FORMAT FOR BRIEF OF COMPLETION REPORTS
(This brief report should not exceed 2 pages)

1. **Title of the project:** *Exploring the effects of peroxisome proliferator-activated receptor beta/delta activation in lipid induced adipocyte inflammation and insulin resistance*

2. **Sanction Order No., date, duration, total budget of the project:**
   Sanction Order No. & date: YSS/2014/000067 dated 20.10.2015
   Duration: Three Years
   Total budget: Rs. 25.20 lakhs

3. **Name & contact details (tel., mob. & email) of the Principal Investigator:**
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4. **Aims & objectives of the project/study (maximum 150 words):**
   • Objective 1: To study the underlying mechanism involved in PPAR beta/delta mediated inhibition of lipid induced insulin resistance. To check whether PPAR beta/delta agonists inhibit the activity of serine kinases, JNK and/or IKK and thereby suppresses lipid induced IRS-1 serine phosphorylation and insulin resistance.
   • Objective 2: To examine the role of PPAR beta/delta in regulation of adipose tissue inflammation, and does stimulation of PPAR beta/delta inactivates NF-κB though the activation of AMPK or inhibition of lipid induced TLR-4 signaling or novel PKC activation?
   • Objective 3: To evaluate the role of PPAR beta/delta in macrophage polarization and its effect on adipocyte function. Does PPAR beta/delta agonist inhibit lipid induced activation of macrophage M1 state and adipocyte dysfunction?

5. **Outcome and salient achievements (maximum 250 words) under the project:**
   Increased annual rate and prevalence of type 2 diabetes (T2D) is suggests that existing therapeutic measures are insufficient. Therefore, to find out a suitable drug target is imminent. Our findings shows the importance of PPARβ/δ activation in adipocytes, and sketeletal muscle cells, two major target cells for insulin action, that attenuates lipid induced insulin resistance. We have observed that activation of PPARβ/δ in adipocytes and macrophages notably inhibits lipid-induced adipose tissue inflammation by the inhibition of TLR4 activation. We also noticed that PPARβ/δ activation notably reduced the expression of PPAR-γ and C/EBP-β which inhibits massive lipid deposition in adipocytes and thus maintain the adipocyte insulin sensitivity. Prolong existence of insulin resistance in human being leads to the development of future pathogenesis of type 2 diabetes, therefore, our observation shows importance of PPARβ/δ as a therapeutic target against this insidious disease. Therefore, it would be ideal to explore different molecules potential to activate PPARβ/δ without imposing any toxic side effects.

S. Dasgupta

PROJECT COMPLETION REPORT

Title of the project:

Exploring the effects of peroxisome proliferator-activated receptor beta/delta activation in lipid induced adipocyte inflammation and insulin resistance.

File number:

YSS/2014/000067 dated 20.10.2015

Name and address of the investigation:

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Annexure-IX

PROJECT COMPLETION REPORT

1. **Title of the project:**
   Exploring the effects of peroxisome proliferator-activated receptor beta/delta activation in lipid induced adipocyte inflammation and insulin resistance.

2. **Principal Investigator(s) and Co-Investigator(s):**
   Dr. Suman Dasgupta (Principal Investigator)

3. **Implementing Institution(s) and other collaborating Institution(s):**
   Tezpur University (Implementing Institution)

4. **Date of commencement:** 16.11.2015

5. **Planned date of completion:** 15.11.2018

6. **Actual date of completion:** 15.02.2019

7. **Objectives as stated in the project proposal:**

   • Objective 1: To study the underlying mechanism involved in PPAR beta/delta mediated inhibition of lipid induced insulin resistance. To check whether PPAR beta/delta agonists inhibit the activity of serine kinases, JNK and/or IKK and thereby suppresses lipid induced IRS-1 serine phosphorylation and insulin resistance.

   • Objective 2: To examine the role of PPAR beta/delta in regulation of adipose tissue inflammation, and does stimulation of PPAR beta/delta inactivates NF-κB though the activation of AMPK or inhibition of lipid induced TLR-4 signaling or novel PKC activation?

   • Objective 3: To evaluate the role of PPAR beta/delta in macrophage polarization and its effect on adipocyte function. Does PPAR beta/delta agonist inhibit lipid induced activation of macrophage M1 state and adipocyte dysfunction?

8. **Deviation made from original objectives if any, while implementing the project and reasons thereof:** None

9. **Experimental work giving full details of experimental set up, methods adopted, data collected supported by necessary table, charts, diagrams & photographs:**

Methodology:

Cell culture, treatments and transfection: The mouse 3T3-L1, rat L6 skeletal muscle cell and human THP-1 monocyte cell lines were procured from the National Centre for Cell Science, Pune, India and were cultured in a similar manner described previously by us (1). Briefly, 3T3L1 preadipocyte and L6skeletal muscle cells were cultured in DMEM medium supplemented with 10% fetal calf serum, 25 mM glucose, 1% penicillin-streptomycin (Invitrogen) in a humidified 95% O2/5% CO2 atmosphere at 37 °C. Two days after confluence, 3T3L1 preadipocytes were stimulated to differentiate over 5 d in differentiation medium supplemented with 5 μg ml−1 insulin, 0.5 mmol per liter 3-isobutyl-1-methylxanthine and 1 μmol per liter dexamethasone. For L6 myotube formation, cells attaining 60–70% confluence were differentiated in DMEM containing 2% fetal bovine serum and 1% pen-strep for 4–6 days prior to all experiments. Cells were incubated for 6 h with 0.75mM of palmitate conjugated with 5% BSA with or without PPARβ/δ activators, GW501516 and GW0742 in absence or presence of inhibitors of TLR4 (CLI-095), AMPK (Compound C) or PKC (Rottlerin). THP-1 monocytes were cultured in RPMI 1640 containing penicillin (100 U/ml) and streptomycin (100 μg/ml) and supplemented with 10% FBS in a humidified 5% CO2 environment at 37 °C. Two days after confluence, THP-1 monocytes were differentiated to macrophages by the treatment with PMA (5 ng/ml) for 48 h. For coculture experiments, macrophages and adipocytes were placed in transwell chamber. On termination of incubations, cells were washed twice with ice-cold PBS and harvested with trypsin (0.25%)–EDTA (0.5 mM). Cell pellets were either resuspended in lysis buffer (1% NP-40, 20 mM HEPES (pH 7.4), 2 mM EDTA, 100 mMNaF, 10 mMsodium pyrophosphate, 1 mM sodium orthovanadate, 1 μg/ml leupeptin, 1 μg/ml aprotinin, 1 μg/ml pepstatin and 1 mM PMSF), sonicated on ice and lysates were centrifuged for 10 min at 10,000 g and protein concentrations of supernatant were determined by the method of Lowry et al (2) for Western blot analysis or harvested cells were subjected RNA isolation followed by semi-quantitative PCR or real-time quantitative PCR analysis for various gene expression study. To determine the inflammatory status in response to palmitate and its rescue by GW0742, we performed κB luciferase assay. κB luciferase vector was transfected to 3T3-L1 adipocytes (2×10^5 cell/well) by using Lipofectamine 2000 (Invitrogen, Carlsbad, CA) following manufacturer's protocol. After 48 h of transfection, cells were washed with DMEM. Transfected cells were then incubated without or with palmitate in absence or presence of GW0742 followed by the determination reported activity using multi-mode detector.

