Annexure-VIII

UNIVERSITY GRANTS COMMISSION

BAHADUR SHAH ZAFAR MARG

NEW DELHI - 110 002

Annual Report of the work done on the Major Research Project.

(Report to be submitted within 6 weeks after completion of each year)

1. Project report No. 1st/2nd/3rd /Final : Final

2. UGC Reference No. F. : 43-87/2014(SR) dated 22.08.15

3. Period of report : **From -** 08.10.2015 **to -** 30.06.2018

4. Title of research project : "Role of fetuin-A gene and protein expression in obesity induced impairment of adipogenesis and stimulation of adipose tissue inflammation implementing insulin resistance and type-2 diabetes".

5. (a) Name of the Principal Investigator : Dr. Suman Dasgupta

(b) Deptt. : Molecular Biology & Biotechnology

(c) University where work has progressed: Tezpur University

6. Effective date of starting of the project : 08.10.2015

7. Grant approved and expenditure incurred during the period of the report:

a. Total amount approved Rs. : 8,52,500/-

b. Interest Earned Rs. : 12,308/-

c. Total expenditure Rs. : 8,58,045/-

d. Report of the work done (Please attach a separate sheet): Attached

i. Brief objective of the project :

(i) To study the underlying mechanism of white, brown and beige or brite (brown-in-white) adipocytes differentiation.

(ii) To study the mechanism of fetuin-A induced impairment of PPARγ and to analyse whether impairment of PPARγ by fetuin-A generally or selectively attenuates white, brown and beige fat adipogenesis?

(iii) Study of the role of fetuin-A in modulating the expression of various adipokines and inflammatory cytokines. Study of the underlying mechanism of obesity induced overexpression of fetuin-A.

(iv) To evaluate the underlying mechanism obesity induced macrophage polarization from anti-inflammatory M2 state to proinflammatory M1 state and its infiltration in the adipose tissue and also to study whether fetuin-A induces the differentiation of preadipocytes into the macrophage like cells?

ii. Work done so far and result achieved and publications, if any, resulting from the work (give details of the papers and names of the journals in which it has been published or accepted for publication)

To study the involvement of Fetuin-A (FetA) in regulating the expression of various cytokines and adipokines from the adipocytes, we incubated 3T3L1 adipocytes with FetA for 6h. FetA strongly induces inflammatory states in adipocytes by upregulation of proinflammatory cytokines expression such as MCP1, TNFα and IL6 (Fig. 1A) whereas the expression of adipokines known to stimulate insulin sensitivity such as adiponectin and adipsin were downregulated (Fig. 1B).

