Scientific and Technical Progress Report (STPR)[FINAL]
(R&D projects)

BRIEF COMPLETION REPORT

1. Project Title: To investigate the role of toll-like receptor 4 (TLR4) in lipid induced impairment of adipogenesis and adipose tissue function implementing insulin resistance.

2. DBT Sanction Order No. & Date: BT/08/IYBA/2014-12 dated 01.09.2015

3. Name of Principal Investigator: Dr. Suman Dasgupta

Name of Co-PI/Co-Investigator: None

4. Institute: Tezpur University

5. Address with Contact Nos. (Landline & Mobile) & Email :

Department of Molecular Biology and Biotechnology,
Tezpur University, Tezpur – 784028, Napaam,
Dist: Sonitpur, Assam
Phone: +91-3712-275441 (O); +91-9954332278 (M)
Email: suman.dsut@gmail.com ; suman@tezu.ernet.in

6. Total Cost: Rs. 42.30 lakh

7. Duration: Three years

8. Approved Objectives of the Project:

Objective 1: To study the role of TLR4 in lipid induced impairment of white and brown fat adipogenesis.
Objective 2: To investigate the role of TLR4 in lipid induced adipocyte dysfunction and insulin resistance.
Objective 3: To evaluate the role of TLR4 in lipid induced macrophage infiltration and macrophage M1 polarisation state in the adipose tissue.

9. Summary and Conclusions:

We have found that TLR4 play a pivotal role in mesenchymal stem cell to adipocyte conversion. The morphological changes associated with lipid accumulation and gene expression profile clearly demonstrated importance of TLR4 signaling in the differentiation of mesenchymal stem cell to adipocyte. However, future in-vivo study with TLR4 knockout mice is needed to confirm this observation. Moreover, we found that the TLR4 mediated inflammatory signaling communicated with mTORC2-Akt-mTORC1 metabolic cascade in macrophage and thereby promoting lipid uptake and foam cell formation. Mechanistically, LPS treatment markedly upregulates TLR4 mediated inflammatory pathway which by activating mTORC2 induces Akt phosphorylation at serine 473 and that aggravate mTORC1 dependent scavenger receptors expression and consequent lipid accumulation in THP-1 macrophages. Inhibition of mTORC2 either by silencing Rictor expression or inhibiting its association with mTOR notably prevents LPS induced Akt activation, scavenger receptors expression and macrophage lipid accumulation. Although suppression of mTORC1 expression by genetic knockdown of Raptor did not produce any significant change in Akt S473 phosphorylation, however, incubation with Akt activator in Rictor silenced cells failed to promote scavenger receptors expression and macrophage foam cell formation. Thus, present research explored the signaling pathway involved in inflammation induced macrophage foam cells formation and therefore, targeting this pathway might be useful for preventing macrophage foam cell formation.

Scientific and Technical Progress Report (STPR)[FINAL]
(R&D projects)

Section-A : Project Details

A1. **Project Title:** To investigate the role of toll-like receptor 4 (TLR4) in lipid induced impairment of adipogenesis and adipose tissue function implementing insulin resistance.

A2. **DBT Sanction Order No. & Date:** BT/08/IYBA/2014-12 dated 01.09.2015

A3. **Name of Principal Investigator:** Dr. Suman Dasgupta
Name of Co-PI/Co-Investigator: None

A4. **Institute:** Tezpur University

A5. **Address with Contact Nos. (Landline & Mobile) & Email :**
Department of Molecular Biology and Biotechnology,
Tezpur University, Tezpur – 784028, Napaam,
Dist: Sonitpur, Assam
Phone: +91-3712-275441 (O); +91-9954332278 (M)
Email: suman.dsut@gmail.com ; suman@tezu.ernet.in

A6. **Total Cost:** Rs. 42.30 lakh

A7. **Duration:** Three years

A8. **Approved Objectives of the Project:**

Objective 1: To study the role of TLR4 in lipid induced impairment of white and brown fat adipogenesis.

Objective 2: To investigate the role of TLR4 in lipid induced adipocyte dysfunction and insulin resistance.

Objective 3: To evaluate the role of TLR4 in lipid induced macrophage infiltration and macrophage M1 polarisation state in the adipose tissue.

A9. **Specific Recommendations made by the Task Force (if any):** None

Section-B : Scientific and Technical Progress

B1. **Progress made against the Approved Objectives, Targets & Timelines during the Reporting Period** (1500-2500 words; 2500-3500 words for final report; data must be included in the form of 2-7 figures and/or tables).

To examine the involvement of toll-like receptor 4 (TLR4) in the impairment of adipogenesis, we treated mesenchymal stem cells and 3T3-L1 preadipocytes with TLR4 inhibitor Cli-095 and analysed the cellular differentiation under the influence of adipocyte differentiation medium (ADM). Interestingly, we noticed that Cli-095 treatment considerably impaired the ADM-induced adipogenesis and cellular glucose uptake, however, Cli-095 treatment was unable to attenuate the conversion of 3T3-L1 preadipocytes to adipocytes and glucose uptake in response to ADM (**Fig. 1A,B**).

