Progress Report for R&D Projects [2018 to 2021]*

Section-A: Project Details

A1. Project Title: **Search for novel anti-platelet and anti-thrombin peptides from Indian viper venom (*Daboia russelii*): Purification, characterization and evaluation of its anti-thrombotic potential**

A2. DBT Sanction Order No. & Date: **BT/PR24531/NER/95/755/2017 Date 30.09.2018**

A3. Name of Principal Investigator: **Prof. Robin Doley (Tezpur University)**
Prof. Mohammad Ashraf (Jamia Milia Islamia University)

Name of Co-PI/Co-Investigator: **Dr. Rupak Mukhopadhyay (Tezpur University)**
Dr. Syed Mansoor Ali (Jamia Milia Islamia University)

A4. Institute: **Tezpur University**
Jamia Milia Islamia University

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A6. Total Cost: **Rs.6079600/-**

A7. Duration: **3 years**

A8. Approved Objectives of the Project:

1. *To isolate anti-platelet and anti-thrombin peptides from Daboia russelii venom (Tezpur University)*
2. *To characterize the biochemical and biophysical properties of purified anti-platelet and anti-thrombin peptides. (Tezpur University)*
3. *To evaluate the antithrombotic potential of purified peptides using animal models of thrombosis. (Jamia Milia Islamia University)*
4. *To identify the molecular mechanism involved in ant- thrombotic potential of purified peptides using proteomics approach. (Jamia Milia Islamia University)*

A9. Specific Recommendations made by the Task Force (if any): NA

Section-B: Scientific and Technical Progress

B1. Summary of the project (500 words) : *attached in Annexure I*

- **Background for the Project (including rationale of the project):**
- **Objectives, Methodology & Work plan:**
- **Outcome of the Project (Expected outcome and the Outcome obtained):**
- **Discussions:**

B2. Achievements till date (Objective-wise): *attached in Annexure II*

B3. Challenges, if any: Due to lockdown purification of samples could not be carried out

B4. Action taken on the earlier recommendations of the TEC / STAG / Review Committee: None

B5. Details of New Leads Obtained, if any: None

B6. Details of Publications & Patents, if any: Manuscript under preparation

Section-C: Details of Grant Utilization (Tezpur University)#

C1. Equipment Acquired or Placed Order with Actual Cost: Acquired Rs. 13.13707 lakhs

C2. Manpower Staffing and Expenditure Details: Rs. 4.53334 lakhs

C3. Details of Recurring Expenditure: 2.75771 lakhs

C4. Financial Requirements for the Next Year with Justifications:

Manpower: Due: 3.75 lakhs (Jan 2020 to March 2021 @25,000 per month)
Due: 4.2 lakhs (April 2021 to March 2022 @35,000 per month)
Total: 7.95 lakhs

Consumable: 3.0 lakhs (For purchase of chemicals and consumables)
Contingency: 0.5 lakhs (for purchase of stationeries)
Travel : 0.5 lakhs (To visit Jamia for experiment)
Overhead : 1.0 Lakhs
Total : 12.95 lakhs

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

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

Instructions:

(i) All the information needs to be provided; otherwise the Progress Report will be treated as incomplete. In case of 'Nil' / 'Not Applicable' information, the same may be indicated.

(ii) In case of multicentre project, a combined Progress Report should be submitted incorporating the progress of all components. The Project Co-coordinator/ PI will be responsible for this.

*(iii) *Please indicate the reporting period [i.e. Year 1/2/3/4/5].*

(iv) Submission of Progress Report by the end of the 11th month of grant sanction is linked with further continuation of the project and timely release of funds for the next year

B1. Summary of the project (500 words)

Background for the Project (including rationale of the project):

Snake venom is a mixture of pharmacologically active proteins and polypeptides working independently or synergistically for inducing various pharmacological effects, such as neurotoxicity, cardiotoxicity, pro-coagulant and/or anticoagulant effects, tissue necrosis and even death in prey or victim. Apart from its toxic effect, this highly evolved venom proteins have contributed towards the development of life saving drugs such as captopril, eptifibatide, tirofiban and others. Selectivity of these toxins and peptides towards its target makes them potential molecules for therapeutic use as it reduces side effects. Therefore understanding the structure and mechanism of toxicity of these proteins have served as research tools as well as prototype for development of drugs.

The haemostatic system of prey/victim is a common target of all the snakes for capturing prey. For instance, Russell's viper envenomation mainly causes excess bleeding leading to death if not treated immediately. These proteins disrupt the components of primary and secondary haemostasis for such pathological manifestations. Recently we have reported the proteome profile of Indian *Daboia russelii* venom which is a complex mixture of proteins and polypeptides. However the bioactivity of some of these proteins needs to be deciphered. Isolating and characterizing anti-platelet and anti-thrombin peptides would lead to development of antithrombotic drugs.