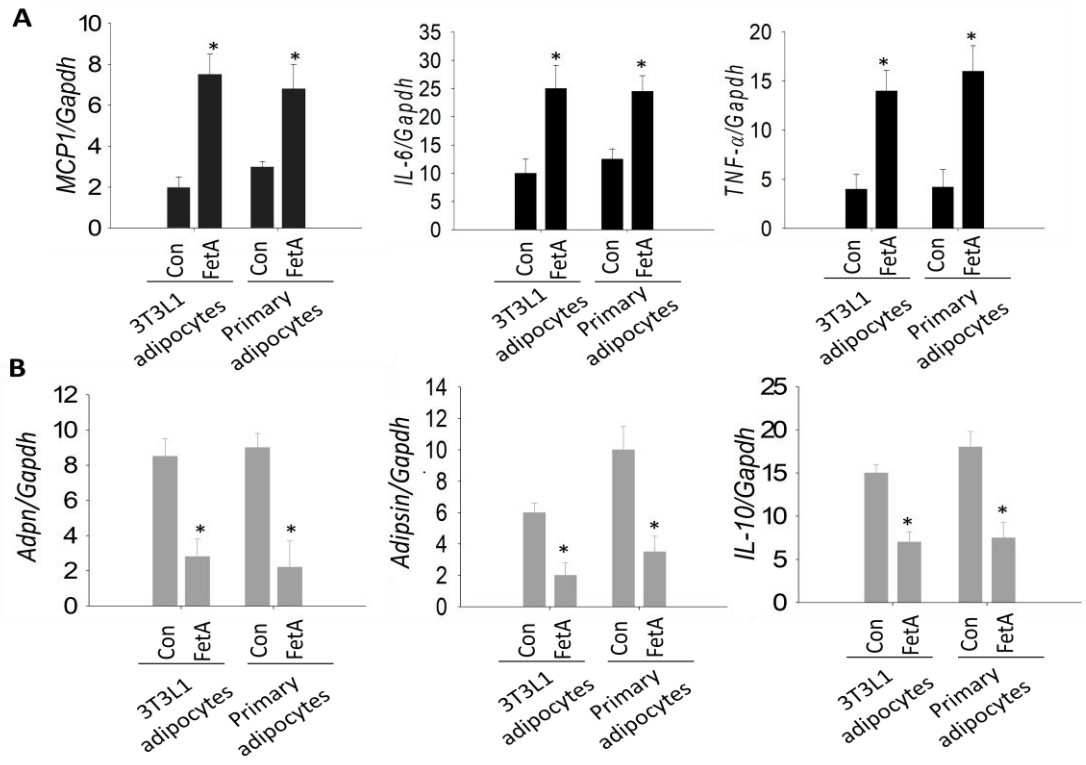

Fig 1: Fetuin-A provoke proinflammatory cytokine milieu in adipocytes. Incubation of FetA in 3T3L1 and primary adipocytes upregulates proinflammatory cytokines expression, namely MCP1, TNFa and IL6 (A), while downregulation was observed in beneficial adipokines level such as Adpn, Adipsin and IL10 (B).

We also investigated the MCP1 and adiponectin protein level by immunoblotting which also showed the similar trend (Fig. 2A), however, effect of FetA is more pronounced in comparison to palmitate (Fig. 2A). Interestingly, in comparison to preadipocytes, mature adipocytes are major source of FetA (Fig. 2B).

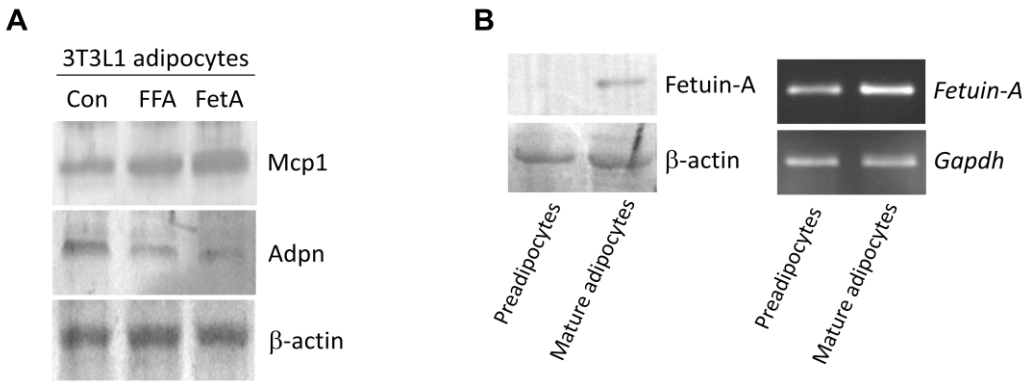

Fig 2: Fetuin-A synthesis and its effect on 3T3L1 adipocytes. (A) Incubation of FFA or FetA in 3T3L1 adipocytes notably upregulates MCP1 expression whereas adiponectin expression was downregulated (B) Fetuin-A protein and gene expression was analysed by immunoblotting and RT-PCR, respectively, in in 3T3L1 preadipocytes and mature adipocytes.