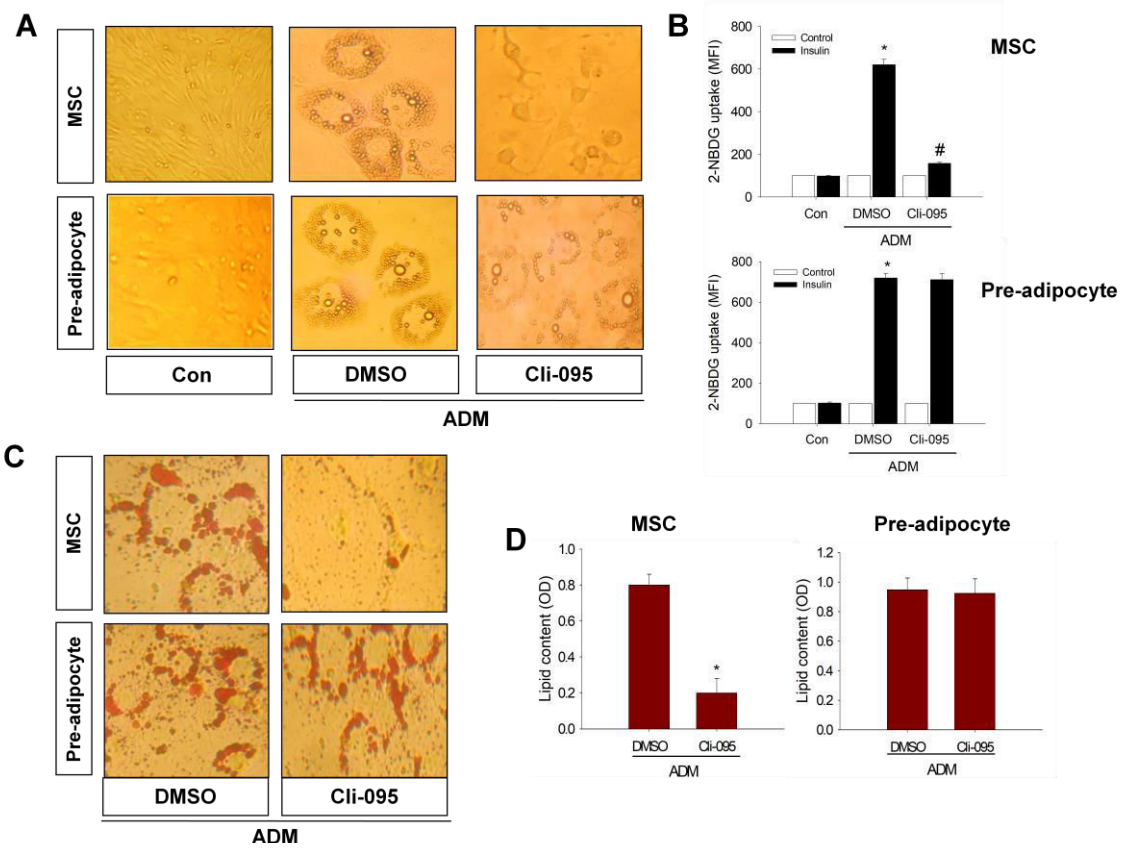

Fig. 1. TLR4 signalling regulates adipogenesis. (**A**) Representative bright-field images showing morphological alteration of mesenchymal stem cells and 3T3-L1 preadipocytes when treated with Cli-095 and subjected to ADM incubation. (**B**) The 2-NBDG uptake assay demonstrating cellular glucose uptake by mesenchymal stem cells and 3T3-L1 preadipocytes when treated without or with Cli-095 in presence of ADM incubation. (**C,D**) Representative Oil-Red O staining images (C) and their quantifications (D) showing lipid accumulation in mesenchymal stem cells and 3T3-L1 preadipocytes when treated with Cli-095 and subjected to ADM incubation. Each value is the mean±SEM of three independent experiments, **p<0.01 vs Con/DMSO.

This was further conformed by Oil-red O staining, as the impairment of lipid accumulation was evident when mesenchymal stem cells were treated with Cli-095 and subjected to ADM-induction, whereas, treatment of Cli-095 was unable to inhibit ADM-induced adipogenesis (**Fig. 1A,B**). Conversion of mesenchymal stem cells and 3T3-L1 preadipocytes to adipocytes was further assessed by investigating the cell specific markers, such as Sca-1, CD34, CD105, FABP4, FATP and TLE3 in mesenchymal stem cells and DLK1, FABP4 for preadipocytes by qPCR analysis. This corroborated with our observation that Cli-095 treatment reduced the mesenchymal stem cells to adipocyte differentiation but failed in the conversion of preadipocytes to adipocytes (**Fig. 2A,B**).

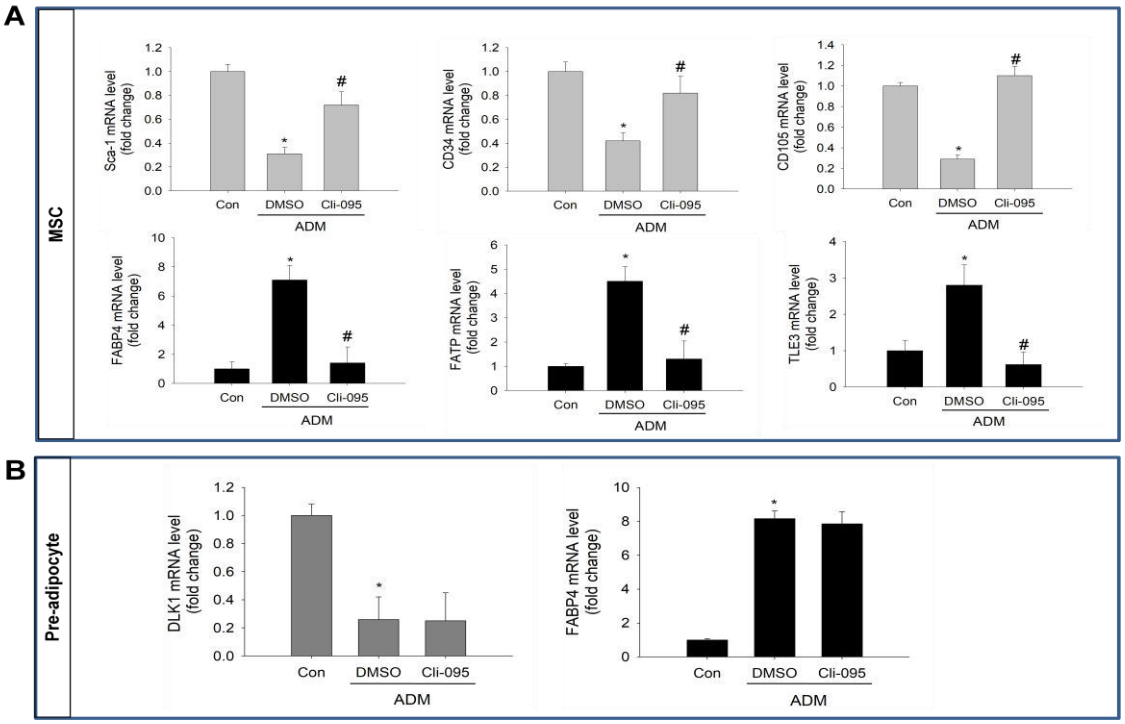

Fig. 2. TLR4 signalling inhibition altered the gene expression profile of adipogenesis. (**A**) RT-qPCR analysis showing abundance of Sca-1, CD34, CD105, FABP4, FATP, and TLE3 mRNA levels in mesenchymal stem cells treated without or with ADM in the presence of vehicle control (DMSO) or Cli-095. β-actin was used as loading control for RT-qPCR analysis. *p<0.05 vs Con, #p<0.05 vs DMSO. (**B**) RT-qPCR analysis showing abundance of DLK1, and FABP4 mRNA levels in 3T3-L1 preadipocytes treated without or with ADM in the presence of vehicle control (DMSO) or Cli-095. β-actin was used as loading control for RT-qPCR analysis. *p<0.05 vs Con, #p<0.05 vs DMSO.

Interestingly, we observed that TLR4 signaling also play a critical role in macrophage lipid accumulation and foam cell formation. We therefore aimed to investigate the role of TLR4 mediated inflammatory milieu in macrophage foam cell formation. For this, differentiated THP-1 macrophages were incubated with LPS in presence of TLR4 pathway inhibitor Cli-095 or PI3K inhibitor LY294002 followed by the treatment of oxLDL for 16 h. LPS incubation strongly induces accumulation of oxLDL as indicated by Oil-Red O staining which was prevented when cells were pre-treated with Cli-095 or LY294002 (**Fig. 3A**). Estimation of lipid accumulation in Oil-Red O stained cells by measuring fluorescent intensity further confirms our observation (**Fig. 3B**).