Glucose uptake assay: 2-NBDG (2-deoxy-2-[(7-nitro-2, 1, 3-benzoxadiazol-4-yl) amino]-D glucose) uptake assay (Cayman, USA) was performed according to manufacturer's instruction. Briefly, 3T3-L1 adipocytes ($1x10^6$ cells/ml) were serum starved overnight in glucose free DMEM supplemented with 2% FBS and then treated without or with GW501516 or GW0742 for 1h followed by 6h palmitate (0.75mM) incubation. Cells were then treated with or without 100nM of porcine insulin for 30 min and 10 min prior to the termination of experiment 2-NBDG (100µg/ml) was added to each of the incubations. Cellular uptake of 2-NBDG was measured using a fluorimeter at excitation and emission wavelengths of 485 and 535 nm, respectively.

Immunofluorescence anlysis: L6 cells were cultured on sterile Millicell EZ-SLIDES (EMD-Millipore, Germany) and treated with different conditions. On termination of incubations, cells were fixed with 4% paraformaldehyde for 10 min followed by blocking with 2% BSA in PBS for 1 h at room temperature. Cells were then incubated with anti-Glut4 antibody (1:50 dilution) in 2% BSA in PBS overnight at 4°C in a rotating platform. After washing with ice-cold PBS, cells were incubated with AlexaFluor 488-conjugated goat anti-rabbit secondary antibody (1:200 dilution) for 1 h at room temperature. Cells were then washed thrice with ice-cold PBS and mounted in Vectashield anti-fade mounting medium containing DAPI (Vector Laboratories, USA). Cellular images were taken in inverted fluorescent microscope (Leica DMi8, Germany) using LAS X software.

Immunoblotting: Immunoblot analysis was performed by following our previously described method (1). Briefly, cell lysates (60 or 40 µg of protein) were subjected to either 10% or 12.5% SDS-PAGE and transferred on to Immobilon-P PVDF membranes (Millipore, Bedford, MA) with the help of either Wet/Tank Blotting System or Semi-Dry trans-blot apparatus (Bio-Rad Laboratories, Hercules, CA). Membranes were first blocked with 10% non-fat dried milk or with 5% BSA in TBST (Tris-buffered saline) buffer for 1 h followed by the overnight incubation with primary antibodies (1:500 or 1:1000 dilutions) in a rotating shaker at 4°C. The membranes were then washed three times with TBST (TBS containing 0.1% Tween 20) buffer for 10 min interval and incubated with either ALP (alkaline phosphatase) or HRP (horse-reddish peroxidise) conjugated goat anti-rabbit or goat anti-mouse or mouse anti-goat IgG secondary antibodies (1:2000 dilution) for 2 h at room temperature. Membranes were then washed three times with TBST for 10 min interval and subjected to either 5-bromro 4-chloro 3-indolyl phosphate/nitroblue tetrazolium (BCIP/NBT) incubation for protein band visualization or exposed to Super Signal West Pico Chemiluminescent Substrate incubation for 5 min at room temperature followed by visualization and quantification in Chemidoc XRS+ System (Bio-Rad Laboratories, USA) using Image Lab Software.

RNA isolation, Semi-quantitative RT-PCR and real-time quantitative PCR: Total RNA was extracted from the cells of different incubations using RNeasy Lipid Tissue Mini Kit

(Qiagen, Germany) according to the manufacturer's instruction. RNA was treated with DNase I and reverse transcribed using the iScript Reverse Transcription Supermix. We used 2X PCR Master Mix for semi-quantitative RT-PCR in BioRad C-1000 Thermal Cycler and iTaq™ Universal SYBR® Green Supermix or DyNAmo color-flash SYBR green qPCR kit were used for real-time quantitative PCR in ABI-7500 system using gene-specific primers. The real-time quantitative PCR analysis was performed in following cycling conditions: 50°C for 2 min, 95°C for 10 min, 40 cycles of 95°C for 15 s, 55°C for 30 s, and 72°C for 30 s. A melt curve analysis was performed in real-time quantitative PCR after the final extension to ensure the specificity of the products. The fold changes in expression were determined using $2^{\Delta\Delta Ct}$ and the expression of target genes were normalized to GAPDH expression.

kB luciferase reporter assay: 3T3-L1 adipocytes (2×105 cells/well) were transfected with κB-luciferase expression plasmid (0.25 mg/well) using Lipofectamine 2000 Transfection Reagent (Invitrogen, USA) following manufacturer's protocol. Briefly, 7.5 µl of Lipofectamine 2000 reagent and 6 µl of 0.25 mg κB luciferase plasmid were added separately into 100 µl of Opti-MEM medium. After 5 min incubation, both solutions were mixed and incubated for 30 min. The transfection mixture was added to the cells containing 0.8 ml of 2% FBS containing DMEM without antibiotics. After incubation at 37°C for 6 h, culture medium was changed to DMEM containing 10% FBS. After 48 h of transfection, cells were washed with DMEM and used for different incubations. On termination of incubations, cells were lysed and luciferase activity was measured using Steady-Glo Luciferase Assay System (Promega, USA) with the help of Varioskan LUX Multimode Microplate Reader (Thermo Scientific, Finland).

Adipocyte Differentiation and Oil red O staining: Differentiated 3T3-L1 adipocytes were treated with palmitate in absence or presence of PPARβ/δ activator, GW0742. On termination of incubations, cells were fixed in 4% paraformaldehyde for 10 min and stained with Oil-Red O stain for 30 min at room temperature. Cells were then rinsed in 60% isopropanol followed by washed in PBS for three times. Cellular images were taken using inverted fluorescent microscope (Leica DMi8, Germany).

***Statistical analysis*:** All data were derived from at least three independent experiments and analyzed by one-way analysis of variance (ANOVA); where the F value indicated significance, means were compared by a post hocmultiple range test. All values were means±SEM. A level of $p < 0.05$ was considered significant. Statistical analyses were conducted using Sigma Plot 10.0 software.

Results:

To study the involvement of PPARβ/δ in preventing lipid induced insulin resistance, we incubated 3T3L1 adipocytes with PPARβ/δ agonist (GW501516 or GW0742) for 1 h followed by the incubation with a inducer of insulin resistance, palmitate (0.75mM) a saturated free fatty acid, for 6h in absence or presence of insulin to assess the effect of PPARβ/δ agonists in preventing lipid induced insulin resistance. Interestingly, both GW501516 and GW0742 prevents lipid induced impairment of insulin stimulated glucose uptake in 3T3L1 adipocytesas indicated by increased uptake of fluorescent labelled deoxyglucose (2-NBDG), however, the effect was more notable when cells treated with GW0742 (**Figure 1A**). Addition of increasing concentrations of GW0742 (5-20μM) to 3T3L1 adipocytes incubation showed a dose dependent improvement of insulin activity suppressed by palmitate (**Figure 1B**). Insulin binding to its receptor on target cell surface transduces a signal cascade which is initiated with insulin receptor tyrosine kinase phosphorylation and ultimately to protein kinase B or Akt through several signaling molecules. It could be seen from **Figure 2A** that all these signaling molecules activation were attenuated in 3T3L1 adipocytes by palmitate which was waived when incubated with PPARβ/δ agonist, GW0742. We also investigated Glut4 migration to cell membrane in response to PPARβ/δ agonist treatment in L6 myotubes as it is an important marker for insulin action which helps in transporting glucose into the cells. Insulin effected Glut4 migration from cytosol to membrane was prevented by palmitate while in cells treated with GW0742, palmitate did not inhibit insulin stimulated Glut4 migration (**Figure 2B**). These results indicate activation of PPARβ/δ in 3T3L1 adipocytes and L6 myotubes prevents lipid induced insulin resistance.