To have more insight, specifically to observe whether the FetA is a key regulator for the expression of cytokines and adipokines from adipocytes, we suppressed expression of FetA by siRNA. Inhibition of FetA expression prevents FFA induced upregulation of MCP1 and downregulation of adiponectin protein and gene expression, respectively (Fig. 3A). Interestingly, forced expression of FetA in 3T3L adipocytes upregulates MCP1 gene and protein expression while adiponectin gene and protein expression was downregulated (Fig. 3B). All these results indicate that FetA notably upregulates inflammatory cytokines in concomitant downregulation of beneficial adipokines expression.

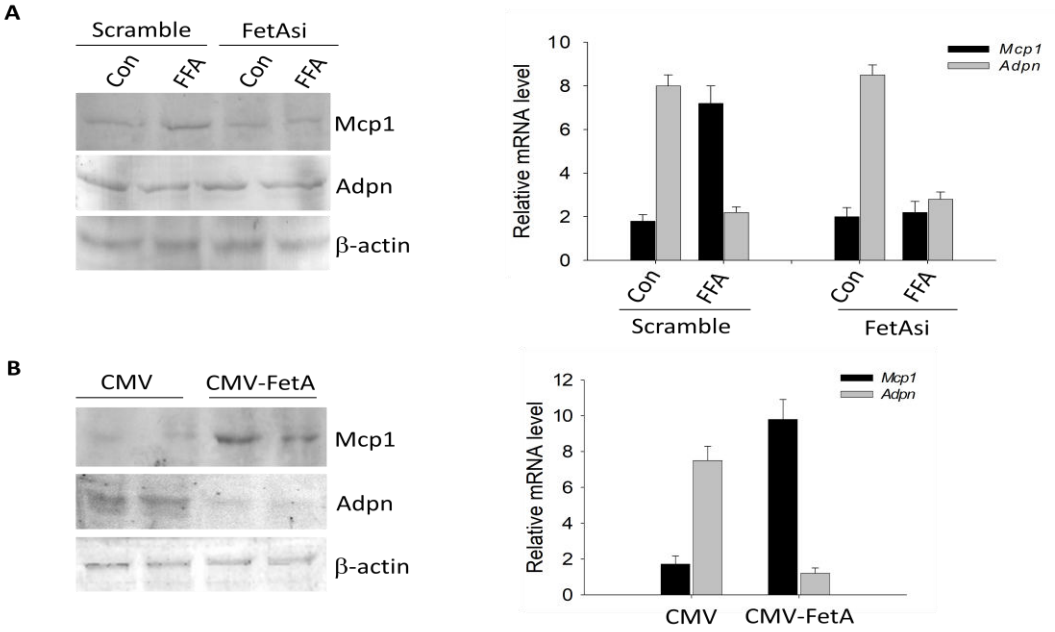

Fig. 3: Alteration of Fetuin-A expression modulates MCP1 and Adiponectin expression in 3T3L1 adipocytes. (A) FFA unable to induce MCP1 or reduce Adpn protein (left) and gene (right) expression in FetA silenced adipocytes. (B) FetA overexpressed cells shows significant upregulation of MCP1 and downregulation Adpn protein (left) and gene (right) expression.

To investigate the effect of FetA in insulin stimulated glucose uptake, we observed that FetA strongly inhibits insulin stimulated glucose uptake in 3T3L1 adipocytes as indicated by impaired uptake of fluorescent labelled deoxyglucose (2-NBDG) (Fig. 4A). 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 Fig. 4B that all these signaling molecules activation were attenuated in 3T3L1 adipocytes by FetA. These results indicate that FetA strongly inhibits insulin action on 3T3L1 adipocytes. Our investigation in this direction is still going on and upon completion of this work we will submit for its publication.