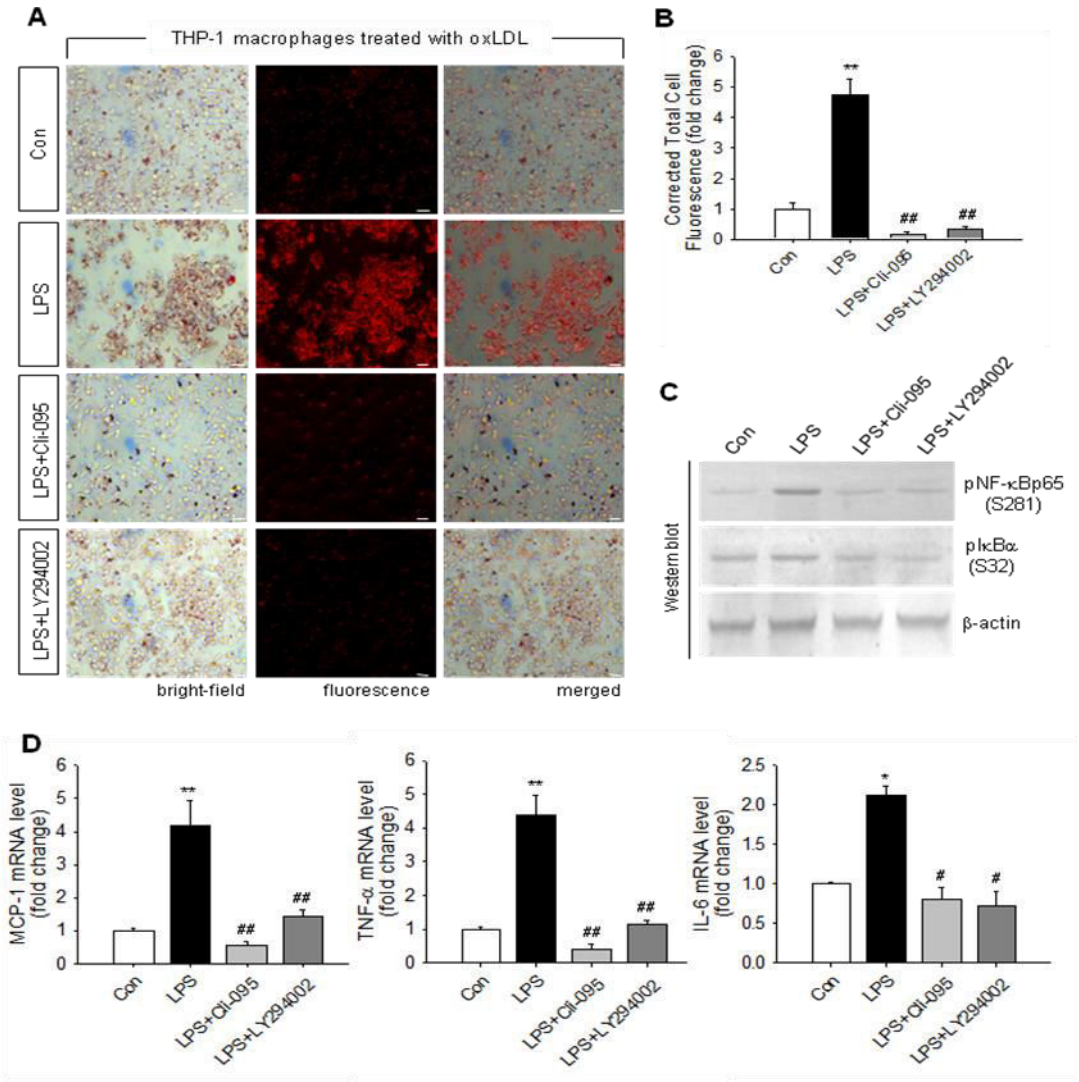

Fig. 3. LPS induced TLR4-PI3K signalling promotes macrophage foam cell formation. (**A**) Representative bright-field (left), fluorescent (middle) and merged (right) images of Oil-Red O staining (**A**) and the quantification of corrected total cell fluorescence (**B**) of THP-1 macrophages incubated with oxLDL (50 μg/ml) for 16 h in absence or presence of LPS (100 ng/ml) with or without TLR4 pathway inhibitor, Cli-095 (3 μM) or PI3K signaling antagonist, LY294002 (50 μM). Scale bar, 75 μm. Corrected total cell fluorescence (CTCF) was calculated using following formula with the help of image J software. CTCF = Integrated Density – (Area of selected cell X Mean fluorescence of background readings). Each value is the mean±SEM of three independent experiments, **p<0.01 vs Con, ##p<0.01 vs LPS. (**C**) Western blot showing abundance of pNF-κBp65 (S281) and pIκB-α (S32) level and (D) Real time quantitative PCR analysis indicates fold changes in MCP-1, TNF-α and IL-6 gene expression in indicated incubations. GAPDH served as an internal control. Each value is the mean±SEM of three independent experiments, **p<0.01, *p<0.05 vs Con, ##p<0.01, #p<0.05 vs LPS.

Although, previous reports highlighted that PI3K play a critical role in inflammatory induced atherosclerosis development, the underlying mechanisms of its involvement in macrophage foam cell formation is yet not clear. LPS treatment markedly enhanced the activation of TLR4-NF-κB pathway as indicated by increased abundance of phospho-NF-κBp65 and phospho-IκB-α level, whereas Cli-095 or LY294002 incubation prevented this activation (**Fig. 3C)**.

Analysis of proinflammatory cytokines gene expression also revealed that LPS stimulated MCP-1, TNF-α and IL-6 gene expression was attenuated when cells were pre-treated with Cli-095 or LY294002 (**Fig. 3D**). Collectively, these results indicate that inhibition of TLR4 or PI3K signaling in macrophages significantly prevents LPS induced foam cell formation suggesting a strong correlation between TLR4 and PI3K activation in LPS induction of macrophage lipid accumulation.