Recent studies showed that obesity-associated chronic low-grade inflammation in adipocytes play a crucial role in the pathogenesis of insulin resistance and T2DM. Palmitate strongly induces inflammatory states in adipocytes by the activation of JNK and IKK which induces serine phosphorylation in IRS and thus mitigates insulin action. To have more insight, specifically to observe whether the effect of PPARβ/δ agonist is mediated through the suppression of JNK and/or IKK, we analysed the pJNK and pIKKβ level in GW0742 treated cells. Palmitate induced upregulation of pJNK and pIKKβ level were significantly inhibited in cells treated with GW0742 (**Figure 3A**). To investigate further, we tested the activation status of NF-κB, a key transcription factor involved in inflammation. Increased abundance of κB luciferase activity in response to palmitate treatment was inhibited by the incubation with GW0742 (**Figure 3B**). All these results suggest that increased inflammatory milieu imposed by palmitate was effectively prevented by the activation of PPARβ/δ.

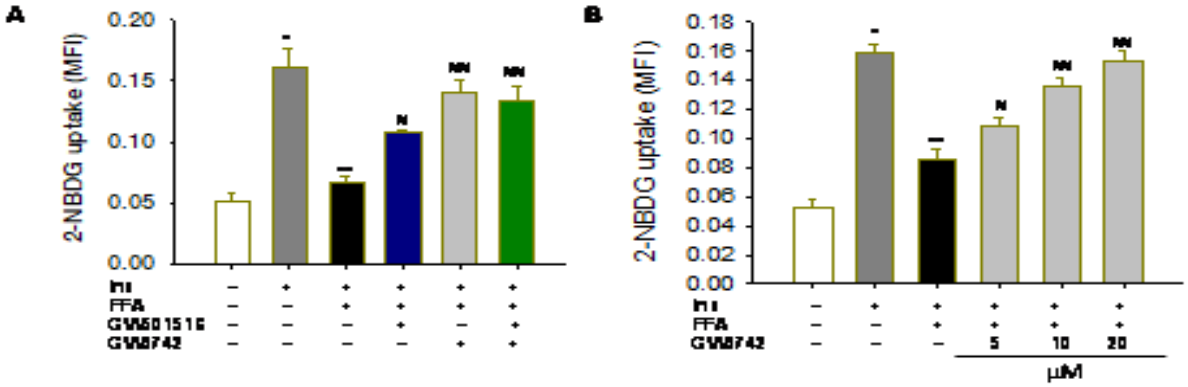


Figure 1: Palmitate (FFA) inhibition of insulin stimulated glucose uptake was prevented by PPARβ/δ agonists. (A)2NBDG uptake by 3T3L1 adipocytes incubated without or with insulin or insulin + FFA in presence or absence of GW501516 or GW0742. (B) 2NBDG uptake by 3T3L1 adipocytes in presence of increasing concentrations of GW0742. Each value is the mean ± SEM of three independent experiments, *p < 0.001 vs Con, **p < 0.001 vs Ins, #p < 0.01, ##p < 0.001 vs Ins + FFA.

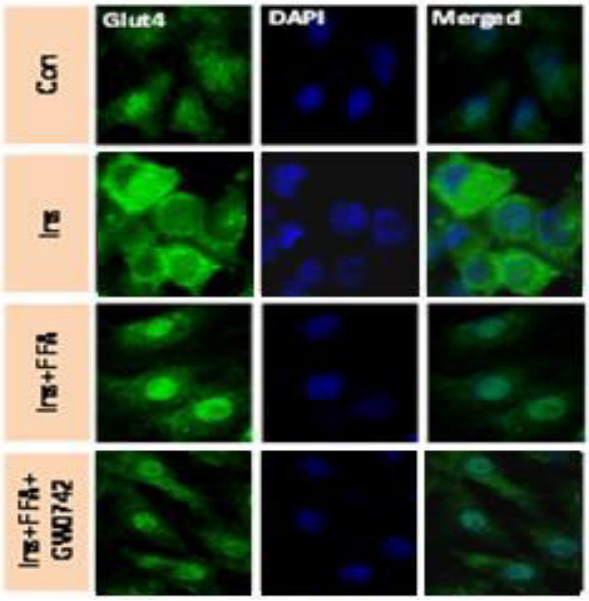


Figure 2: Effect of PPARβ/δ agonists on the lipid induced impairment of insulin signalling. Representative images of immunofluorescence analysis of Glut-4migration in L6 myotubes in response to various incubations as indicated above.

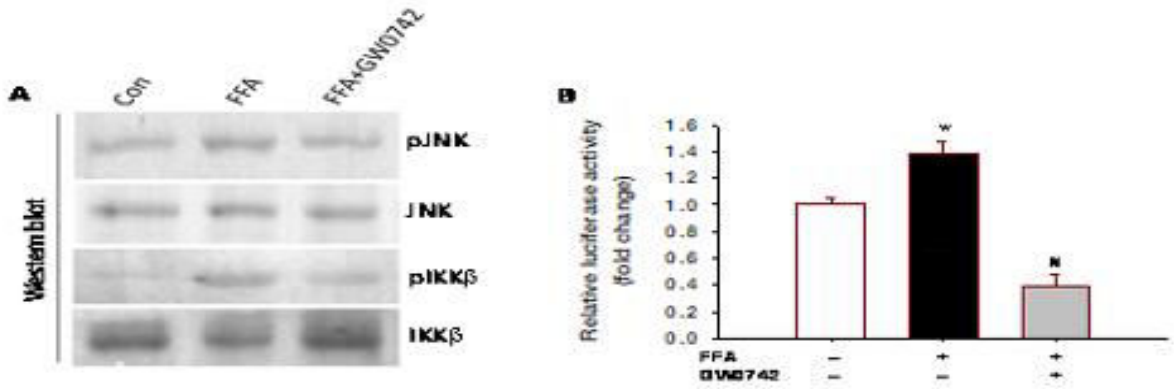


Figure 3: Suppression of adipocyte inflammation by PPARβ/δ agonist. (A) Western blot showing pJNK and pIKKβ abundance in 3T3L1 adipocytes incubated without or with FFA in absence or presence of GW0742. Total JNK and IKKβ were used as loading control. (B) kB-luciferase activity in adipocytes in response to indicated incubations. Each value is the mean ± SEM of three independent experiments, *p < 0.01 vs Con, #p < 0.001 vs FFA.

In addressing the ***objective 2*** of our project, we incubated 3T3L1 adipocytes with TLR4, AMPK and PKC inhibitors, CLI-095, Compound C and Rottlerin, respectively, for 1 h followed by the incubation with a inducer of insulin resistance, palmitate (0.75mM) a saturated free fatty acid, for 6h to investigate the involvement of TLR4, AMPK and PKC pathway in promoting lipid induced insulin resistance. Interestingly, TLR4 antagonist CLI-095 strikingly inhibits lipid induced NF-κB activation, a master regulator of inflammatory signalling (**Figure 4**). AMPK inhibitor did not produce any significant changes, however, treatment with PKC inhibitor showed meagre effect on NF-κB inactivation (**Figure 4**). All these indicate TLR4 signaling play a crucial role in lipid induced adipocyte inflammation. To analyse the effect of PPARβ/δ signaling in the prevention of lipid induced adipocyte inflammation, we incubated 3T3-L1 adipocytes with FFA in presence or absence of PPARβ/δ agonist GW0742 and on termination of incubations, cells were subjected to real time qPCR analysis of various proinflammatory cytokines gene expression. Marked induction of TNF-α, IL-1β, IL-6 and MCP-1 proinflammatory gene expression was observed in FFA treated cells which were significantly reduced when cells pretreated with GW0742 (**Figure 5**). Our results suggest that activation of PPARβ/δ notably prevents lipid-induced adipocyte inflammation by inhibiting TLR4 activation. Recent studies showed that obesity-associated chronic low-grade inflammation in adipocytes play a crucial role in the pathogenesis of insulin resistance and T2DM. Previously, we observed that palmitate induced activation of JNK and IKK promotes serine phosphorylation in IRS and mitigates insulin action. However, when cells were treated with GW0742, it notably suppressed JNK and IKK activation and prevents adipocytes insulin resistance.