The limitations of present thrombotic therapies and recent developments on snake venom research have provided new tools to decipher molecular details of various physiological processes and inspiration to design a number of therapeutic agents. Although significant progress has been made in understanding the structure–function relationships and the mechanisms of some of these antithrombotics, still these leads fail to reach the market as a new drug. Mainly because of the toxicity associated with the venom and lack of information regarding their mechanism of action, and signalling cascade associated with it. **Thus we hypothesized that evaluation of antithrombotic potential of venom peptides along with signalling pathways using proteomics approach will lead from bench to bedside.**

Objectives, Methodology & Work plan:

Overall Objectives:

1. *Purification of anti-platelet and anti-thrombin peptides from venom using chromatographic approach*
2. *Biochemical and biophysical characterization of the purified anti-platelet and anti-thrombin peptides.*
3. *Evaluation of antithrombotic potential of purified peptides using in vitro and in vivo animal model systems.*
4. *Platelet proteome analysis to elucidate the signalling cascade induced by anti-platelet and anti-thrombin peptides.*

Work plan:

OBJECTIVE 1

Purification of anti-platelet proteins/ peptides from Indian *Daboia russelii* venom

1. Fractionation of crude venom using chromatographic approach

OBJECTIVE 2

Biochemical and biophysical characterization of the purified anti-platelet protein/peptide

1. Blood coagulation factor assay
2. Determination of primary structure by sequencing/ LC MS-MS
3. Determination of secondary structure

OBJECTIVE 3

Evaluation of anti-thrombotic effect of purified anti-platelet protein/ peptide

1. In-vitro clotting time
2. Thrombin generation assay
3. Thrombin-induced fibrin polymerization assay
4. Clot retraction assay
5. Assay in thrombosis model
6. Bleeding time
7. In-vivo toxicity assays

OBJECTIVE 4

Platelet proteome analysis to elucidate the signaling cascade induced by anti-platelet protein/ peptide

1. Isolation of platelets from blood of control and peptide treated thrombotic animals
2. Preparation of total platelet protein for platelet proteome analysis and iTRAQ labeling

Outcome of the Project:

Expected outcome:

a. Understanding the mechanism of anti-platelet and anti-thrombin peptides from the venom of Indian *Daboia russelii* and its contribution towards the venom toxicity.
b. Characterization of the anti-thrombotic peptide obtained from the venom.
c. Elucidating the pharmacokinetics and pharmacodynamics of anti-platelet and anti-thrombin peptides
d. Identification of molecular mechanism(s) involved in antithrombotic action of anti-platelet and anti-thrombin peptides

e. Effect of anti-platelet on Platelet proteome and elucidation of the signalling cascade induced by anti-platelet and anti-thrombin peptides.

Outcome obtained:

a. Identification and isolation of anti platelet protein from *Daboia russelii* venom and biochemical characterization
b. Identification and isolation of thrombin like protein from *Trimerusurus erythrurus* venom and biochemical characterization (Additional work done)

Discussions: Crude venom of *Daboia russelii* showed anti-platelet activity. In order to identify the anti-platelet components, it was fractionated by RP-HPLC and a total of 37 fractions were revealed, out of which P29 exhibited maximum inhibition of platelet aggregation induced by collagen. P29 further fractionated into 4 fractions and among them F4 exhibited the highest inhibition of platelet aggregation. However, it was much less compared to that of the parent fraction. P29 possesses PLA_2 activity but has no effect on coagulation assays and thrombin inhibition.
Crude venom of *Trimeresurus erythrurus* was found to possess thrombin-like activity which was subjected to fractionation for isolation of the component responsible for the activity. RP-HPLC crude venom gives a total of 20 peaks which were screened for thrombin-like activity and P15 showed most activity among them. Mass spectrometric analysis of peak 15 identifies 4 proteins belonging to SVSPs which might be acting as thrombin-like enzyme either independently or synergistically.

Further studies comprising mechanism of action and structural details of these proteins might give a better understanding of thrombin-like enzymes and unveil the therapeutic potentials of these enzymes.

Objectives:

1. *Purification of anti-platelet and anti-thrombin peptides from venom using chromatographic approach:*

2. *Biochemical and biophysical characterization of the purified anti-platelet and anti-thrombin peptides:*

Objective 1: *Purification of anti-platelet and anti-thrombin peptides from venom using chromatographic approach:*

METHODOLOGY:

1.1: ASSAY OF CRUDE VENOM FOR ANTI-PLATELET ACTIVITY

Preparation of platelet rich plasma (PRP) and platelet poor plasma (PPP):

For preparation of PRP fresh human blood was collected in tubes containing 3.8% tri-sodium citrate (9:1, blood: tri-sodium citrate) and allowed to stand vertically for 10 mins. PRP was prepared by centrifuging the standing whole blood tubes at 160g for 6 mins. The yellowish supernatant containing PRP was collected by pipetting out and used immediately within 4 hours.

For preparation of PPP fresh goat blood collected in tubes containing 3.8% tri-sodium citrate (9:1, blood: tri-sodium citrate) or the blood left after PRP separation was subjected to another centrifugation step at 3000 rpm for 20 minutes. The yellowish, clear supernatant was collected by pipetting out and used immediately or stored at -20oC for future use.