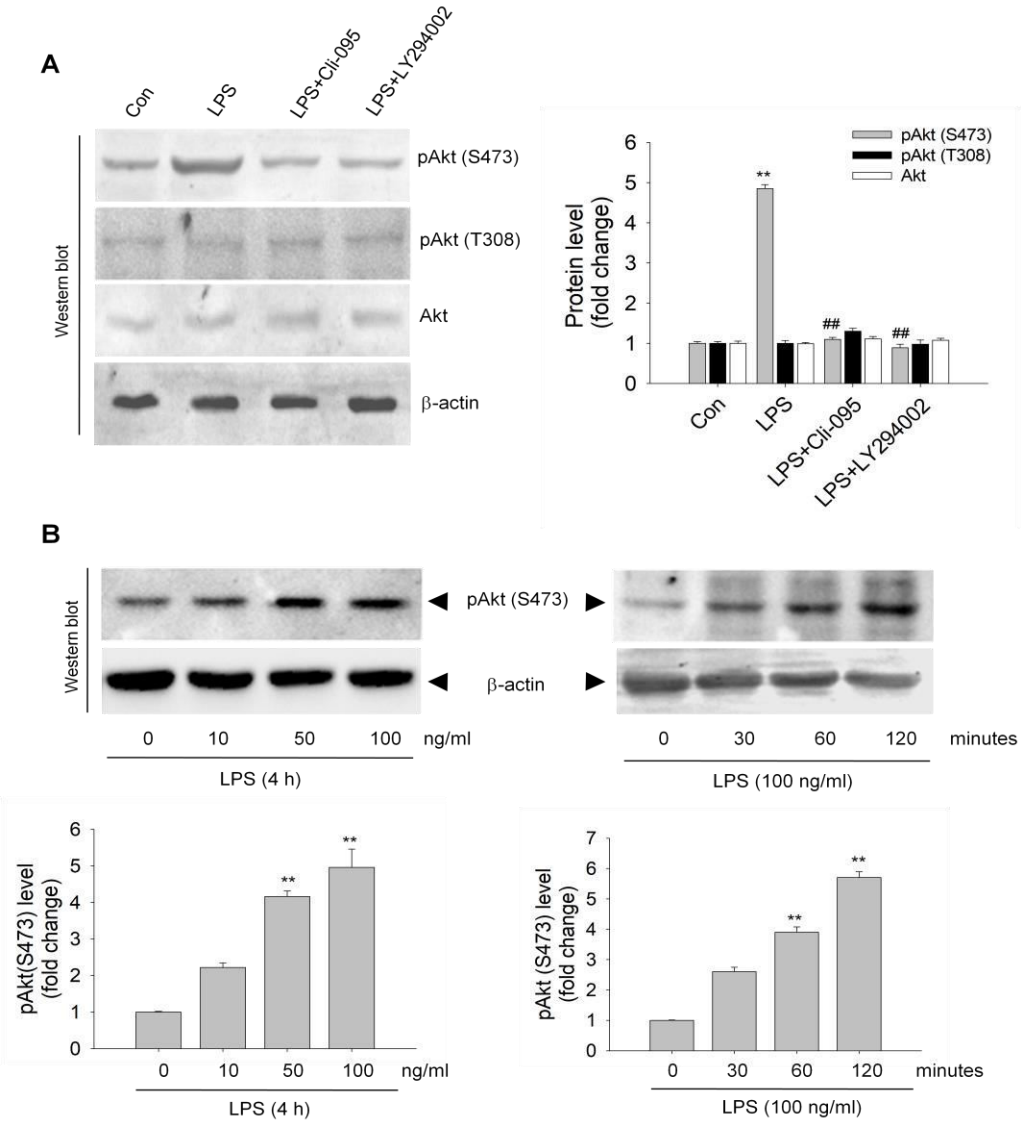

Fig. 4. LPS stimulates PI3K dependent Akt phosphorylation at serine 473.
(**A**) Western blot images (left) and quantifications (right) showing abundance of pAkt (S473), pAkt (T308) and total Akt level in THP-1 macrophages incubated with LPS (100 ng/ml) in absence or presence of TLR4 pathway inhibitor, Cli-095 (3 μM) or PI3K signaling antagonist, LY294002 (50 μM). β-actin used as loading control. Each value is the mean±SEM of three independent experiments, **p<0.01 vs Con, ##p<0.01 vs LPS. (**B**) Western blot images (left) and quantifications (right) showing dose and time dependent effect of LPS on pAkt (S473) level in THP-1 macrophages. β-actin used as loading control. Each value is the mean±SEM of three independent experiments, *p<0.05, **p<0.01 vs Con.

To explore further about the downstream signaling of TLR4 dependent PI3K pathway, we examined the activation status of Akt because of two reasons, (i) several studies highlighted the importance of Akt in its involvement for the progression of atherosclerosis and (ii) Akt activation depends on its phosphorylation at T308 and S473 residues by PI3K and mTORC2, respectively. Surprisingly, we observed that LPS treatment did not produce any significant change in Akt phosporylation at T308. However, more unexpectedly we have detected a strong induction of Akt S473 phosporylation without any significant alteration of its total protein level (**Fig. 4A)**. Interestingly, inhibition of TLR4 or PI3K pathway, subdued Akt S473 phosporylation level (**Fig. 4A)**. This result indicates that PI3K confers an important role in TLR4 induced Akt S473 phosporylation in THP-1 macrophages. This is quite an unusual observation as previous reports indicated that growth factor induced PI3K activation leads to Akt activation at T308 and therefore it could be possible that depending on the type of signal and its cognate receptor activation, the PI3K molecule may act differently on its substrate Akt. Moreover, dose and time dependent analysis of Akt S473 phosphorylation showed maximum stimulation in response to 50 ng/ml of LPS for 2 h of incubation (**Fig. 4B**).

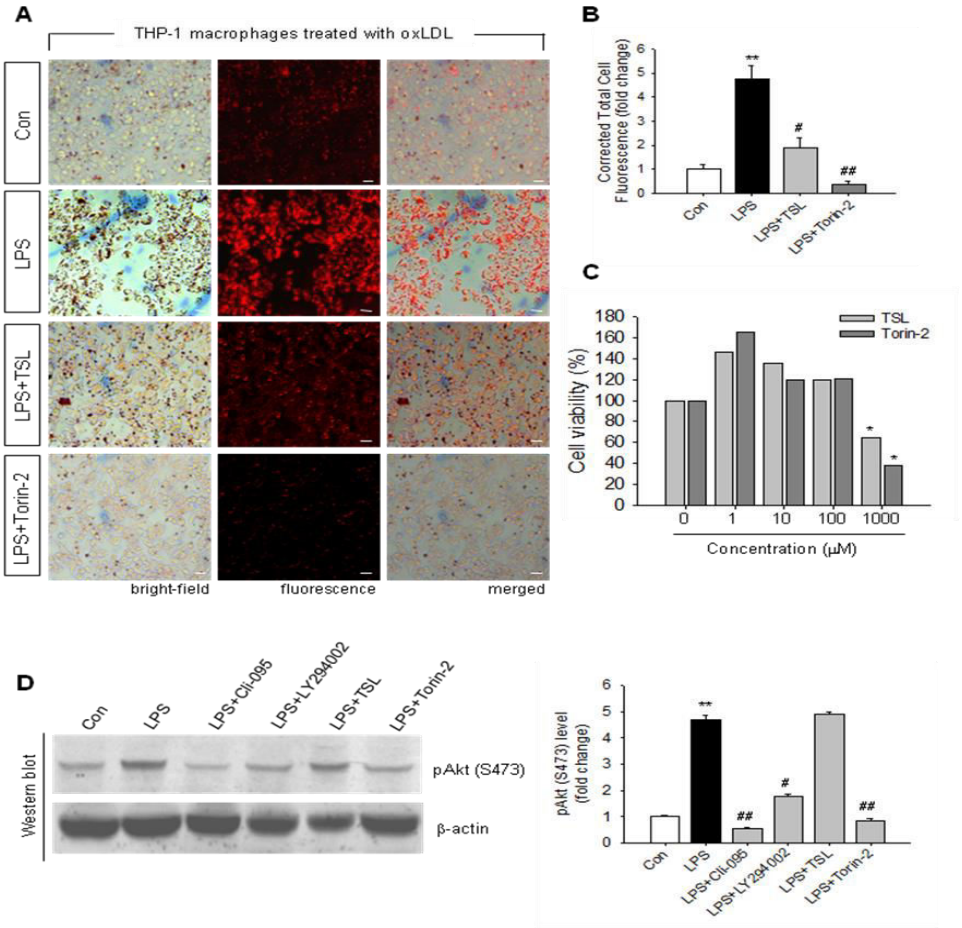

Fig. 5. Inhibition of mTOR prevents TLR4 induced Akt S473 phosphorylation and macrophage foam cell formation. (**A,B**) Representative bright-field (left), fluorescent (middle) and merged (right) images of Oil-Red O staining (**A**) and the quantification of corrected total cell fluorescence (**B**) of THP-1 macrophages incubated with oxLDL (50 μg/mL) for 16 h in absence or presence of LPS (100 ng/ml) without or with a rapalog, Temsirolimus (TSL, 10 μM) or second-generation ATP-competitive dual inhibitor of mTORC1 and mTORC2, Torin-2 (100 μM). Scale bar, 75 μm. Corrected total cell fluorescence (CTCF) was calculated using following formula with the help of image J software. CTCF = Integrated Density – (Area of selected cell X Mean fluorescence of background readings). Each value is the mean±SEM of three independent experiments, **p<0.01 vs Con, ##p<0.01 vs LPS. (**C**) THP-1 macrophages were exposed to indicated concentrations of Temsirolimus or Torin-2 for 24 h followed by the addition of MTT in each well and incubated for additional 4 h at 37°C. After solubilization in 1 (N) isopropanol/0.04 (N) HCl, absorbance was measured at 570 nm in a microplate spectrophotometer and data was

expressed as % of cell viability relative to control. Each value is the mean±SEM of three independent experiments. *p<0.05 vs Con. (**D**) Western blot image (left) and quantification (right) showing abundance of phospho-Akt S473 level in THP-1 macrophages incubated with LPS (100 ng/ml) in absence or presence of indicated inhibitors. β-actin used as loading control. Each value is the mean±SEM of three independent experiments, **p<0.01 vs Con, ##p<0.01, #p<0.05 vs LPS.