To have more insight, specifically to observe whether the effect of PPARβ/δ agonist is mediated through the inhibition of lipid induced TLR4 activation, we incubated cells with PPARβ/δ agonist in presence of FFA and observed that NF-κB and IκB-α activation by FFA was significantly prevented by PPARβ/δ agonist GW0742 and TLR4 antagonist, CLI-095. This was confirmed by κB luciferase assay. Increased abundance of κB luciferase activity in response to palmitate treatment was inhibited by GW0742 and CLI-095 (**Figure 6**). These results suggest that increased inflammatory milieu imposed by lipid was effectively prevented by the activation of PPARβ/δ. Therefore, targeted activation of PPARβ/δ in adipocytes could be beneficial for lowering inflammatory cues in adipose tissue microenvironment and thus promoting insulin sensitivity and glucose homeostasis.

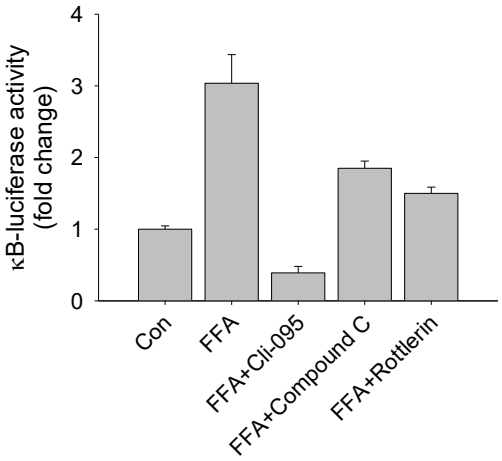


Figure 4: Palmitate (FFA) induced activation of NF-κB, a master regulator of inflammatory signalling, was prevented by TLR4 antagonist, CLI-095. The kB-luciferase activity analysis showing abundance of κB promoter activity in 3T3L1 adipocytes treated without or with FFA in presence or absence of TLR4, AMPK and PKC inhibitors, CLI-095, Compound C and Rottlerin, respectively.

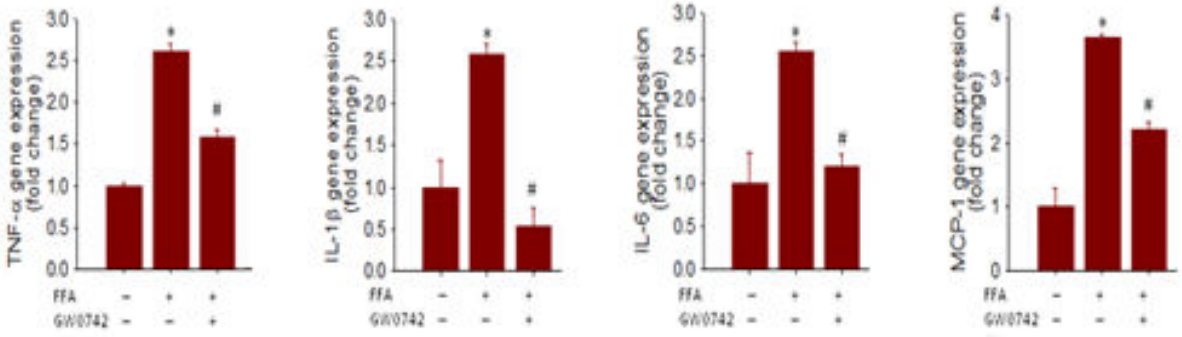


Figure 5: Lipid induced inflammatory cytokines expression was prevented by PPARβ/δ agonists, GW0742. Real time qPCR analysis of proinflammatory cytokines gene expression analysis depicts marked induction of TNF-α, IL-1β, IL-6 and MCP-1 gene expression in 3T3-L1 adipocytes in response to FFA incubation were notably suppressed by PPARβ/δ agonists, GW0742. Each value is the mean ± SEM of three independent experiments, *p < 0.001 vs Con, #p < 0.01 vs FFA.

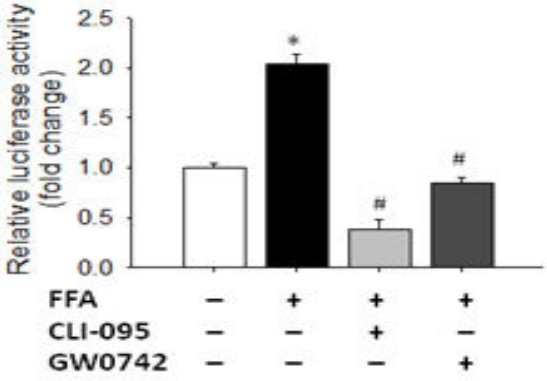


Figure 6: Prevention of FFA induced adipocytes NF-κB activation by TLR4 antagonist and PPARβ/δ agonist. Increased kB luciferase activitydue to FFA incubated cells was waived by GW0742 and CLI-095. Each value is the mean ± SEM of three independent experiments, *p < 0.001 vs Con, #p < 0.01 vs FFA.

In addressing the ***Objective 3*** of our project, we incubated THP-1 macrophages with PPARβ/δ activator, GW0742, for 1h followed by the 6h incubation of palmitate (0.75mM), a saturated free fatty acid and known inducer of insulin resistance, to investigate the effect of PPARβ/δ activation on macrophage polarization status. Palmitate treatment for 6h significantly induces the M1 polarization status as indicated by the increased abundance of iNOS, TNF-α, IL-1β, and MCP-1 gene expression (**Figure 7**). Interestingly, pretreatment of PPARβ/δ activator, GW0742 markedly prevents lipid-induced proinflammatory cytokines gene expression (**Figure 7**).

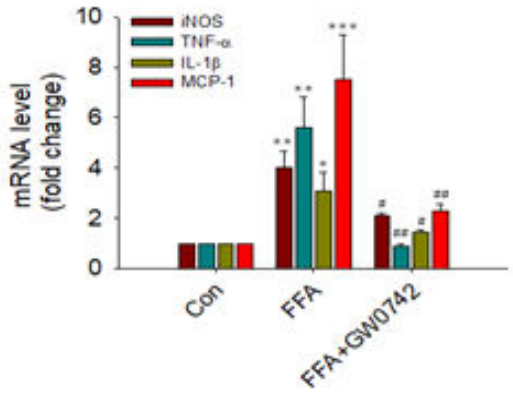


Figure 7: THP-1 macrophages were treated with palmitate in absence or presence of PPARβ/δ activator, GW0742. On termination of incubations, cells were harvested for RNA extraction followed by RT-PCR analysis of various M1 proinflammatory (iNOS, TNF-α, IL-1β, and MCP-1) gene expression. Each value is the mean ± SEM of three independent experiments, ***p < 0.001, **p < 0.01, *p < 0.05 vs Con, ##p < 0.01, #p < 0.05 vs FFA.

Obesity is known to regulate the macrophage polarization which favours its phenotypic switch from an anti-inflammatory M2 polarization state to a proinflammatory M1 polarization state in the adipose tissue and contributes for the development of insulin resistance. We therefore examined the M2 anti-inflammatory gene expressions particularly IL-4, IL-10, IL-13 and TGF-β in macrophages treated with palmitate in absence or presence of PPARβ/δ activator, GW0742. Interestingly, we observed that GW0742 notably upregulates the M2 antiinflammatory gene expression (**Figure 8**). It would be interesting to note that although we observed a massive upregulation of M1 proinflammatory gene expression in response to palmitate, however, we did not notice any significant alteration of M2 anti-inflammatory gene expressions in response to palmitate and that may be due to the

selected dose and time of palmitate incubation. However, GW0742 incubation leads to a massive stimulation of M2 markers with concomitant decrease of M1 markers in THP-1 macrophages indicate that cellular activation of PPARβ/δ could able to revert the macrophage polarization from M1 to M2 state that favours insulin sensitivity.

