Annexure –VIII

UNIVERSITY GRANTS COMMISSION

BAHADUR SHAH ZAFAR MARG

NEW DELHI – 110 002

FINAL REPORT OF THE WORK DONE ON THE MAJOR RESEARCH PROJECT

1. Project report number Final
2. UGC Reference No. & Date No. F. 41-1284/2012(SR) dated 25th July 2012
3. Period of report 01-07-2012 to 31-12-2015
4. Title of research project **"Antagonism of platelet activating factor (PAF) – mediated inflammatory responses by *Tylophora asthmatica*"**
5. (a) Name of the Principal Investigator : **Dr. Gopal Marathe K**

   (b) Department Department of Studies in Biochemistry

   (c) University/ College where the project was undertaken University of Mysore
6. Effective date of starting of the project
7. Grant approved and expenditure incurred during the period of the report:
   a) Total amount approved Rs: **15, 01, 281/-**
   b) Total amount released Rs: **13, 91, 533/-**
   c) Total expenditure Rs. **13, 91, 369/-**
   d) Total amount to be release Rs: **1, 09, 748/-**
   e) Report of the work done

i.	Objectives of the Project	1. Establishing bioassays for PAF-mediated responses at Dept of Biochemistry, Manasagangothri, University of Mysore. 2. Isolating the active anti-PAF component(s) of *T.asthmatica* root using solid phase extraction columns, HPLC and establishing structures of active components. 3. Chemical synthesis of active component(s) and its/their validation as anti-PAF agent(s)
ii.	Work done so far	➢ Animal model (Swiss albino) for PAF induced lethality was developed ➢ Effect of *Tylophora asthmatica* root extract on PAF induced death in Swiss albino mice and histological examination of lungs and liver was carried out. ➢ Inhibitory activity of *Tylophora asthmatica* root

		extract on PAF induced human platelet aggregation was checked. ➢ Inhibition of PAF induced β2-integrin mediated human neutrophils adhesion by *T. asthmatica* root extract was checked. ➢ PAF induced foot paw Edema in Wister rats was restored by *T. asthmatica* root extract pretreatment. ➢ *T. asthmatica* root extract individual components were separated by HPLC. (Details are mentioned in the progress report)
	Publications, if any, resulting from the work	➢ Jacob SP, Lakshmikanth CL, Chaithra VH, Kumari TR, Chen CH, McIntyre TM and Marathe GK. Lipopolysaccharide Cross-Tolerance Delays Platelet-Activating Factor-Induced Sudden Death in Swiss Albino Mice: Involvement of Cyclooxygenase in Cross-Tolerance. PLOS ONE DOI:10.1371/journal.pone. eCollection 2016. ➢ Lakshmikanth CL, Jacob SP, Chaithra VH, Castro-Faria-Neto HC and Marathe GK. Sepsis – In search of cure. Inflamm Res. 65(8):587-602. doi: 10.1007/s00011-016-0937-y. Epub 2016. ➢ Lakshmikanth CL, Jacob SP, Divya A, Appu Rao AG, Marathe GK. Interference of phenol during quantification of a bacterial lipoprotein. Biokemistri. 27; 123-128. 2015 ➢ Marathe GK, Pandit C, Lakshmikanth CL, Chaithra VH, Jacob SP, D'Souza CJM. To hydrolyze or not to hydrolyze: the dilemma of platelet-activating factor acetylhydrolase. Journal of Lipid Research.55; 1847-1854. 2014
iii.	Has the progress been according to original plan of work and towards achieving objectives if not, state reasons	Yes
iv.	Please indicate the difficulties, if any, experienced in implementing the project	Second installment of the grant was not released in time.
v.	If project has not been completed, please indicate the approximate time by which it is likely to be completed.	Project has been completed
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 work done may also be sent to University Grants Commission.	A summary of the work done for the period (Annual basis) may please be sent to the Commission on a separate sheet.