In-vitro platelet aggregation: In-vitro platelet aggregation was studied according to method developed by Born (1962) using Lumi- Aggregometer (CHRONO-LOG Corporation, USA). For dose dependent assay, different concentrations of crude venom (0.01, 0.1, 1.0 and 10 µg/ml) were pre-incubated with 500 µl of PRP in glass cuvettes for 3 minutes at 37ºC. Following this platelet aggregation was induced by addition of agonists, i.e. collagen (2µg/ml). The maximum platelet aggregation rate was recorded within 6 minutes with continuous stirring at 37ºC. The light transmittance was calibrated with PPP. Platelet aggregation of PRP induced by agonists was taken as control.

1.2: FRACTIONATION OF CRUDE VENOM TO ISOLATE ANTI-PLATELET PROTEINS

Reverse Phase HPLC (RP-HPLC): 1ml (2 mg) of crude RVV was subjected to RP-HPLC using Jupiter C18 with particle size of 5µm, pore size 300A˚ and dimensions 4.6×250mm. The column was pre-equilibrated with milli Q containing 0.1% trifluoro acetic acid (TFA) and fractionated using 80% acetonitrile (MeCN) containing 0.1%TFA. The flow rate was maintained at 1ml/min. A gradient of 5-70% MeCN in 20-140 minutes was used for separation of proteins. The total time of run was set for 170 minutes. Protein was monitored at 215 and 280 nm. The fractions were collected manually.

SDS-PAGE: RP-HPLC fractions were subjected to electrophoresis according to the method developed by Laemmeli (1970). 12.5% resolving poly acrylamide gel and 5% stacking poly acrylamide gel was prepared. 5µl loading buffer was mixed with 15µl sample (5µg) and loaded into wells. For reducing condition, β-ME was added in the ratio of 1:9 to loading buffer and the mixture was boiled for 3 minutes at 95°C before loading into the wells. The electrophoresis was performed at constant Volt (80V) for stacking and 120 V for resolving. For detection of protein bands in gel, silver staining was performed.

1.3: SCREENING FOR ANTI-PLATELET ACTIVITY:

In-vitro platelet aggregation: In-vitro platelet aggregation was studied using 1µg of RP-HPLC fraction as described earlier.

RECHROMATOGRAPHY OF P29:

P29 (100ug) was re-chromatographed using Acclaim 300 column. The flow rate was maintained at 1ml/min. TFA (0.1%) and acetonitrile (80%) were used to create a gradient of 40-50% B in 15-65 minutes. The total time of run was set for 90 minutes. Protein was monitored at 215 and 280 nm. The fractions were collected manually.

1.4: ASSAY OF P29 FRACTIONS FOR ANTI-PLATELET ACTIVITY

Preparation of platelet rich plasma (PRP) and platelet poor plasma (PPP):

For preparation of PRP fresh human blood was collected in tubes containing 3.8% tri-sodium citrate (9:1, blood: tri-sodium citrate) and allowed to stand vertically for 10 mins. PRP was prepared by centrifuging the standing whole blood tubes at 160g for 6 mins. The yellowish supernatant containing PRP was collected by pipetting out and used immediately within 4 hours.

For preparation of PPP the blood left after PRP separation was subjected to another centrifugation step at 3000 rpm for 20 minutes. The yellowish, clear supernatant was collected by pipetting out and used immediately or stored at -20ºC for future use.

In-vitro platelet aggregation: In-vitro platelet aggregation was studied according to method developed by Born (1962) using Lumi- Aggregometer (CHRONO-LOG Corporation, USA). Briefly, 1 µg of each fraction was pre-incubated with 500 µl of PRP in glass cuvettes for 3

minutes at 37ºC. Following this platelet aggregation was induced by addition of agonists, i.e. collagen (2µg/ml). The maximum platelet aggregation rate was recorded within 6 minutes with continuous stirring at 37ºC. The light transmittance was calibrated with PPP. Platelet aggregation of PRP induced by agonists was taken as control.
For dose dependent assay, different concentrations of P29_4 (0.01, 0.1, 1.0 and 10 µg/ml) were pre-incubated with 500 µl of PRP in glass cuvettes for 3 minutes at 37ºC prior to addition of agonist.