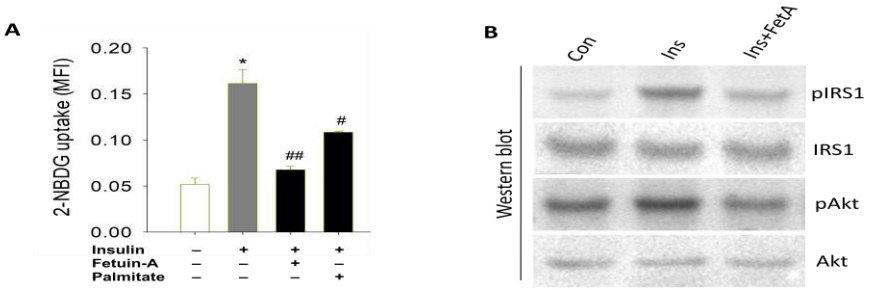

Fig 4: Inhibition of insulin signaling pathway by FetA. (A) 2-NBDG uptake by 3T3L1 adipocytes incubated without or with insulin or insulin + FetA or FFA. (B) Western blot showing pIRS-1 (Tyr-989) and pAkt (Thr-308) abundance in 3T3L1 adipocytes incubated without or with Ins or Ins+FetA.

FetA incubation in 3T3-L1 adipocytes significantly decrease in adiponectin expression and its secretion (Fig. 5A). We then hypothesized that between FetA and adiponectin there may be an involvement of Wnt signalling because Wnt has been indicated to suppress PPARγ expression in preadipocytes and adiponectin is one of the PPARγ targeted genes. It is also known that during obesity Wnt is involved in negative regulation of adipogenesis when decrease of adiponectin also occurs. However, it remained unclear what makes excess of Wnt expression in obesity. Addition of increasing concentration of FetA to 3T3L1 adipocytes effected a dose dependent increase in Wnt3a expression. These results indicate an association of FetA with Wnt3a upregulation. Interestingly there was a corresponding decrease of PPARγ expression in accordance with the FetA induced Wnt3a increase in the adipocytes (Fig. 5B). To examine it further we used Wnt3a gene knockout adipocytes and observed that FetA failed to produce inhibition on PPARγ and adiponectin in the absence of Wnt3a support (Fig. 5C,D).