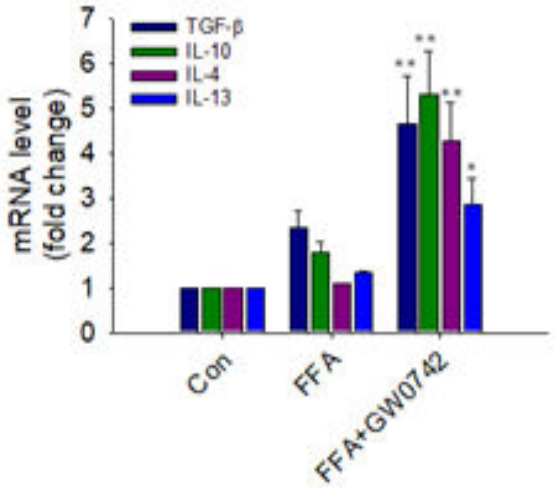


Figure 8: 3T3-L1 cells were treated with palmitate in absence or presence of GW0742. On termination of incubations, cells were harvested for RNA extraction followed by RT-PCR analysis of IL-4 and TGF-β gene expression. Each value is the mean ± SEM of 3 independent experiments, **p< 0.01, *p< 0.05 vs Con.

Therefore, our results suggest that PPARβ/δ activation notably prevents lipid-induced M1 proinflammatory gene expression and promotes M2 antiinflammatory gene expression in macrophages. Thus, our study clearly depicts that PPARβ/δ activation have profound effect on the attenuation of lipid-induced adipose tissue inflammation.

To explore the role of PPARβ/δ activation on macrophage-adipocyte cross-talk for inhibiting lipid-induced adipocytes insulin resistance, we coculture macrophage and adipocytes in Boyden chamber. Macrophages treated with PPARβ/δ activator, GW0742 for 1h were exposed to adipocytes incubated with palmitate, a saturated free fatty acid, for 4h and subjected to glucose uptake assay. Insulin stimulated increased uptake of 2-NBDG, a fluorescent labeled glucose analog, by adipocytes were strikingly inhibited when exposed to palmitate incubation. Interestingly, we observed that when macrophages exposed to PPARβ/δ activator, GW0742 and was cocultured with adipocytes, the lipid induced impairment of 2-NBDG uptake was waived (**Figure 9**). This result indicates that GW0742 incubated macrophages produced beneficial effect on lowering the lipid-induced adipocytes insulin insensitivity.

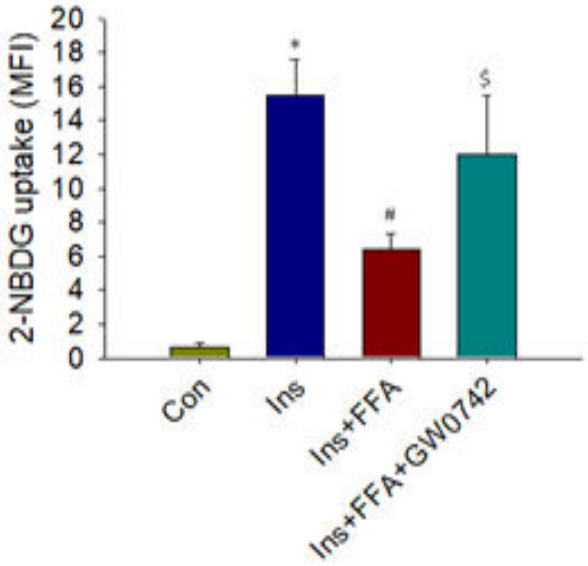


Figure 9: Analysis of 2-NBDG uptake by 3T3-L1 adipocytes in response to Insulin (Ins) or Ins + FFA (palmitate) in presence or absence of GW0742 treated macrophages cocultured in Boyden chamber. Each value is the mean ± SEM of three independent experiments. *$p < 0.01$ vs Con; #$p < 0.05$ vs Ins; $^{\$}p < 0.05$ vs Ins+FFA.

Adipose tissue was once considered an inert tissue, functioning solely as an energy storage depot for triglycerides. More recently, it has been viewed as a master regulatory tissue in controlling both glucose and lipid homeostasis in humans. The importance of adipose tissue in controlling whole-body metabolism is reinforced by the observation that a lack of adipose tissue leads to the development of insulin resistance and type 2 diabetes in mice and humans. The formation of mature adipocytes from preadipocytes, a process called adipogenesis, plays a crucial role in controlling normal adipose tissue function which not only stores energy in the form of triglycerides but it secretes a variety of adipokines that mainly govern cellular metabolism.

We therefore investigated whether PPARβ/δ activation might play a role in adipogenesis. We examined the effect of PPARβ/δ activator, GW0742 on the adipogenesis in-vitro. Oil red O staining for detection of adipocyte differentiation was investigated. Incubation of PPARβ/δ activator, GW0742, notably reduced lipid droplets in the cytoplasm from cultured 3T3-L1 cells (**Figure 10**).

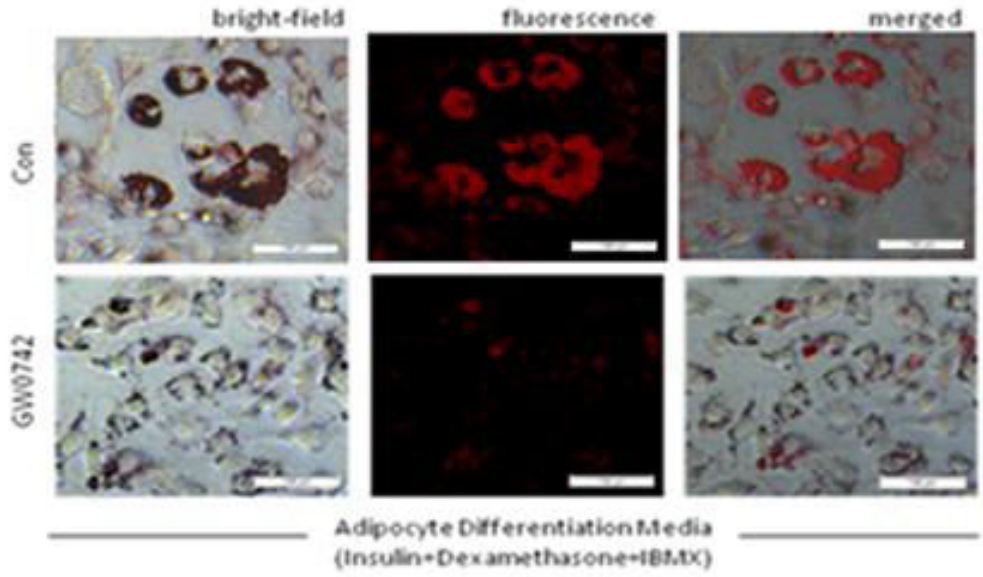


Figure 10: Images showing Oil-red O staining of 3T3-L1 adipocytes incubated without or with PPARβ/δ activator GW0742. Scale bar, 20 μm.

Moreover, we also analysed the gene expression and protein level of PPAR-γ and C/EBP-β, the master regulators of adipogenesis. We noticed that incubation of PPARβ/δ activator GW0742 strikingly lower the gene and protein expression of PPAR-γ and C/EBP-β as indicated by RT-PCR and Western blot analysis (**Figure 11**).