Investigating the underlying mechanism of TLR4 stimulated Akt S473 phosphorylation and its participation in macrophage lipid accumulation; we treated THP-1 macrophages with LPS in absence or presence of mTOR inhibitors, Temsirolimus or Torin-2, followed by the incubation of oxLDL for 16 h. Temsirolimus is a rapamycin analog which effectively inhibits mTOR whereas Torin-2 is a second-generation ATP-competitive inhibitor of mTOR that inhibits both mTORC1 and mTORC2. Interestingly, Oil-Red O staining showed that incubation with Torin-2 markedly prevents LPS induced oxLDL uptake in macrophages whereas pre-treatment of Temsirolimus produced meagre effect on LPS stimulated lipid accumulation (**Fig. 5A**). This result was corroborated with the estimation of fluorescence intensity in Oil-Red O stained cells (**Fig. 5B**). Since, mTOR signaling is known to play a crucial role in regulating cellular proliferation and survivability, we analysed the effect of Temsirolimus and Torin-2 on macrophage cell viability. MTT cell viability assay clearly indicates that incubation of Temsirolimus or Torin-2 upto 100 μM concentrations for 24 h did not produce any notable toxic effect on THP-1 cells; however, concentration at 1000 μM significantly impaired macrophage cell viability (**Fig. 5C**). Further, investigating the effect of mTOR inhibition on Akt S473 phophorylation it was noted that incubation of Torin-2 significantly suppressed the LPS induced Akt S473 phophorylation in THP-1 macrophages (**Fig. 5D**).

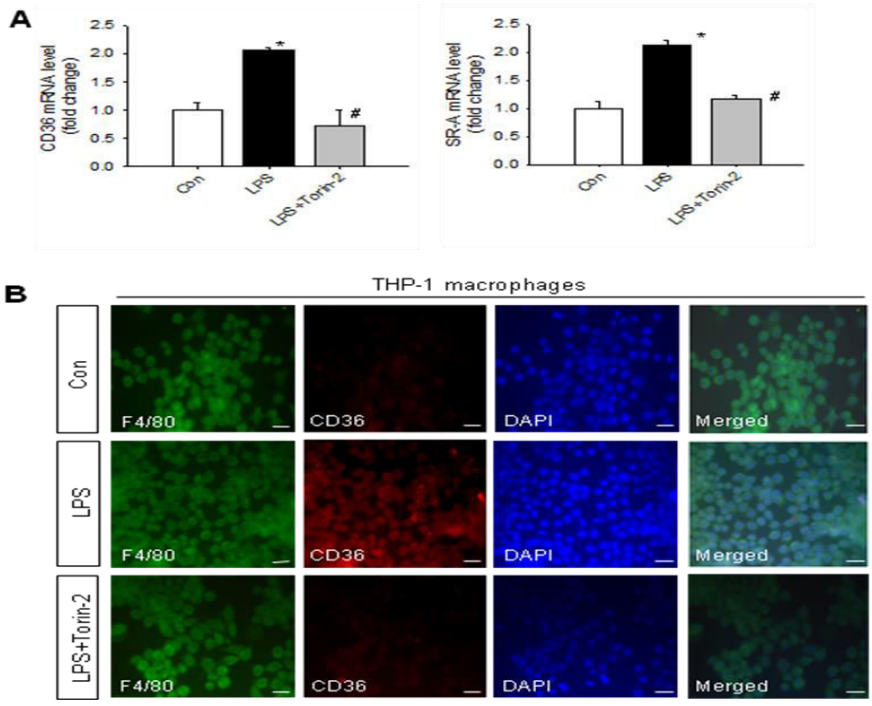

Fig. 6. Involvement of mTOR-Akt signaling cascade in LPS induced macrophage scavenger receptors expression.
(**A**) Real-time quantitative PCR analysis showing fold changes of CD-36 and SR-A gene expression in THP-1 macrophages incubated without or with LPS (100 ng/ml) in absence or presence of mTOR antagonist, Torin-2 (100 μM). GAPDH served as an internal control. Each value is the mean±SEM of three independent experiments, *p<0.05 vs Con, #p<0.05 vs LPS. (**B**) Representative immunofluorescence images showing increased abundance of CD-36 level in LPS (100 ng/ml) incubated THP-1 macrophages which were prevented when pre-treated with Torin-2 (100 μM). DAPI was used for nuclear counterstaining. Scale bar, 75 μm.

Although it is well established that macrophage foam cell formation is dependent on the effective accumulation of modified LDL through the scavenger receptor-mediated endocytosis, however, involvement of scavenger receptors such as CD-36 and SR-A in this process is controversial. To investigate the effect of LPS induced Akt S473 phosphorylation on CD-36 and SR-A gene expression, THP-1 macrophages were incubated with LPS in absence or presence of Torin-2. LPS incubation significantly upregulates CD-36 and SR-A gene expressions and this was inhibited by Torin-2 (**Fig. 6A**). Immunofluorescence study further confirmed our observation; increased abundance of CD-36 in response to LPS was abrogated when cells were pre-treated with Torin-2 (**Fig. 6B**). All these data indicate the participation of mTOR dependent Akt activation in TLR4 induced scavenger receptors expression.