1.5: RESULTS AND DISCUSSION:
Inhibition of collagen induced platelet aggregation was observed in a minimum dose of 0.1µg/ml of crude RVV. 0.1 and 1µg/ml RVV inhibited 3% platelet aggregation and 10µg/ml inhibited 10% platelet aggregation (Fig 1A and 1B). To identify and isolate the anti-platelet components, crude RVV was fractionated and the fractions were screened for anti-platelet activity. Fractionation of crude RVV on the basis of hydrophobicity revealed a total of 37 peaks (Fig 2) out of which 8 major peaks were run on a 12.5% SDS-PAGE and stained with silver stain (Fig 3) to check the purity. To identify the anti-platelet components, the fractions were screened for anti-platelet activity. The effect of 1µg RP-HPLC fractions on % aggregation of PRP induced by collagen was determined. The fractions P3, P4, P9, P10, P11, P12, P16, P17, P22, P23, P24 and P29 inhibited collagen induced platelet aggregation. Out of these P29 exhibited maximum inhibition (Fig 4A and 4B). P29 further fractionated on the basis of hydrophobicity gave 4 peaks (Fig 5A). The peaks were numbered as F1 - F4. To assess the purity and determine the molecular weight of the proteins, SDS PAGE was performed (Fig 5B). Subsequently, each of these was screened for anti-platelet activity and it was observed that the highest inhibition of platelet aggregation was exhibited by F4 among the RP-HPLC fractions of P29 (Fig 5C and5). However, it was much less compared to that of the parent fraction. Dose dependent effect of F4 on platelet aggregation induced by collagen was studied and no dose dependent effect was observed (Fig 5E and 5F). Biochemical characterization of the parent fraction, P29 was then performed to study its characteristics.

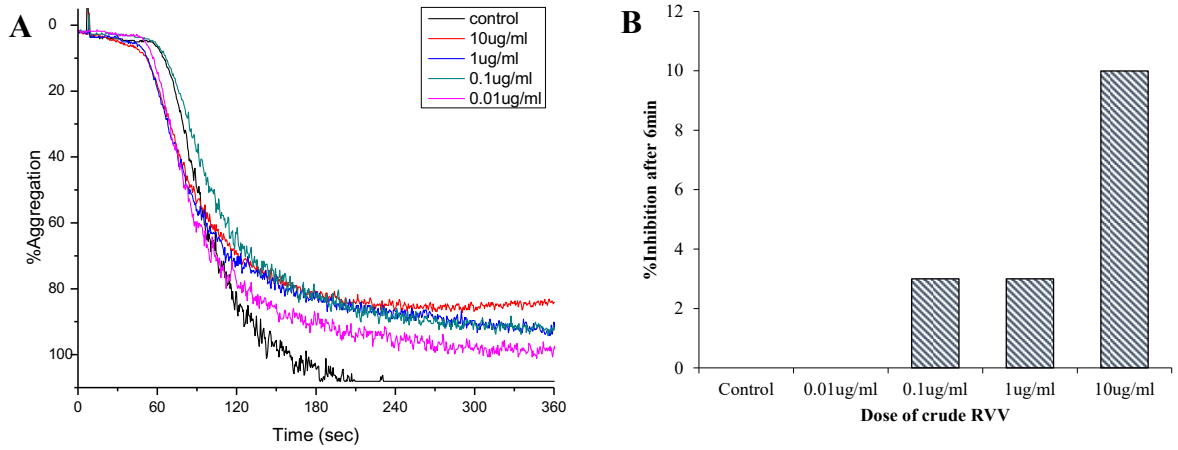


Figure 1: Dose dependent effect of crude *Daboia russelii* venom on platelet aggregation induced by collagen (A) Progressive curve (B) Bar diagram