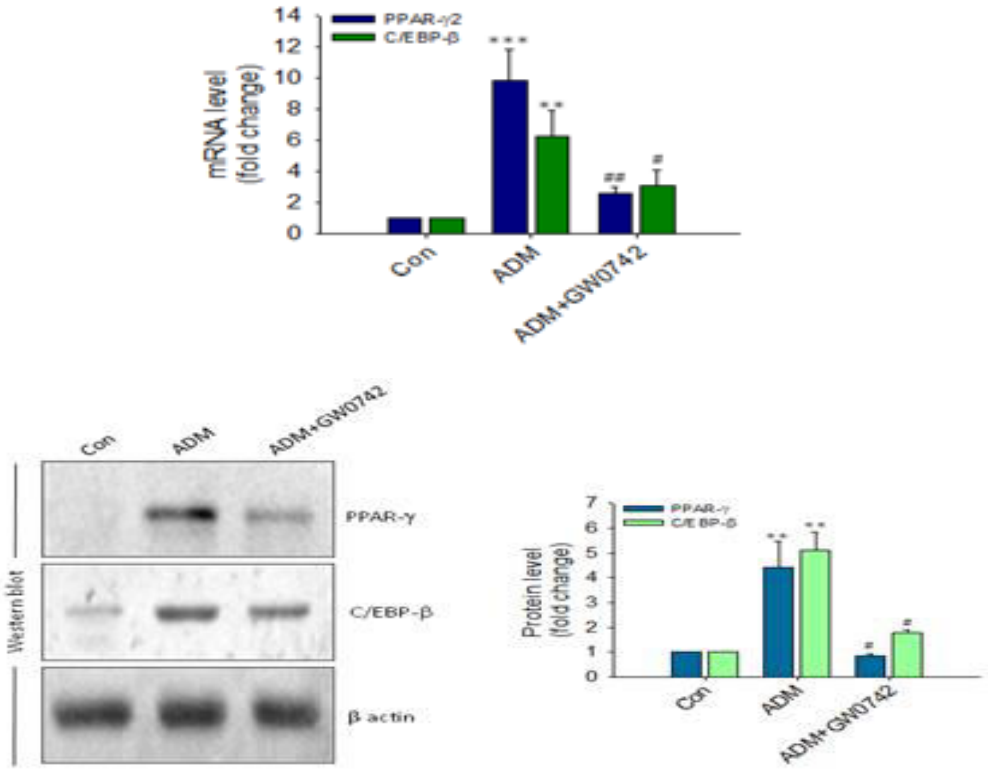


Figure 11: 3T3-L1 preadipocytes were treated with adipocyte differentiation medium (ADM) in absence or presence of PPARβ/δ activator, GW0742, for 7 days. On termination of incubations, cells were harvested for RNA extraction followed by RT-PCR analysis of PPAR-γ2 and C/EBP-β gene expression (top). In a separate set of same incubation, cells were subjected to Western blotting to observe the PPAR-γ and C/EBP-β level (bottom). Each value is the mean ± SEM of 3 independent experiments, **p < 0.01, *p < 0.05 vs Con, ##p < 0.01, #p < 0.05 vs ADM.

10. **Detailed analysis of results indicating contributions made towards increasing the state of knowledge in the subject:**

Over the last decade, an abundance of evidence has emerged demonstrating a close link between a state of chronic low-level inflammation and obesity induced insulin resistance (IR) and type 2 diabetes (T2D). It is well established that peroxisome proliferator-activated receptors (PPARs), members of the nuclear receptor superfamily of ligand-inducible transcription factors, play a key role in maintaining body metabolic homeostasis by linking lipid metabolism and innate immunity (3). Of the three PPAR isotypes found in mammals, PPARα and PPARγ are the targets for hypolipidemic (fibrates) and anti-diabetic (thiazolidinediones) drugs, respectively. In recent years, peroxisome proliferator-activated receptor β/δ (PPARβ/δ) activation has been proposed as a potential treatment for IR and metabolic syndrome (4-8). Activation of PPARβ/δ by high-affinity ligands enhances fatty acid catabolism in adipose tissue and skeletal muscle, with displaying a lean phenotype and is protected from high fat diet-induced obesity (9). However, there are no data showing involvement of PPARβ/δ and also the potential mechanism of suppressing inflammation and stimulating insulin sensitivity in adipocytes, a major insulin target cells.

Investigating the role of PPARβ/δ in preventing the lipid induced insulin resistance, we observed that activation of PPARβ/δ by GW0742 in 3T3L1 adipocytes and L6 myotubes strongly prevents palmitate-induced impairment of glucose uptake and insulin signalling pathway molecule inactivation and Glut4 migration. It was previously reported that lipid induced adipose tissue inflammation attenuates of insulin signaling pathway by the stimulation of serine phosphorylation in IRS1. Interestingly, we observed that PPARβ/δ agonist treatment markedly inhibits lipid induced activation of serine kinases such as JNK and IKKβ which are known to promote inhibitory serine phosphorylation at IRS1 that attenuate insulin signaling. Since prolong existence of insulin resistance in human being leads to the development of future pathogenesis of T2DM, our observation shows importance of PPARβ/δ as a possible therapeutic target against this insidious disease. To address the role of PPARβ/δ in preventing lipid-induced adipocyte inflammation, we observed that activation of PPARβ/δ in 3T3L1 adipocytes markedly prevents palmitate induced TLR4 activation and proinflammatory signalling cascade. Interestingly, we observed that PPARβ/δ agonist prevents NF-κB activation and various proinflammatory cytokines gene expression such as TNF-α, IL-1β, IL-6 and MCP-1 which

are known to cause inflamed adipocyte, the major causal factor for promoting adipocyte insulin resistance. Since prolong existence of insulin resistance in human being leads to the development of future pathogenesis of T2DM, activation of adipocytes PPARβ/δ could be a valuable option to inhibit adipose tissue inflammation and insulin resistance.

Obesity is known to regulate the macrophage polarization which favours its phenotypic switch from an M2 polarization state to a M1 polarization state in the adipose tissue and that contributes for the development of insulin resistance. We therefore interested to examine the effect of PPARβ/δ activator GW0742 on macrophage polarization status particularly whether lipid-induced overexpression of M1 markers could be reduced by PPARβ/δ activation with an induction of M2 anti-inflammatory gene expressions in macrophages. We have observed that lipid-induced overexpression of M1 macrophage markers were notably subdued by GW0742. It would be interesting to note that although we observed a massive upregulation of M1 proinflammatory gene expression in response to palmitate, however, we did not notice any significant alteration of M2 anti-inflammatory gene expressions in response to palmitate and that may be due to the selected dose and time of palmitate incubation. However, we noticed that GW0742 treatment significantly upregulates the M2 macrophage markers expression which indicate that cellular activation of PPARβ/δ could able to revert the macrophage polarization from M1 to M2 state that favours insulin sensitivity. Moreover, to explore the role of PPARβ/δ activation on macrophage-adipocyte cross-talk for inhibiting lipid-induced adipocytes insulin resistance, we incubated macrophages with PPARβ/δ activator, GW0742, and coculture with adipocytes exposed to palmitate, and analysed the adipocytes glucose uptake. Insulin stimulated increased uptake of glucose was strikingly inhibited when exposed to palmitate incubation and it was intriguing to note that adipocytes insulin sensitivity was induced when it was coculture with GW0742 treated macrophages. This result indicates that GW0742 incubated macrophages produced beneficial effect on lowering the lipid-induced adipocytes insulin insensitivity.

Adipose tissue now considered as a master regulatory tissue in regulating insulin sensitivity. Several studies have shown that adipogenesis, a process where mature adipocytes were formed from preadipocytes, plays a critical role in controlling normal adipose tissue function that govern cellular metabolism. We observed that PPARβ/δ activator, GW0742, notably reduced the adipogenesis process as indicated by reduction of lipid droplets and PPAR-γ and C/EBP-β gene and protein expression in 3T3-L1 cells. Therefore, the results we obtained from this study clearly depict the importance of PPARβ/δ activation in adipocytes as a possible therapeutic target against lipid-induced adipose tissue inflammation and insulin resistance.