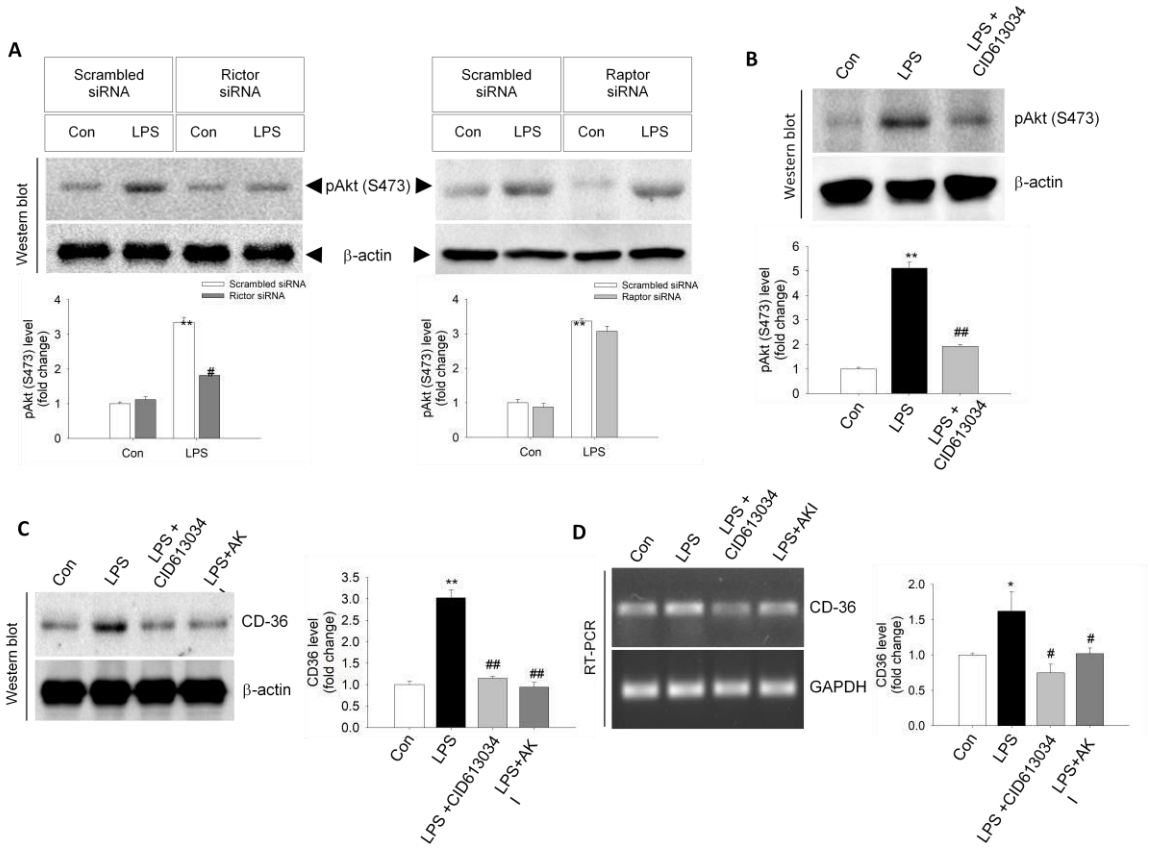

Fig. 7. Attenuation of mTORC2 prevents LPS induced Akt S473 phosphorylation and macrophage lipid accumulation. (**A**) Western blot image (upper) and quantification (lower) showing abundance of phospho-Akt S473 level in Scrambled and Rictor siRNA (left) or Scrambled and Raptor siRNA (right) transfected THP-1 cells in response to LPS incubation (100 ng/ml). β-actin used as loading control. Each value is the mean±SEM of three independent experiments, *p<0.01 vs Scrambled siRNA transfected control cells, #p<0.05 vs Scrambled siRNA transfected LPS treated cells. (**B**) Western blot image (upper) and quantification (lower) showing phospho-Akt S473 level in LPS treated THP-1 macrophages in absence or presence of Rictor-mTOR association inhibitor CID613034 (100 nM). β-actin used as loading control. Each value is the mean±SEM of three independent experiments, **p<0.01 vs Con, ##p<0.01 vs LPS. (**C,D**) Western blot image (left) and quantification (right) (**C**) and RT-PCR analysis (left) and quantification (right) (**D**) showing CD-36 abundance in response to LPS (100 ng/ml) incubation in absence or presence of Rictor-mTOR association inhibitor CID613034 (100 nM) or Akt kinase inhibitor (AKI, 10 μM). β-actin and GAPDH used as loading control for Western blot and RT-PCR, respectively. Each value is the mean±SEM of three independent experiments, **p<0.01, *p<0.05 vs Con, ##p<0.01, #p<0.05 vs LPS.

To investigate the involvement of mTOR complexes mTORC1 and mTORC2 in Akt activation we silenced Raptor or Rictor expression, respectively. Interestingly, we observed that knockdown of Rictor, but not Raptor, notably inhibits LPS induced Akt S473 phosphorylation (**Fig. 7A**). Moreover, THP-1 macrophages treated with pharmacological inhibitor of Rictor-mTOR association, CID613034, also curtail the LPS instigated Akt S473 phosphorylation (**Fig. 7B**). These results indicate the specificity of mTORC2 in regulating inflammation induced Akt S473 phosphorylation in THP-1 macrophages. To have direct evidence on the involvement of mTORC2 dependent Akt signaling on macrophage scavenger receptors expression and foam cell formation, THP-1 cells were incubated with LPS in absence or presence of Rictor-mTOR association inhibitor CID613034 or Akt1/2 kinase inhibitor (AKI) followed by the analysis of macrophage CD-36 expression and lipid accumulation. LPS effected induction of CD-36 protein and gene expression was compromised in CID613034 or AKI treated cells (**Fig. 7C,D**). Collectively, all these observations unveil a crucial role of mTORC2 mediated Akt S473 phosphorylation in inflammation induced scavenger receptor expression that facilitates macrophage foam cells formation.