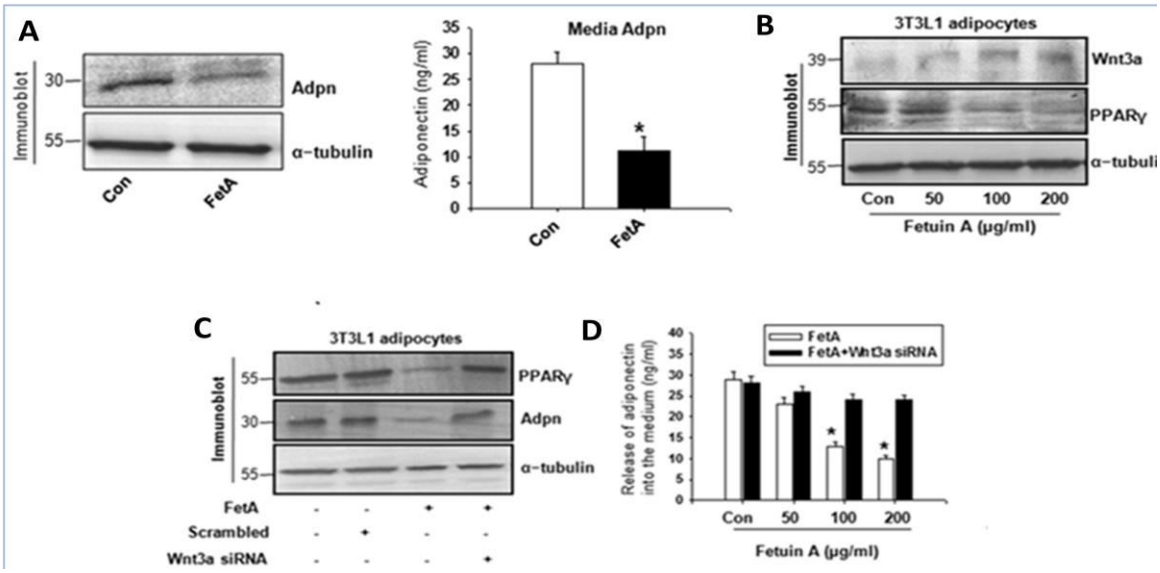

Fig 5: Inhibition of adiponectin expression by FetA. (A) Adiponectin expression and its release into the medium was determined by immunoblot analysis and ELISA respectively, from 3T3L1 adipocytes incubated without (Con) or with FetA for 4 h (n = 3). *P < 0.05 versus Con. Data represents mean ± s.e.m. (B) Immunoblot showing effect of increasing concentration of FetA onWnt3a and PPARγ protein levels in 3T3L1 adipocytes. (C) Expression of PPARγ and adiponectin was evaluated through immunoblot in the presence or absence of FetA inWnt3a silenced 3T3L1 adipocytes. (D) Adiponectin released into the medium from Con, FetA or FetA+Wnt3a silenced 3T3L1 adipocytes in response to varied concentrations of FetA was determined by ELISA (n=3). *P b 0.05 versus FetA+Wnt3a siRNA.

iii. Has the progress been according to original plan of work and towards achieving the objective. if not, state reasons : Yes, part of the project work completed as we only received the first year grant of this project.

iv. Please indicate the difficulties, if any, experienced in implementing the project: We only received the first year grant from the UGC.

v. If project has not been completed, please indicate the approximate time by which it is likely to be completed. A summary of the work done for the period (Annual basis) may please be sent to the Commission on a separate sheet: Project tenure completed. However, due to the unavailability of fund we could not able to complete the project work. This is because we only received the first year grant of this project.

vi. If the project has been completed, please enclose a summary of the findings of the study. One bound copy of the final report of the work done may also be sent to University Grants Commission.: Attached.

vii. Any other information which would help in evaluation of work done on the project. At the completion of the project, the first report should indicate the output, such as (a) Manpower trained (b) Ph.D. awarded (c) Publication of results (d) other impact, if any:

(a) Manpower trained - 1
(b) Ph.D. awarded - Nil
(c) Publication of results - Nil
(d) Other impact - Nil

SIGNATURE OF THE PRINCIPAL INVESTIGATOR

Annexure - III

UNIVERSITY GRANTS COMMISSION
BAHADUR SHAH ZAFAR MARG
NEW DELHI – 110 002

STATEMENT OF EXPENDITURE IN RESPECT OF MAJOR RESEARCH PROJECT

1. Name of Principal Investigator : Dr. Suman Dasgupta

2. Dept. of Principal Investigator : Department of Molecular Biology and Biotechnology

   University : Tezpur University

3. UGC approval Letter No. and Date : 43-87/2014(SR) dated: 22.08.2015

4. Title of the Research Project : *"Role of fetuin-A gene and protein expression in obesity induced impairment of adipogenesis and stimulation of adipose tissue inflammation implementing insulin resistance and type 2 diabetes"*

5. Effective date of starting the project : 08.10.2015

6. a. Period of Expenditure : From 08.10.2015 to 30.06.2018

   b. Details of Expenditure :

