile of key transcription factors required for islet differentiation. B shows temporal protein expression in islet differentiation of certain key proteins.

### 6. Functional Characterization of ILCCs

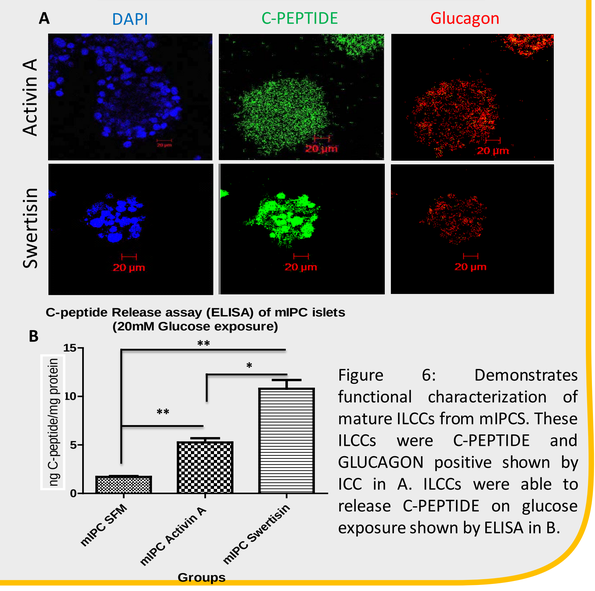



Figure 6: Demonstrates functional characterization of mature ILCCs from mIPCS. These ILCCs were C-PEPTIDE and GLUCAGON positive shown by ICC in A. ILCCs were able to release C-PEPTIDE on glucose exposure shown by ELISA in B.

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**Presented at , Till and McCulloch Meetings, Stem Cell Network, Toronto, Ontario; 26th to 28th October, 2015**