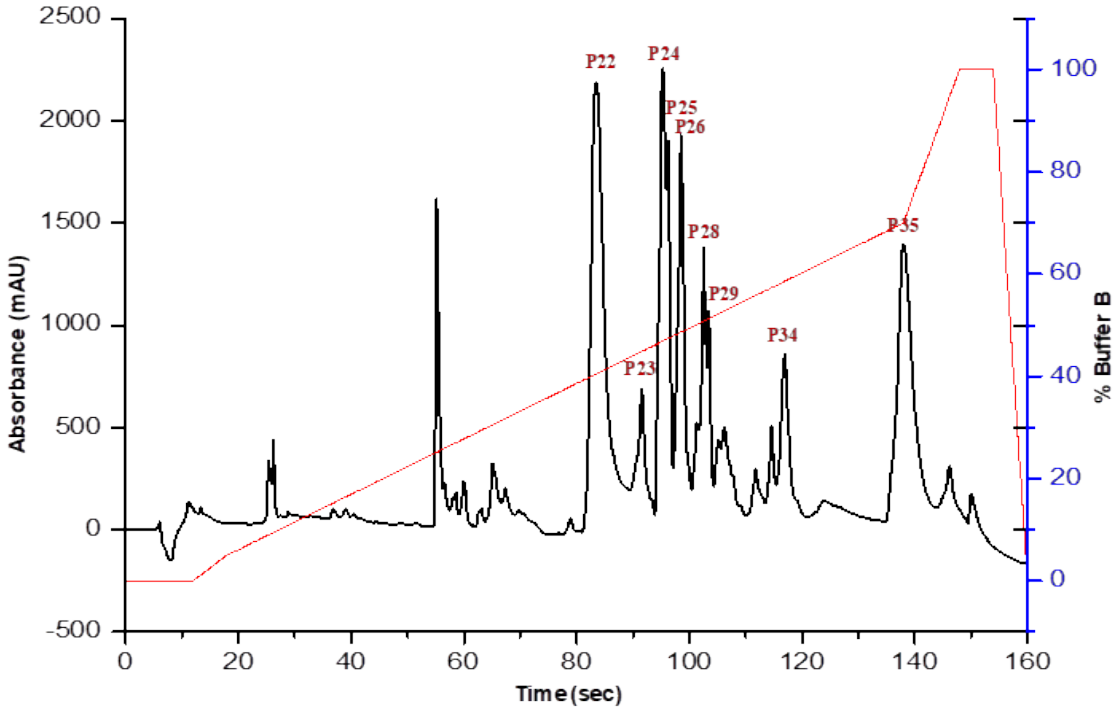


Figure 2: Reverse Phase HPLC profile of crude venom of *Daboia russelii.*

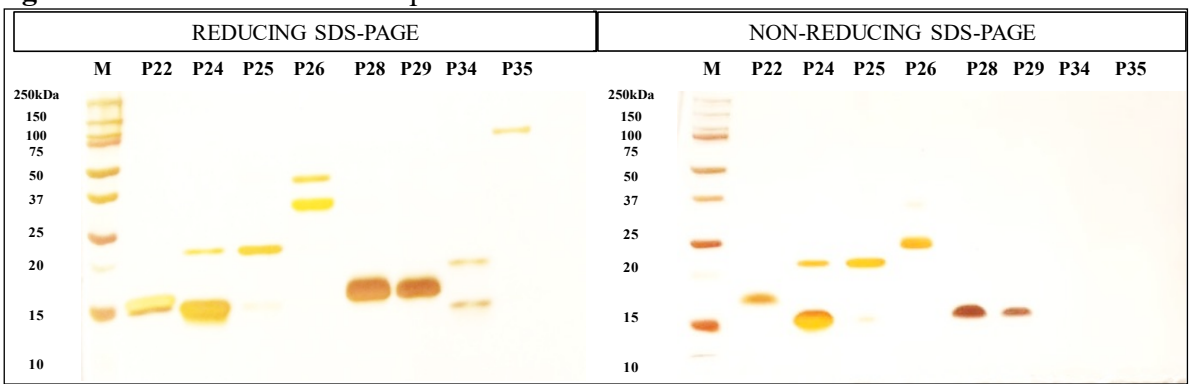


Figure 3: Reducing and non-reducing silver stained 12.5% SDS-PAGE of RP-HPLC fractions (M: Page ruler plus protein marker, P22, P24, P25, P26, P28, P29, P34, P35 are RP-HPLC peaks