Sl No.	Item	Fund received (Rs.)			Expenditure (Rs)				Remarks (Rs)
		1st instalment	2nd instalment	Total (Rs) = **A**	I (2015-2016)	II (2016-2017)	III (2017-2018)	Total: I+II+III = **B**	**A-B**
i.	Books & Journals	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil
ii.	Equipment	2,92,000	Nil	2,92,000	Nil	2,91,570	Nil	2,91,570	430
iii.	Contingency	25,000	Nil	25,000	Nil	20,580	1909	22,489	2511
iv.	Field work/Travel	25,000	Nil	25,000	Nil	14,895	26,701	41,596	- 16596
v.	Hiring Services	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil
vi.	Chemicals & Glassware	2,00,000	Nil	2,00,000	1,97,894	Nil	Nil	1,97,894	2106
vii.	Overhead	10,500	Nil	10,500	8163	Nil	Nil	8163	2337
viii	Manpower	3,00,000	Nil	3,00,000	58,333	1,68,000	70,000	2,96,333	3667
	Total (Rs)	8,52,500	Nil	8,52,500	2,64,390	4,95,045	98,610	8,58,045	- 5545
	Interest earned (Rs)	For the period of 2015-2018 = 12,308							
	Total Balance (Rs)	[Balance (A-B): - 5545 + Interest earned: 12,308] = **6763**							

Finance Officer
Tezpur University

c. Staff: Date of Appointment : 25.11.2015

Sl No.	Items	From	To	Amount Approved during 2015-2018 (Rs.)	Expenditure Incurred during 2015-2018 (Rs.)	Remarks
1.	Honorarium to PI	Nil	Nil	Nil	Nil	None
2.	a) NET/GATE qualified-Rs. 16,000/- p.m. for initial 2 years and Rs. 18,000/- p.m. for the third year.					
	b)Non-GATE/Non-NET: Rs. 14,000/- p.m. for initial 2 years and Rs. 16,000/- p.m. for the third year.	25/11/2015	31/07/2017	3,00,000/-	2,96,333/-	Balance of Rs. 3667/- **(Project Fellow did not get fellowship from 31/07/2017 to 30/06/2018 due to the unavailability of fund)**

1. It is certified that the appointment(s) have been made in accordance with the terms and conditions laid down by the Commission.

2. If as a result of check or audit objection some irregularly is noticed at later date, action will be taken to refund, adjust or regularize the objected amounts.

3. Payment @ revised rates shall be made with arrears on the availability of additional funds.

4. It is certified that the **grant of Rs. 8,52,500/- (Rupees Eight Lakhs Fifty Two Thousand Five Hundred only)** received from the University Grants Commission under the scheme of support for **Major Research Project** entitled ***"Role of fetuin-A gene and protein expression in obesity induced impairment of adipogenesis and stimulation of adipose tissue inflammation implementing insulin resistance and type 2 diabetes"*** vide UGC letter No. F 43-87/2014(SR) dated 22.08.2015, along with the **interest earned from the bank of Rs. 12,308/- (Rupees Twelve Thousand Three Hundred Eight only). An amount of Rs. 8,58,045/- (Rupees Eight Lakhs Fifty Eight Thousand Forty Five only) has been utilized** for the purpose for which it was sanctioned and accordance with the terms and conditions laid down by the University Grants Commission. The **unspent balance of Rs. 6763/- (Rupees Six Thousand Seven Hundred Sixty Three only)** remained at the end of the project was **returned to the University Grants Commission by RTGS No. SBIN118256718019 dated 13.09.2018.**

S. Dasgupta 24/9/18

SIGNATURE OF THE PRINCIPAL INVESTIGATOR

Dr S. Dasgupta
PI, UGC- MRP Project
UGC Ref. No.. F 43-87/2014 (SR)
Univ. Ref. No.: DORD/MBBT/SD/20-253
Dept. of MBBT, Tezpur University
Assam- 784028

REGISTRAR
(Seal)
Registrar
Tezpur University

Month - wise detailed statement of expenditure towards salary and HRA of project fellow

Name of Project Fellow : Ms. Sayani Mazumder

Project Ref. No. : 43-87/ 2014 (SR) dated 22.8.2015

Period of Expenditure : 08.10.2015 to 30.06.2018

Title of the research project : "Role of fetuin-A gene and protein expression in obesity induced impairment of adipogenesis and stimulation of adipose tissue inflammation implementing insulin resistance and type-2 Diabetes".