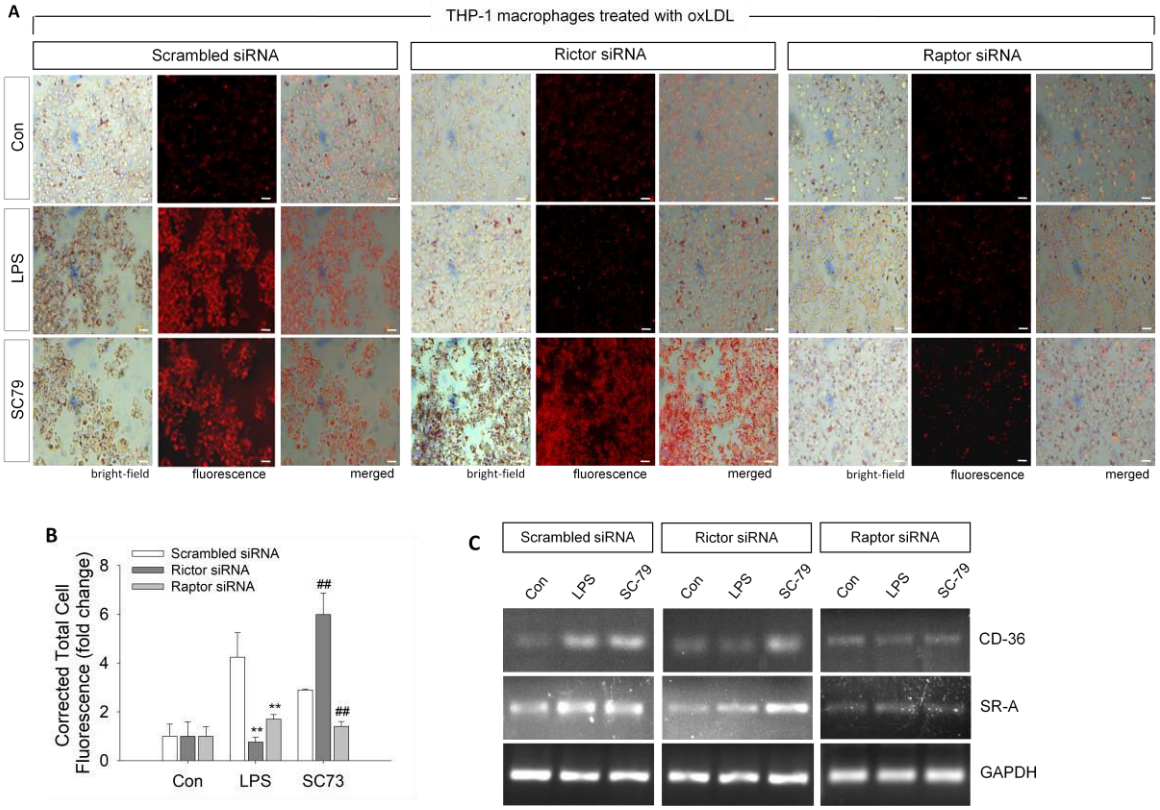

Fig. 8. mTORC2 dependent Akt activation promotes mTORC1 mediated scavenger receptor expression and macrophage foam cell formation.
(**A,B**) Representative images of Oil-Red O staining (**A**) and the quantification of corrected total cell fluorescence (**B**) in Scrambled (left) or Rictor siRNA (middle) or Raptor siRNA (right) transfected THP-1 macrophages incubated with oxLDL (50 µg/mL) for 16 h in absence or presence of LPS (100 ng/ml) or SC79 (10 µM). Scale bar, 75 µm. Corrected total cell fluorescence (CTCF) was calculated using following formula with the help of image J software. CTCF = Integrated Density – (Area of selected cell X Mean fluorescence of background readings). Each value is the mean±SEM of three independent experiments, *p<0.05 vs Scrambled siRNA transfected LPS treated cells, #p<0.05 vs Scrambled siRNA transfected SC79 treated cells. (**C**) RT-PCR analysis showing CD-36 and SR-A gene expression in Scrambled (left) or Rictor siRNA (middle) or Raptor siRNA (right) transfected THP-1 macrophages incubated without or with LPS (100 ng/ml) or SC79 (10 µM) for 16 h. GAPDH used as loading control.

To gain further insight into the potential role of mTORC1 and mTORC2 in macrophage lipid accumulation, Raptor or Rictor silenced THP-1 macrophages were incubated without or with LPS or Akt activator SC79. As expected, LPS induced macrophage oxLDL uptake was significantly inhibited in Rictor knockdown cells, however, incubation of SC79 markedly augmented lipid accumulation in Rictor silenced macrophages (**Fig. 8A,B**). Surprisingly, we observed a profound attenuation of LPS induced lipid droplet accumulation in Raptor silenced macrophages, and more intriguingly, Raptor silenced cells demonstrated significant recede of lipid accumulation even in the presence of SC79 (**Fig. 8A,B**). Moreover, analysis of scavenger receptors expression revealed subdued expression of CD-36 and SR-A in SC79 treated Raptor knockdown cells (**Fig. 8C**). These results indicate that mTORC2-Akt-mTORC1 signaling is instrumental in inflammation induced scavenger receptors expression and macrophage foam cells formation.

B2. Summary and Conclusions of the Progress made so far (minimum 100 words, maximum 200 words)

We have found that TLR4 play a pivotal role in mesenchymal stem cell to adipocyte conversion. The morphological changes associated with lipid accumulation and gene expression profile clearly demonstrated importance of TLR4 signaling in the differentiation of mesenchymal stem cell to adipocyte. However, future in-vivo study with TLR4 knockout mice is needed to confirm this observation. Moreover, we found that the TLR4 mediated inflammatory signaling communicated with mTORC2-Akt-mTORC1 metabolic cascade in macrophage and thereby promoting lipid uptake and foam cell formation. Mechanistically, LPS treatment markedly upregulates TLR4 mediated inflammatory pathway which by activating mTORC2 induces Akt phosphorylation at serine 473 and that aggravate mTORC1 dependent scavenger receptors expression and consequent lipid accumulation in THP-1 macrophages. Inhibition of mTORC2 either by silencing Rictor expression or inhibiting its association with mTOR notably prevents LPS induced Akt activation, scavenger receptors expression and macrophage lipid accumulation. Although suppression of mTORC1 expression by genetic knockdown of Raptor did not produce any significant change in Akt S473 phosphorylation, however, incubation with Akt activator in Rictor silenced cells failed to promote scavenger receptors expression and macrophage foam cell formation. Thus, present research explored the signaling pathway involved in inflammation induced macrophage foam cells formation and therefore, targeting this pathway might be useful for preventing macrophage foam cell formation.

B3. Details of New Leads Obtained, if any:

Interestingly, we observed that inhibition of TLR4 signaling promotes mesenchymal stem cell to adipocyte conversion. Moreover, TLR4 signaling in macrophages play a key role in macrophage conversion to lipid-laden foam cell.

B4. Details of Publications & Patents, if any:

Banerjee, A. Sinha, S. Saikia, B. Gogoi, A.K. Rathore, A.S. Das, D. Pal, A.K. Buragohain, **S. Dasgupta*** (2018) Inflammation induced mTORC2-Akt-mTORC1 signaling promotes macrophage foam cell formation. ***Biochimie*** 151, 139-149. [DOI: 10.1016/j.biochi.2018.06.001] *Corresponding author. ***(IF: 4.372)***

Section-C : Details of Grant Utilization#

C1. Equipment Acquired or Placed Order with Actual Cost:

Equipment Acquired: Gel Documentation System: Rs. 9.99915 lakh. Asset Certificate already submitted to DBT, New Delhi.

C2. Manpower Staffing and Expenditure Details:

One JRF/SRF, Mr. Dipanjan Banerjee, received Rs. 8.76633 lakh.

C3. Details of Recurring Expenditure:

Under the Consumable, Travel and Contingency head:

Consumable: 15.05749 lakh.
Travel: 0.49893 lakh.
Contingency: 0.57957 lakh.

Under the Overhead: 0.87496 lakh.

Under the Cash Award head: 3.00 lakh.

C4. Financial Requirements for the Next Year with Justifications:

Project completed with a negative balance of Rs. -0.44621 and therefore the DBT to kindly adjust the amount by releasing the aforementioned amount to the Tezpur University.

#Grant utilization details (UC&SE, Assets Certificate & manpower details) also required to be submitted separately as per the prescribed format

16/09/19

[Signature(s) of the Investigator(s)]

Instructions:

(i) All the information needs to be provided, otherwise STPR will be treated as incomplete. In case of 'Nil' / 'Not Applicable' information, the same may be indicated.
(ii) Incase of multicentric project, combined STPR requires to be submitted incorporating the progress of all components. The Project Co-coordinator/ PI will be responsible for this.
(iii) STPR need to be submitted by post (02 copies) and also by email.
*(iv) *Please indicate the reporting period [i.e. 1st year **(I)** / 2nd year **(II)** / 3rd year **(III)**].*
(v) Submission of STPR is linked with further continuation of the project and release of grant.