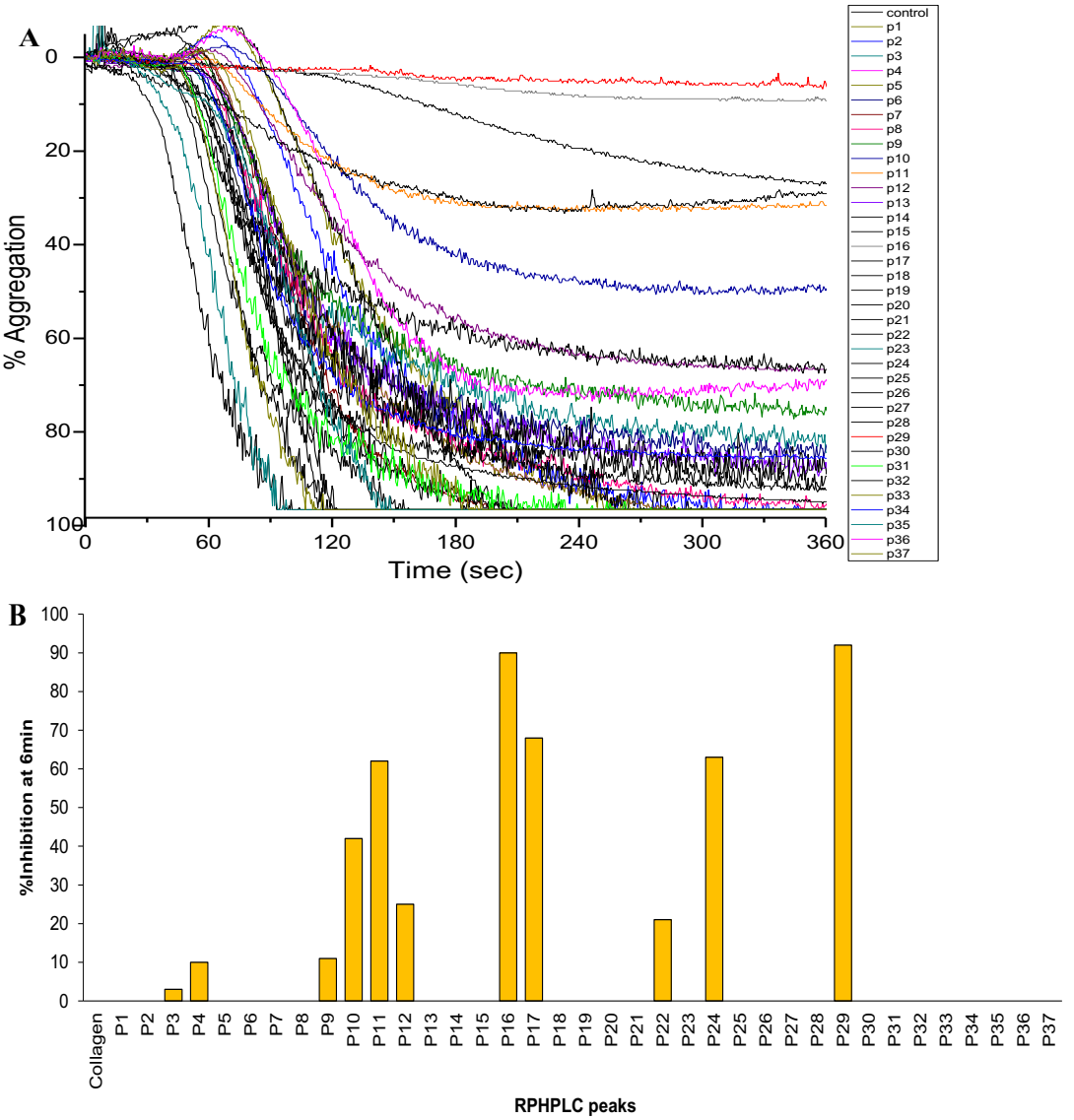
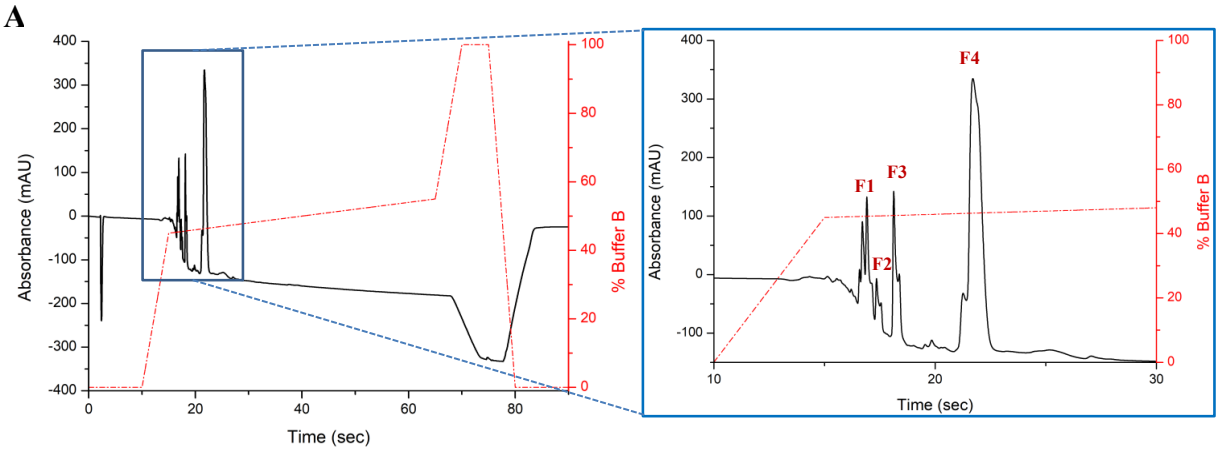


Figure 4: Effect of RP HPLC peaks (1ug each) on platelet aggregation induced by collagen. (A) Progressive curve, (B) Bar Graph