11. **Conclusions summarizing the achievements and indication of scope for future work:**

Increased annual rate and prevalence of type 2 diabetes (T2D) is suggests that existing therapeutic measures are insufficient. Therefore, to find out a suitable drug target is imminent. Our findings shows the importance of PPARβ/δ activation in adipocytes, and sketeletal muscle cells, two major target cells for insulin action, that attenuates lipid induced insulin resistance. We have observed that activation of PPARβ/δ in adipocytes and macrophages notably inhibits lipid-induced adipose tissue inflammation by the inhibition of TLR4 activation. We also noticed that PPARβ/δ activation notably reduced the expression of PPAR-γ and C/EBP-β which inhibits massive lipid deposition in adipocytes and thus maintain the adipocyte insulin sensitivity. Prolong existence of insulin resistance in human being leads to the development of future pathogenesis of type 2 diabetes, therefore, our observation shows importance of PPARβ/δ as a therapeutic target against this insidious disease. Therefore, it would be ideal to explore different molecules potential to activate PPARβ/δ without imposing any toxic side effects.

12. **S&T benefits accrued:**

i. List of Research publications

S No	Authors	Title of paper	Name of the Journal	Volume	Pages	Year
1.	S.A. Choudhary, N. Bora, D. Banerjee, L. Arora, A.S. Das, R. Yadav, K-N. Klotz, D. Pal,, A.N. Jha, S. Dasgupta	A novel small molecule A_{2A} adenosine receptor agonist, indirubin-3'-monoxime, alleviates lipid-induced inflammation and insulin resistance in 3T3-L1 adipocytes	*Biochemical Journal*	DOI: 10.1042/BCJ20190251	-	2019

ii. Manpower trained on the project

a) Research Scientists or Research Associates : Nil
b) No. of Ph.D. produced : Nil
c) Other Technical Personnel trained : Two

iii. Patents taken, if any : Nil

13. **Financial Position:**

No	Financial Position/Budget Head	Funds Sanctioned	Expenditure	% of Total cost
I	Salaries/Manpower costs	NIL	NIL	NA
II	Equipment	Rs. 15,60,000	Rs. 15,60,000	100%
III	Consumables	Rs. 6,60,000	Rs. 6,31,616.34	104.6%
IV	Contingencies		Rs. 34,891	
V	Travel		Rs. 24,153	
VI	Overhead Expenses	Rs. 3,00,000	Rs. 2,53,130	84.4%
VII	Others, if any	NIL	NIL	NA
	Total	Rs. 25,20,000	Rs. 25,03,790.34	99.36%

14. **Procurement/ Usage of Equipment**

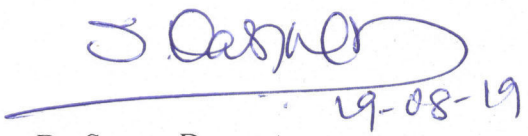
a)

S No	Name of Equipment	Make/Model	Cost (Rs)	Date of Installation	Utilization Rate (%)	Remarks regarding maintenance
1.	UV-Visible spectrophotometer	Make: Eppendorf Model: Biospectrometer, Kinetic	5,19,750.00	13.06.2016	100	None
2.	Table top cold centrifuge	Make: Eppendorf Model:5810-R	5,69,625.00	13.06.2016	100	None
3.	Thermal cycler (PCR machine)	Make: BioRad Model:C1000 Touch	4,69,981.00	29.03.2016	100	None

b) Plans for utilizing the equipment facilities in future

We are utilizing all the equipment purchased under this project to perform research work in our laboratory. We have not asked these instruments to perform the research work funded by other Govt. funding agencies and therefore request the SERB to kindly permit us for utilizing these instruments to perform our research work at Tezpur University.

Name and Signature with Date:



Dr. Suman Dasgupta
(Principal Investigator)

Annexure-II

REQUEST FOR ANNUAL INSTALMENT WITH UP-TO-DATE STATEMENT OF EXPENDITURE

1. SERB Sanction Order No and date : YSS/2014/000067 dated 20/10/2015

2. Name of the PI : Dr. Suman Dasgupta

3. Total Project Cost : Rs. 25,20,000/-

4. Revised Project Cost : Not Applicable

5. Date of Commencement : 16.11.2015

6. Statement of Expenditure :
(Month wise expenditure incurred during current financial year)

Month & year	**Expenditure incurred/ committed**
June 2018	Rs. 1,37,003 (Consumable expenditure incurred)
December 2018	Rs. 24,376 (Consumable expenditure incurred)
February 2019	Rs. 42,267 (Consumable expenditure incurred)
March 2019	Rs. 27,712.34 (Consumable expenditure incurred)

7. Grant received in each year:

a. 1st Yéar : Rs. 18,80,000/-

b. 2nd Year : Rs. 2,50,000/-

c. 3rd Year : Rs. 2,50,000/-

e. 4th Year : Rs. 80,000/-

d. Interest, if any : Rs. 17,115/-

e. Total (a + b + c + d + e) : Rs. 24,77,115/-

Statement of Expenditure [From 16.11.2015 (DOS*) to 15.02.2019 for the financial year 2018-19]

Sr. No	Sanctioned Heads	Total Funds Allocated (indicate sanctioned or revised)	Expenditure Incurred				Total Expenditure till 31.03.2018	Balance as on (15.02.2019)	Requirement of Funds	Remarks (if any)
			1st Year (16.11.2015 DOS to 31.03.2016)	2nd Year (01.04.2016 to 31.03.2017)	3rd Year (01.04.2017 to 31.03.2018)	4th Year (01.04.2018 to 15.02.2019)				
(I)	(II)	(III)	(IV)	(V)	(VI)		(VII = IV + V+ VI)	(VIII = III –VII)		
1.	Manpower costs	NIL	NA	NA	NA	NA	NA	NA	NA	
2.	Consumables	Rs. 6,00,000	Rs. 2,13,134	Rs. 1,30,864	Rs. 51,260	Rs. 2,36,358.34	Rs. 6,90,660.34	Rs. -90,660.34	NIL	
3.	Travel		NIL	Rs. 24,153	NIL	NIL				
4.	Contingencies		NIL	NIL	Rs. 34,891	NIL				
5.	Others, if any Interest earned	Rs. 17,115	NIL	NIL	NA	NIL	NIL	Rs. 17,115	NIL	
6.	Equipment	Rs. 15,60,000	NIL	Rs. 15,59,356	Rs. 644	NIL	Rs. 15,60,000	NIL	NIL	
7.	Overhead expenses	Rs. 3,00,000	Rs. 62,500	Rs. 1,28,130	Rs. 62,500	NIL	Rs. 2,53,130	Rs. 46,870	NIL	
8.	Total	Rs. 24,77,115	Rs. 2,75,634	Rs. 18,42,503	Rs. 1,49,295	Rs. 2,36,358.34	Rs. 25,03,790.34	Rs. -26,675.34	Rs. 26,675.34	

S. Dasgupta

(Dr. Suman Dasgupta)
Name and Signature of Principal Investigator:
Date: 09.07.19

Signature of Competent financial authority:
(with seal) Date:

Finance Officer
Tezpur University

* DOS – Date of Start of project :**16.11.2015**

Note: 1. Expenditure under the sanctioned heads, at any point of time, should not exceed funds allocated under that head, without prior approval of SERB i.e. Figures in Column (VIII) should not exceed corresponding figures in Column (III) 2. Utilization Certificate (Annexure III) for each financial year ending 31st March has to be enclosed along with request for carry- forward permission to the next financial year.