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: 4 M. Sc Students carried out their project work on the topics related to the MRP and were trained in the project. b) PhD: One candidate registered for Ph.D (DOR.9.9/Ph.D/CVH/0498/2013-14). c) Publications: 4 papers published d) Other impact: Findings from this project will be utilized as preliminary data by PI for writing next grant(s).

PRINCIPAL INVESTIGATOR

Dr. GOPAL MARATHE. K. MSc..Ph.D.
Associate Professor
Dept. of Studies in Biochemistry
University of Mysore, Manasagangotri
Mysore-570006

REGISTRAR

Registrar
University of Mysore
Mysore-570 005

24/10/16

Annexure – IX

UNIVERSITY GRANTS COMMISSION

BAHADUR SHAH ZAFAR MARG

NEW DELHI – 110 002

PROFORMA FOR SUBMISSION OF INFORMATION AT THE TIME OF SENDING THE FINAL REPORT OF THE WORK DONE ON THE PROJECT

1. **TITLE OF THE PROJECT**: "Antagonism of platelet activating factor (PAF) – mediated inflammatory responses by *Tylophora asthmatica*"
2. **NAME AND ADDRESS OF THE PRINCIPAL INVESTIGATOR**:
   Dr. Gopal Marathe K,
   Department of Biochemistry,
   University of Mysore, Manasagangothri,
   Mysore-570006
3. **NAME AND ADDRESS OF THE INSTITUTION**:
   Department of Biochemistry,
   University of Mysore, Manasagangothri,
   Mysore-570006
4. **UGC APPROVAL LETTER NO. AND DATE**: No. F. 41-1284/2012(SR) dated 25th July 2012
5. **DATE OF IMPLEMENTATION**: **1-07-2012**
6. **TENURE OF THE PROJECT**: **3 years+6 months extension (1-07-2012 to 31-12-2015)**
7. **TOTAL GRANT ALLOCATED**: **Rs. 15, 01, 281/-**
8. **TOTAL GRANT RECEIVED: RS. 13, 91, 533/-**
9. **TOTAL AMOUNT RELEASED RS: 13, 91, 533/-**
10. **FINAL EXPENDITURE: RS.13, 91, 369/-**
11. **TOTAL AMOUNT TO BE RELEASE RS: 1, 09, 748/-**
12. **TITLE OF THE PROJECT**: "Antagonism of platelet activating factor (PAF) – mediated inflammatory responses by *Tylophora asthmatica*"
13. **OBJECTIVES OF THE PROJECT**:
    1. Establishing bioassays for PAF-mediated responses at Dept of Biochemistry, Manasagangothri, University of Mysore.
    2. Isolating the active anti-PAF component(s) of *T. asthmatica* root using solid phase extraction columns, HPLC and establishing structures of active components.

3. Chemical synthesis of active component(s) and its/their validation as anti-PAF agent(s)

14. WHETHER OBJECTIVES WERE ACHIEVED:

Yes. The first objective was to develop bioassays in Dept of Biochemistry, Manasagangothri, University of Mysore for inflammatory responses mediated by PAF. Two assays were developed. Human platelet aggregation and human neutrophil adhesion assay.

The second objective was to isolate the active anti-PAF component(s) of *T. asthmatica* root. The crude ethanolic extract of *T. asthmatica* root showed antagonistic properties both in PAF mediated bioassays and in PAF mediated lethality in Swiss albino mice. Hence, individual components of crude were separated by HPLC.