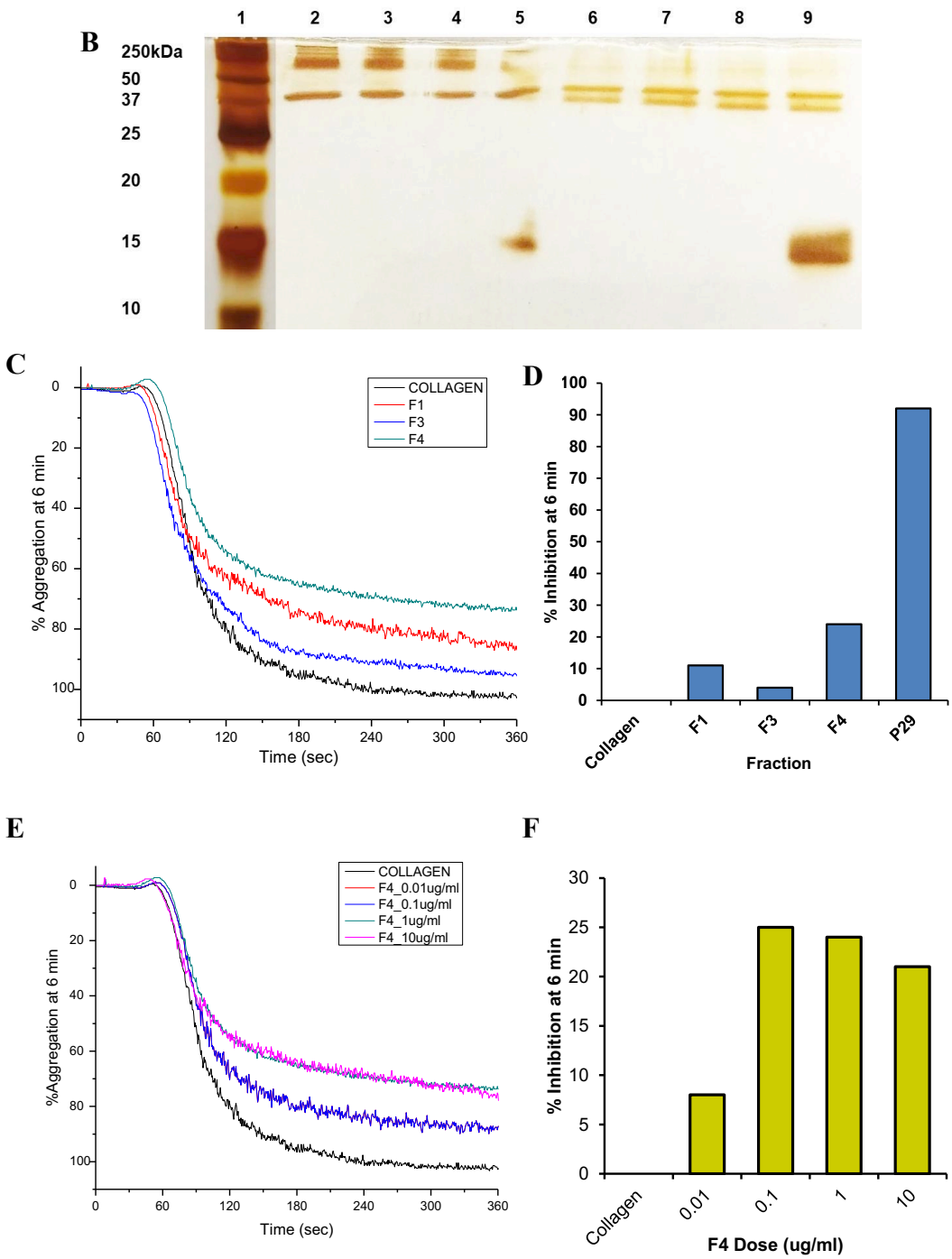


Figure 5: (A) Separation of P29 proteins of *Daboia russelii* on Acclaim 300 column. **(B)** Silver stained 12.5% reducing SDS-PAGE of RP-HPLC peaks (Lane 1: Page ruler plus protein marker, Lane 2-5: RP-HPLC fractions 1 to 4 in non reducing condition Lane 6-9: RP-HPLC fractions 1 to 4 in reducing condition). **(C-D) Effect of RPHPLC fractions of P29 on platelet aggregation induced by collagen** (C) Progressive curve, (D) Bar graph **(E-F) Dose dependent effect of P29_F4on platelet aggregation induced by collagen:** (E) Progressive curve, (F) Bar graph

Objective 2: Biochemical characterization of anti-platelet protein from Daboia russelii venom

1.1: METHODOLOGY:

SDS-PAGE: P29 was subjected to electrophoresis according to the method as described earlier. For detection of protein bands in gel, silver staining was performed.

Phospholipase A_2 (PLA_2) assay: The PLA_2 activity of P29 was determined using $sPLA_2$ assay kit (Cayman, MI, USA) according to the manufacturer's protocol using diheptanoylthio-phosphatidylcholine as substrate. Briefly, different amounts of P29 (0.01, 0.1, 1.0 and 10 µg/ml) were added to reaction mixture containing DTNB [5,5'- dithio-bis-(2-nitrobenzoic acid)] and the reaction volume was adjusted to 20 µl with assay buffer (25 mM Tris-Cl pH 7.5, 10 mM CaCl2, 100 mM KCl, 0.3 mM Triton X-100). Following this, 1.66 mM of the substrate (200 µl) was added to initiate the hydrolytic reaction. As positive control, 0.05µg bee venom PLA2 was used. The rate of hydrolysis was quantified at 405 nm for 10 min MultiSkanGO spectrophotometer (Thermo Scientific, USA). For each set of experiments reaction mixture containing DTNB and assay buffer was taken as blank and 0.05µg bee venom PLA2 was used as positive control. Enzyme activity was expressed in micromoles of substrate hydrolysed per min of enzyme (µM/ min). The results are mean ± SD of three independent experiments.

Recalcification time (RT): Recalcification time of citrated goat PPP was determined according to method developed by Langdell (1953) with slight modifications. Different amounts of P29 (0.01, 0.1, 1.0 and 10 µg/ml) were pre-incubated with 50µl of PPP for 2 minutes at 37ºC and the plasma clot formation was initiated by addition of 25µl of 50mM $CaCl_2$. Following gentle shaking the change in OD was monitored at 405 nm every 15 seconds for 900 seconds using a MultiSkanGO spectrophotometer (Thermo Scientific, USA). For each set of experiment, clotting time of PPP with 20mM Tris-Cl buffer (pH 7.4) was considered as the normal clotting time (NCT).