Name of the Principal Investigator : Dr. Suman Dasgupta

Month wise details :

Sl No.	Month (Year)	Fellowship (Rs.)	HRA*
1	December (2015)	14000	2333
2	January (2016)	14000	NIL
3	February (2016)	14000	NIL
4	March (2016)	14000	NIL
5	April (2016)	14000	NIL
6	May (2016)	14000	NIL
7	June (2016)	14000	NIL
8	July (2016)	14000	NIL
9	August (2016)	14000	NIL
10	September (2016)	14000	NIL
11	October (2016)	14000	NIL
12	November (2016)	14000	NIL
13	December (2016)	14000	NIL
14	January (2017)	14000	NIL
15	February(2017)	14000	NIL
16	March (2017)	14000	NIL
17	April (2017)	14000	NIL
18	May (2017)	14000	NIL
19	June (2017)	14000	NIL
20	July (2017)	14000	NIL
21	August (2017)	14000	NIL
22	September (2017)	NIL#	NIL
23	November (2017)	NIL#	NIL
24	December (2017)	NIL#	NIL
25	January (2018)	NIL#	NIL
26	February (2018)	NIL#	NIL
27	March (2018)	NIL#	NIL
28	April (2018)	NIL#	NIL
29	May (2018)	NIL#	NIL
30	June (2018)	NIL#	NIL
Total		**Rs. 2,96,333/-**	

*Since the fellow availed hostel facility from January 2016 therefore not entitled for HRA.

 # Project fellow did not get fellowship due to the unavailability of fund in the Manpower head.

24/09/18

(Principal Investigator)

Dr S. Dasgupta
PI, UGC- MRP Project
UGC Ref. No.. F 43-87/2014 (SR)
Univ. Ref. No.: DORD/MBBT/SD/20-253
Dept. of MBBT, Tezpur University
Assam- 784028

(Finance Officer)

Finance Officer
Tezpur University

(Registrar)

Registrar
Tezpur University

Annexure - V

UNIVERSITY GRANTS COMMISSION
BAHADUR SHAH ZAFAR MARG
NEW DELHI – 110 002

UTILIZATION CERTIFICATE

Certified that from the grant of **Rs. 8,52,500/- (Rupees Eight Lakhs Fifty Two Thousand Five Hundred only)** received as 1st instalment from the University Grants Commission under the scheme of support for **Major Research Project** entitled ***"Role of fetuin-A gene and protein expression in obesity induced impairment of adipogenesis and stimulation of adipose tissue inflammation implementing insulin resistance and type 2 diabetes"*** vide UGC letter No. F 43-87/2014(SR) dated 22.08.2015, along with the **interest earned from the bank of Rs. 12,308/- (Rupees Twelve Thousand Three Hundred Eight only). An amount of Rs. 8,58,045/- (Rupees Eight Lakhs Fifty Eight Thousand Forty Five only) has been utilized** for the purpose for which it was sanctioned and accordance with the terms and conditions laid down by the University Grants Commission. The **unspent balance of Rs. 6763/- (Rupees Six Thousand Seven Hundred Sixty Three only)** remained at the end of the project was **returned to the University Grants Commission by RTGS No. SBIN118256718019 dated 13.09.2018.**

S. Dasgupta 24/09/18

SIGNATURE OF THE
PRINCIPAL INVESTIGATOR
Dr S. Dasgupta
PI, UGC- MRP Project
UGC Ref. No.. F 43-87/2014 (SR)
Univ. Ref. No.: DORD/MBBT/SD/20-253
Dept. of MBBT, Tezpur University
Assam- 784028

REGISTRAR
(Seal)
Registrar
Tezpur University

For SURAJIT CHAKRABORTY & CO.
CHARTERED ACCOUNTANTS
28.10.2018
CA. SURAJIT CHAKRABORTY
M. No.- 305054

STATUTORY AUDITOR
(Seal)