Annexure I

UTILISATION CERTIFICATE

(For the financial year 1st April 2018 to 31st March 2019)

(Rs. In lakhs)

1. Title of the project/scheme: ***"To investigate the role of toll-like receptor 4 (TLR4) in lipid induced impairment of adipogenesis and adipose tissue function implementing insulin resistance"***

2. Name of the Organisation : Tezpur University

3. Principal Investigator : Dr. Suman Dasgupta

4. Deptt. of Biotechnology sanction order No. & date of sanctioning the project : BT/08/IYBA/2014-12, dated 01/09/2015

5. Amount brought forward from the previous financial year quoting DBT sanction No. & date in which the authority to carry forward the said amount was given : Rs. 5.22167 BT/08/IYBA/2014-12, dated 04.09.2019

6. Amount received from DBT during the financial year (*Please give No. & dates of Sanction order showing the amounts*) : Rs. 0.56

7. Other receipts/interest earned, if any, on the DBT grants : Rs. 0.15665 (bank interest)

8. Total amount that was available for expenditure during the financial year (Sl. Nos. 5, 6 and 7) :Rs. 5.93832

9. Actual expenditure (excluding commitments) incurred during the financial year (Statement of Expenditure is enclosed) : Rs. 6.38453 (Attached)

10. Unspent balance refunded, if any (*Please give the details of Cheque No. etc.*) : Nil

11. Balance amount available at the end of the financial year : Rs. -0.44621

12. Amount allowed to be carried forward to the next financial year vide letter No. & date : Nil

1. Certified that the amount of **Rs. 6.38453 lakhs** mentioned against col. 9 has been utilized on the project/ scheme for the purpose for which it was sanctioned and that the negative balance of **Rs. - 0.44621 lakhs** remaining at the end of the year.

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

Kinds of checks exercised:

1. Rs. 2.49133 lakhs for Manpower
2. Rs. 3.84973 lakhs for Consumables
3. Rs. 0.04347 lakhs for Contingency

(PROJECT INVESTIGATOR)

Dr. S. Dasgupta
PI, DBT- IYBA Project
DBT Ref. No.. BT/08/IYBA/2014-12
Univ. Ref. No.: DORD/MBBT/SD/20-254
Dept. of MBBT, Tezpur University
Assam- 784028

(FINANCE OFFICER)

Finance Officer
Tezpur University
21/08/19

(HEAD OF THE INSTITUTE)

(To be countersigned by the DBT Officer-in-charge)

Registrar
Tezpur University

Annexure II

STATEMENT OF EXPENDITURE REFERRED TO IN PARA 9 OF THE UTILISATION CERTIFICATE

Showing grants received from the Department of Biotechnology and the expenditure incurred during the period from 1st April 2018 to 31stMarch 2019.

(Rs. In lakhs)

	Item	Unspent balance carried for-ward from previous year	Grants received from DBT during the year	Other receipts/ interest earned, if any, on the DBT grants	Total of Col. (2+3+4)	Expenditure (excluding commitments incurred during the year)	Balance (5-6)	Remarks
	1	2	3	4	5	6	7	8
1. Non-Recurring i) Equipment's	Nil	Nil	Nil	Nil	Nil	Nil	Nil	
2. Recurring (i) Human Resource	One JRF	Rs. 1.75	0.56	Nil	Rs. 2.31	Rs. 2.49133	Rs. -0.18133	
(ii) Consumables (iii) Travel (iv) Contingency		Rs. 3.34663	Nil	Rs. 0.15665	Rs. 3.50328	Rs. 3.84973 Nil Rs. 0.04347	Rs. -0.38992	
(v) Overhead (If applicable)		Rs. 0.12504	Nil	Nil	Rs. 0.12504	Nil	Rs. 0.12504	
Cash award @ Rs. 1 lakh per annum for 3 years		Nil	Nil	Nil	Nil	Nil	Nil	
Total		Rs. 5.22167	Rs. 0.56	Rs. 0.15665	Rs. 5.93832	Rs. 6.38453	Rs. -0.44621	

(PROJECT INVESTIGATOR)

Dr. S. Dasgupta
PI, DBT- IYBA Project
DBT Ref. No.. BT/08/IYBA/2014-12
Univ. Ref. No.: DORD/MBBT/SD/20-254
Dept. of MBBT, Tezpur University
Assam- 784028

(HEAD OF THE INSTITUTE)

Registrar
Tezpur University

(FINANCE OFFICER)

Finance Officer
Tezpur University
01/08/19

Annexure A

Manpower Staffing Details for the DBT-IYBA project sanctioned by the Department of Biotechnology (during the period from 1st April 2018 to 31st March 2019)

NAME OF THE PERSON	NAME OF THE POST	DATE OF JOINING	DATE OF LEAVING	TOTAL MONTHLY SALARY	TOTAL SALARY PAID DURING THE FINANCIAL YEAR	TOTAL SALARY PAID DURING PROJECT PERIOD
Mr. Dipanjan Banerjee	SRF	01/12/2017	28/09/2018	Rs. 28,000 /month	Rs. 28,000 X 6 months (January - June 2018)+ arrear of December 2017 (Rs. 3,000)+ Rs 24,000 for July+ Rs. 54,133 for August and September 2018 = Rs. 2,49,133	Rs. 8,76,633

(Signature of Principal Investigator)

Dr. S. Dasgupta
PI, DBT- IYBA Project
DBT Ref. No.. BT/08/IYBA/2014-12
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Assam- 784028

(Signature of Accounts Officer)

Finance Officer
Tezpur University
01/08/19

(SIGNATURE OF HEAD OF THE INSTITUTE)

Registrar
Tezpur University

Annexure B

Manpower Expenditure Details (In financial year wise manner)*:

SANCTIONED POSTS	NUMBER	SCALE OF PAY	ANNUAL OUTLAY	OUTLAY FOR THE ENTIRE PERIOD	REVISED SCALE, IF ANY	REVISED ANNUAL OUTLAY	REVISED PROJECT OUTLAY	ACTUAL RELASES BY DBT	ACTUAL EXPENDITURE	BALANCE
JRF For First & Second Year SRF For Third Year	One(1)	First Two Years: Rs. 25,000 + Rs. 2500 (HRA) = Rs. 27,500 Third Year: Rs. 28,000 + (HRA) = Rs. Rs. 30,800	First two Years: Rs. 6,60,000 Third Year: Rs. 3,69,600	Rs. 10,29,600	NA	NA	NA	Rs. 8,58,500 (Rs. 3,30,000+ Rs.1,02,500 +3,70,000 + 56,000)	Rs. 8,76,633	Rs. -18,133

(Signature of Principal Investigator)

Dr. S. Dasgupta
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(Signature of Accounts Officer)
Finance Officer
Tezpur University
01/08/19

(SIGNATURE OF HEAD OF THE INSTITUTE)
Registrar
Tezpur University

* Details of manpower salary/ fellowship revision alongwith due- drawn statement and arrears requested should be given separately, if applicable.

Due- Drawn Statement

Name of the Project Staff	Month and Year	Due	Drawn	Difference
DIPANJAN BANERJEE	January 2018	Nil	Rs. 28,000	Nil
	February 2018	Nil	Rs. 28,000	Nil
	March 2018	Nil	Rs. 28,000	Nil
	April 2018	Nil	Rs. 28,000	Nil
	May 2018	Nil	Rs. 28,000	Nil
	June 2018	Nil	Rs. 28,000	Nil
	July 2018	Nil	Rs. 28,000	Nil
	August 2018	Nil	Rs. 28,000	Nil
	September 2018	Nil	Rs. 26,133	Nil

(Signature of Principal Investigator)

Dr. S. Dasgupta
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Dept. of MBBT, Tezpur University
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(Signature of Accounts Officer)

Finance Officer
Tezpur University
04/09/19

(SIGNATURE OF HEAD OF THE INSTITUTE)

Registrar
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