15. ACHIEVEMENTS FROM THE PROJECT:

An animal model for PAF induced lethality was developed. Swiss albino strain of mice was used in this study. Exogenous administration (Intraperitonial) of PAF (5 µg/ mouse) causes death of animals within 15-20min. Molecular mechanism exerted by PAF in inducing sudden death in Swiss albino mice is yet to solve. However, agonists for Toll like receptors has been reported to delay the PAF induced death in Swiss albino mice. Crude ethanolic extract of *T. asthmatica* root was effectively antagonized PAF mediated death. The animals treated with 10 mg/mouse of 2h prior to lethal dose of PAF (5 µg/ mouse), 72% survival was observed where as 0% survival was seen in animals injected with PAF (5 µg/ mouse). An antagonistic property of T .asthmatica root extract was confirmed by histology and quantification of tissue MPO levels in lungs and liver. *Invitro* bioassays for PAF mediated inflammation was established, namely human platelet aggregation and human neutrophil adhesion assay. *T. asthmatica* root extract has showed inhibitory effects in the PAF mediated bioaasys. HPLC separation of individual components of *T. asthmatica* root extract was carried out, the chromatogram showed 12 prominent components. Further validation of individual HPLC fractions needs to be characterized for antagonistic properties in PAF mediated inflammation.

16. SUMMARY OF THE FINDINGS:

Inflammation is a complex non-specific regulated self defence immune response initiated by infectious agents. However, when dysregulated has the potential to cause pathological symptoms. Its progression involves many inflammatory mediators, one such most potent pro-inflammatory phospholipid mediators of known is Platelet Activating Factor (PAF: 1-O-alkyl-2-acetyl-sn-glycerophosphocholine) is a unique and potent biologically active ether phospholipid, produced by wide variety of cell types to an appropriate stimuli. PAF acts by binding to a G-protein coupled receptor (PAF-R), uncontrolled activation of PAF-R leads to many pathological responses like asthma,

ischemia, gastric, pulmonary distress, pancreatitis, allergy, sepsis etc. The biosynthesis of PAF is tightly controlled but, oxidation of phospholipids generates molecules which mimic the structure of PAF (PAF-like lipids) and activates PAF receptor. The uncontrolled activation of the PAF receptor is implicated in pathophysiological responses. PAF is degraded by the hydrolysis of acetyl residue at the sn-2 position by the action of PAF-acetylhydrolase (PAF-AH) enzyme to form biologically inactive lyso-PAF. PAF-AH activity is seen in variety of cells and tissues. Alteration in the levels of plasma PAF-AH has been reported in many diseased conditions. Specific PAF-R antagonists would be expected to neutralize PAF and PAF like lipids mediated inflammatory events. Although, several structurally distinct, competitive PAF-R antagonists have made their way up to clinical trials, not all of the clinical trials that have been conducted to date have been promising. Identification of more effective new PAF-R antagonists likely to impact pathophysiology of PAF mediated inflammation.

Tylophora asthmatica (T.asthmatica) is a perennial plant native to south and east India. It belongs to family Asclepidaceae. Traditionally, T.asthmatica has been used in treatment of asthma, dermatitis and rheumatism. The plant has been described as bronchodilator, emetic, expectorant and diaphoretic. The plants from this species reported to exhibit anti-asthmatic activity by the direct stimulation of adrenal cortex, and also posses in vitro anti-amoebic activity against the strains of Entamoeba histolytica, they are also effective in intestinal as well as hepatic amoebiasis in experimental animals. Phenanthroindolizidine alkaloids from Tylophora species are shown to exhibit anti-inflammatory and anti-tumor properties. In this study, it has been shown that the use of *Tylophora asthmatica* root extract antagonized PAF mediated sudden death in Swiss albino mice and also blocked the PAF mediated human platelet aggregation as well as PAF mediated human neutrophil activation.

Our results suggest the possible presence of active component(s) in this plant and if its chemical identity is established and hopefully such active components can be used in animal models of inflammation.

17. CONTRIBUTION TO THE SOCIETY:

- Setting up novel Inflammatory assays for a laboratory.
- Sudden lethality of PAF in Swiss albino mice was established- A readout that can be readily employed to screen anti-inflammatory compounds.
- Preliminary studies strongly indicate the presence of anti-PAF components in *Tylophora asthmatica.*
- Training both Ph.D and M Sc students to take up the research problems

18. WHETHER ANY PH.D. ENROLLED/PRODUCED OUT OF THE PROJECT:

Yes. Ms. Chaithra V H, project fellow working as full time research scholar, registered for Ph.D (DOR.9.9/Ph.D/CVH/0498/2013-14).