UTILISATION CERTIFICATE
FOR THE FINANCIAL YEAR-ENDING 31ST MARCH 2018-19
(To be given separately for each financial year ending on 31st March)

UC pertains to
✔*Appropriate box*

First Release	Second Release	Third Release	Fourth Release ✔	Final Release

Is the UC provisional : YES

1. Title of the Project/Scheme : "Exploring the effects of peroxisome proliferator activated receptor beta/delta activation in lipid induced adipocyte inflammation and insulin resistance".

2. Name of the Principal Investigator : Dr. Suman Dasgupta

3. Implementing Institution : Tezpur University

4. SERB sanction order No.& date : YSS/2014/000067 dated 20/10/2015

5. Amount brought forward from the previous financial year quoting SERB letter number and date in which the authority to carry forward the said amount was given : (i) Amount : NIL
(ii) Letter no. : YSS/2014/000067
(iii) Date : 25.09.2018

6a. Amount received during the financial year (Please give SERB letter/order no.and date for the amount) : (i) Amount : NIL
(ii) Order no. : NA
(iii) Date : NA

6b. Interest earned, if any : NIL

7. Total amount that was available for expenditure Rs (excluding commitments during the financial year (Sr.no.5+6a+6b) : NIL

8. Actual expenditure (excluding commitments) incurred during the financial year (upto 31.03.17) : NIL

9. Balance amount available at the end of the financial year (8-9): OR/ Negative balance (if expenditure incurred is more than the funds released) : NIL

10. Unspent balance, if any, refunded to SERB (give details of cheque/DD no. etc.) : NIL

11. Amount to be carried forward to the next financial year (if any) : NIL

UTILISATION CERTIFICATE

Certified that out of **Rs. 15,60,000/-** of **Non-Recurring** grants-in-aid sanctioned during the year **2015-2016** in favour of **The Registrar, Tezpur University** under **SERB letter/order No. YSS/2014/000067 dated 20/10/2015** was unutilized in the first year and **carried forward** under **SERB letter/order No. YSS/2014/000067 dated 15.11.2016** during the period of **2016-2017**; a sum of **Rs. 15,59,356/-** has been utilized for the purpose for which it was sanctioned and the balance of **Rs. 644/-** remaining unutilized at the end of the year 2017. **The balance amount of Rs. 644/- was refunded to SERB through DD No. 049783 on 30.08.2017.**

Certified that we have satisfied ourselves that the conditions on which the grants-in-aid was sanctioned have been fulfilled / are being fulfilled and that we have exercised the following checks to see that the money was actually utilized for the purpose for which it was sanctioned.

Kinds of checks exercised: Nil

Signature of PI
Date 03.07.19
Dr. S. Dasgupta
PI, DST-SERB Project
SERB Ref. No.- YSS/2014/000067
Univ Ref. No.- DoRD/MBBT/SD/20-258
Dept. of MBBT, Tezpur University
Assam- 784028

Signature of Registrar/Accounts Officers
Date 3/7/19
Finance Officer
Tezpur University

Signature of Head of Institution
Date 3/7/19
Registrar
Tezpur University

Guidelines for preparation of U.C.

1. U.C. should be only for the grants released by the SERB. Please do not account for Security deposits/other matching grants/account opening charges and miscellaneous items.
2. SERB Sanction No. and Dt. should be accurately shown in the U.C.
3. Even if the grant is unutilized in the financial year in which the grant was released by SERB a NIL, U.C. needs to be forwarded to SERB along with a request for carrying forward the grant to the next financial year. Such grants which are carried forward must be shown in the subsequent U.C. as carried forward grant and not amount received in the subsequent year (ref Sl No. 5 on pre-page).

Science and Engineering Research Board

UC accepted has been accepted by

Signature:_____

Name of the SERB Officer:_____

Designation:_____

UTILISATION CERTIFICATE
FOR THE FINANCIAL YEAR-ENDING 31ST MARCH 2018-19

(To be given separately for each financial year ending on 31st March)

UC pertains to ✔*Appropriate box*	First Release	Second Release	Third Release	Fourth Release ✔	Final Release

Is the UC provisional : YES

1. Title of the Project/Scheme : "Exploring the effects of peroxisome proliferator activated receptor beta/delta activation in lipid induced adipocyte inflammation and insulin resistance".

2. Name of the Principal Investigator : Dr. Suman Dasgupta

3. Implementing Institution : Tezpur University

4. SERB sanction order No.& date : YSS/2014/000067 dated 20/10/2015

5. Amount brought forward from the previous financial year quoting SERB letter number and date in which the authority to carry forward the said amount was given : (i) Amount : Rs. 1,25,906/- (ii) Letter no. : YSS/2014/000067 (iii) Date : 25.09.2018

6a. Amount received during the financial year (Please give SERB letter/order no.and date for the amount) : (i) Amount : Rs. 80,000/- (ii) Order no. : YSS/2014/000067 (iii) Date : 25.09.2018

6b. Interest earned, if any : Rs. 3,777/-

7. Total amount that was available for expenditure Rs (excluding commitments during the financial year (Sr.no.5+6a+6b) : Rs. 2,09,683/-

8. Actual expenditure (excluding commitments) incurred during the financial year (upto 31.03.19) : Rs. 2,36,358.34/-

9. Balance amount available at the end of the financial year (8-9): OR/ Negative balance (if expenditure incurred is more than the funds released) : Rs. -26,675.34/-

10. Unspent balance, if any, refunded to SERB (give details of cheque/DD no. etc.) : NIL

11. Amount to be carried forward to the next financial year (if any) : NIL

UTILISATION CERTIFICATE

Certified that out of **Rs. 80,000/-** of **Recurring** grants-in-aid sanctioned during the year **2018-19** in favour of **The Registrar, Tezpur University** under SERB letter/order No. **YSS/2014/000067 dated 25.09.2018** and **Rs. 1,25,906/-** on account of unspent balance of the previous year 2017-18, along with the interest earned from the bank of **Rs. 3777-**; a sum of **Rs. 2,36,358.34/-** has been utilized for the purpose for which it was sanctioned and the negative balance of **Rs. -26,675.34/-** occurred at the end of the year.

Certified that we have satisfied ourselves that the conditions on which the grants-in-aid was sanctioned have been fulfilled / are being fulfilled and that we have exercised the following checks to see that the money was actually utilized for the purpose for which it was sanctioned.

Kinds of checks exercised:

1. Rs. 1,37,003/- for Consumables
2. Rs. 24,376/- for Consumables
3. Rs. 42,267/- for Consumables
4. Rs. 27,712.34/- for Consumables

Signature of PI
Date 09.07.19

Dr. S. Dasgupta
PI, DST-SERB Project
SERB Ref. No.- YSS/2014/000067
Univ Ref. No.- DoRD/MBBT/SD/20-258
Dept. of MBBT, Tezpur University
Assam- 784028

Signature of Registrar/Accounts Officers
Date 31/7/19
Finance Officer
Tezpur University

Signature of Head of Institution
Date 31/7/19
Registrar
Tezpur University

Guidelines for preparation of U.C.

4. U.C. should be only for the grants released by the SERB. Please do not account for Security deposits/other matching grants/account opening charges and miscellaneous items.
5. SERB Sanction No. and Dt. should be accurately shown in the U.C.
6. Even if the grant is unutilized in the financial year in which the grant was released by SERB a NIL, U.C. needs to be forwarded to SERB along with a request for carrying forward the grant to the next financial year. Such grants which are carried forward must be shown in the subsequent U.C. as carried forward grant and not amount received in the subsequent year (ref Sl No. 5 on pre-page).

Science and Engineering Research Board

UC accepted has been accepted by

Signature: _____

Name of the SERB Officer: _____

Designation: _____