Thesis Title: "Characterization of Platelet activating factor antagonist(s) from *Tylophora asthamatica*".

19. NO. OF PUBLICATIONS OUT OF THE PROJECT: 4, attached.

- Jacob SP, Lakshmikanth CL, Chaithra VH, Kumari TR, Chen CH, McIntyre TM and Marathe GK. Lipopolysaccharide Cross-Tolerance Delays Platelet-Activating Factor-Induced Sudden Death in Swiss Albino Mice: Involvement of Cyclooxygenase in Cross-Tolerance. PLOS ONE DOI:10.1371/journal.pone. eCollection 2016.
- Lakshmikanth CL, Jacob SP, Chaithra VH, Castro-Faria-Neto HC and Marathe GK. Sepsis – In search of cure. Inflamm Res. 65(8):587-602. doi: 10.1007/s00011-016-0937-y. Epub 2016.
- Lakshmikanth CL, Jacob SP, Divya A, Appu Rao AG, Marathe GK. Interference of phenol during quantification of a bacterial lipoprotein. Biokemistri. 27; 123-128. 2015
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PRINCIPAL INVESTIGATOR

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Mysore-570 006

REGISTRAR

Registrar
University of Mysore
Mysore-570 006

24/10/16

Annexure – X

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

ASSESSMENT CERTIFICATE

It is certified that the proposal entitled **"Antagonism of platelet activating factor (PAF) – mediated inflammatory responses by *Tylophora asthmatica*"** by **Dr. Gopal Marathe K** Dept. of Biochemistry has been assessed by the two member committee consisting the following members for submission to the University Grants Commission, New Delhi for financial support under the scheme of Major Research Projects:

Details of Expert Committee:

1. **Prof. K. Kemparaju**
   DOS in Biochemistry
   University of Mysore
   Manasagangothri
   Mysore-570006
2. **Prof. Ravishankar Rai**
   DOS in Microbiology
   University of Mysore
   Manasagangothri
   Mysore-570006

Dr. K. KEMPARAJU, M Sc., Ph D.
Professor
Dept. of Studies in Biochemistry
University of Mysore
Mysore - 570 006, INDIA

Dr. RAVISHANKAR RAI.V
Professor
Department of Studies in Microbiology
University of Mysore
Manasagangothri, Mysore-6

The proposal is as per the guidelines.

REGISTRAR
Registrar
University of Mysore
Mysore-570 005

To,

Smt. Suresh Rani
Under secretary
University Grant Commission
Bahadurshah Zafar Marg
New Delhi-110002

Sub: Depositing unspent balance amount respect of UGC-MRP Project F No. 41-1284/2012
Ref. UGC-Letter No. F.41-1284/2012(SR) Dated 10-01-2018

With reference to the above subject, I am enclosing herewith the deposited details of the **unspent balance amount of Rs. 164/-** (one hundred and sixty four only) vide Pay in Slip dated 18-01-2019. UGC-MRP project entitled **"Antagonisim of platelet activating factor (PAF) – mediated inflammatory responses by *Tylophora asthmatica*"** under taken by Dr. Gopal Marathe K Principal Investigator, UGC-MRP DoS in Biochemistry University of Mysore, Mysore-570006 for consideration and issue of **NO DUE CERTIFICATE(NOC)** for further needful action.

केनरा बैंक Canara Bank **Common Pay-in-Slip**

Date : 18-01-2019
Deposited in Jeevalakshmipuram Branch
Account Maintained at UGC office New Delhi-110002 Branch
Name of Account Holder Secretary UGC-Office
A/c No. 8627101100210212
Cash ✓ Cheque ☐ ₹ 164/-
Amount in words One hundred and Sixty four rupees only.

Chq. No. / Date	Drawee Bank & Branch	Amount (Rs.) ₹	P.
		164	00
		164	00
	Total	164	00

18 JAN 2019

Signature of Cashier Signature of Supervisor

Dr. GOPAL MARATHE. K. M.Sc.,Ph.D.,
PROFESSOR
Dept. Of Studies in Biochemistry
University of Mysore, Manasagangotri
MYSURU-570006

ಮೈಸೂರು ವಿಶ್ವವಿದ್ಯಾನಿಲಯ

ಸ್ಥಾಪನೆ. 1916

ಹಣಕಾಸು ಅಧಿಕಾರಿಗಳ ಕಛೇರಿ
ವಿಶ್ವವಿದ್ಯಾನಿಲಯ ಕಾರ್ಯಸೌಧ
ಮೈಸೂರು-570005

ಹಶಾ. 46/2017-18

ದಿನಾಂಕ : 14-03-2018

ಟಿಪ್ಪಣಿ

ವಿಷಯ:- ಡಿ.ಡಿ. ಯನ್ನು ಅನುದಾನ ಸಂಸ್ಥೆಗೆ ರವಾನಿಸುವ ಬಗ್ಗೆ

ಉಲ್ಲೇಖ:-ನಿಮ್ಮ ಟಿಪ್ಪಣಿ ಸಂಖ್ಯೆ:ಡಿ ವಿ 4/200/2012-13 ದಿನಾಂಕ 09-02-2018

*****

ಮೇಲ್ಕಂಡ ವಿಷಯಕ್ಕೆ ಸಂಬಂಧಿಸಿದಂತೆ ಡಾ॥ ಗೋಪಾಲ್ ಮರಾಠೆ, ಸಹ ಪ್ರಾಧ್ಯಾಪಕರು ಮತ್ತು ಪ್ರಧಾನ ಪರಿಶೋಧಕರು , ಜೀವರಸಾಯನ ಶಾಸ್ತ್ರ ಅಧ್ಯಯನ ವಿಭಾಗ ಮಾನಸ ಗಂಗೋತ್ರಿ, ಮೈಸೂರು. ಇವರು UGC-MRP ಅನುದಾನದಿಂದ ಕೈಗೊಂಡಿರುವ ಯೋಜನೆಯಲ್ಲಿ ಬಳಕೆಯಾಗದೆ ಉಳಿದಿದ್ದ ಮೊಬಲಗು ರೂ. 164/- ಗಳನ್ನು ಅನುದಾನ ಸಂಸ್ಥೆಗೆ ಹಿಂದಿರುಗಿಸುವ ಸಂಬಂಧ **"Secretary UGC New Delhi" (DD No. " 535657 " Dated 13-03-2018 Rs. 164/-)** ಇವರ ಹೆಸರಿನಲ್ಲಿ ಡಿ ಡಿ ಯನ್ನು ತಯಾರಿಸಿ ಈ ಪತ್ರದೊಂದಿಗೆ ಲಗತ್ತಿಸಿ ಮುಂದಿನ ಸೂಕ್ತ ಕ್ರಮಕ್ಕಾಗಿ ರವಾನಿಸಲಾಗಿದೆ.

ಹಣಕಾಸು ಅಧಿಕಾರಿ

ಗೆ,

ಕುಲಸಚಿವರು

ಮೈಸೂರು ವಿಶ್ವವಿದ್ಯಾನಿಲಯ

ಮೈಸೂರು.

ಪ್ರತಿ:-

1. ಡಾ॥ ಗೋಪಾಲ್ ಮರಾಠೆ, ಸಹ ಪ್ರಾಧ್ಯಾಪಕರು ಮತ್ತು ಪ್ರಧಾನ ಪರಿಶೋಧಕರು , ಜೀವರಸಾಯನ ಶಾಸ್ತ್ರ ಅಧ್ಯಯನ ವಿಭಾಗ, ಮಾನಸ ಗಂಗೋತ್ರಿ, ಮೈಸೂರು

2.ಕಛೇರಿ ಪ್ರತಿ