Prothrombin time (PT): PT was measured according to manufacturer's protocol. Different amounts of P29 (0.01, 0.1, 1.0 and 10 µg/ml) were added with 50µl of goat PPP and incubated for 2 minutes at 37ºC. Subsequently, 50µl of Uniplastin was added to initiate clot formation which was monitored at 405 nm every 2 seconds for 120 seconds using a MultiSkanGO spectrophotometer (Thermo Scientific, USA). For each set of experiment, clotting time of PPP with 20mM Tris-Cl buffer (pH 7.4) was considered NCT.

Activated partial thromboplastin time (APTT): APTT was determined according to the manufacturer's instructions. Briefly, different amounts of P29 (0.01, 0.1, 1.0 and 10 µg/ml) were pre-incubated with 50 µl goat PPP and 50 µl APTT reagent for 3 min at 37°C. Subsequently, 50µl of 25mM CaCl2 was added to trigger the clotting time of plasma which was monitored at 405 nm every 2 seconds for 300 seconds using a MultiSkanGO spectrophotometer (Thermo Scientific, USA). For each set of experiment, clotting time of PPP with 20mM Tris-Cl buffer (pH 7.4) was considered NCT.

Thrombin like Activity: Thrombin like activity of P29 was assessed for doses 0.01, 0.1, 1 and 10µg/ml in buffer containing 20mM Tris pH7.4, 100mM NaCl and 1mg/ml bovine serum albumin. Each dose was pre-incubated for 2 min followed by addition of substrate S2238 (200mM). The release of colored product p-nitroaniline was monitored at 405 nm for 900 secs at an interval of 10 sec using a MultiSkanGO spectrophotometer (Thermo Scientific, USA). Reaction containing buffer, thrombin and substrate was taken as control.

Thrombin Inhibition Assay: Inhibition of amidolytic activity of thrombin by P29 was assessed for doses 0.01, 0.1, 1 and 10µg/ml in buffer containing 20mM Tris pH7.4, 100mM NaCl and 1mg/ml bovine serum albumin. Each dose was pre-incubated with 0.5nM thrombin for 30 min followed by addition of substrate S2238 (200mM). The release of colored product p-nitroaniline was monitored at 405 nm for 900 secs at an interval of 10 sec using a MultiSkanGO spectrophotometer (Thermo Scientific, USA). Reaction containing buffer, thrombin and substrate was taken as control.

Platelet Aggregation Assay: In-vitro platelet aggregation was studied for different concentrations of P29 (0.01, 0.1, 1.0 and 10µg/ml) as described earlier.

Comparative effect of P29 on platelet aggregation induced by collagen, Ristocetin, ADP and AA: The effect of 1ug of P29 on platelet aggregation induced by various agonists was studied as described earlier.

2.2: RESULTS AND DISCUSSION:

SDS PAGE analysis of P29 revealed single bands of molecular weight ~15kDa in both reducing and non-reducing SDS PAGE (Fig 6) which is in the range of PLA_2 family. This was confirmed by performing PLA_2 activity assay. It was observed that there is an increase in $sPLA_2$ activity with increase in concentration (Fig 7A). The dose dependent effect of P29 on recalcification time, prothrombin time and activated partial thromboplastin time was studied and it was observed that there is no delay or shortening of coagulation time with respect to normal clotting time by any of the doses suggesting that P29 does not exert any effect on the intrinsic as well as extrinsic pathways of blood coagulation (Fig 7B). Dose dependent thrombin-like activity of P29 and its effect on thrombin inhibition was studied. Neither did it show thrombin like activity nor inhibited thrombin when tested with chromogenic substrate (Fig 7C-7D). The dose dependent effect of different concentrations of P29 was studied. Inhibition of platelet aggregation was observed in the minimum dose taken for the study i.e. 0.01µg/ml of P29 and it increased in a dose dependent manner. The percentage inhibition of collagen induced platelet aggregation by 0.01, 0.1, 1 and10µg/ml of P29 were 32%, 44%, 57% and 91% respectively (Fig 8). The effect of 1 µg of P29 on platelet aggregation induced by various agonists was studied. It was found that platelet aggregation induced by collagen was maximum inhibited (92% inhibition by P29, 0% by control) and that by ristocetin was mildly inhibited (45% inhibition by P29, 15% by control). P29 had no effect on platelet aggregation induced by arachidonic acid (0% inhibition by P29, 0% by control). ADP induced platelet aggregation was enhanced (99% aggregation by P29 as compared to 53% by control) (Fig 9).

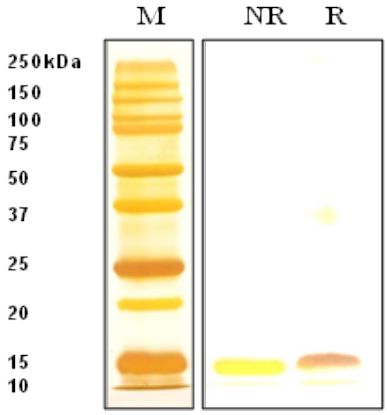


Figure 6: Silver stained 12.5% SDS-PAGE of RPHPLC fraction P29 (M: marker, NR: Non reduced, R: Reduced)

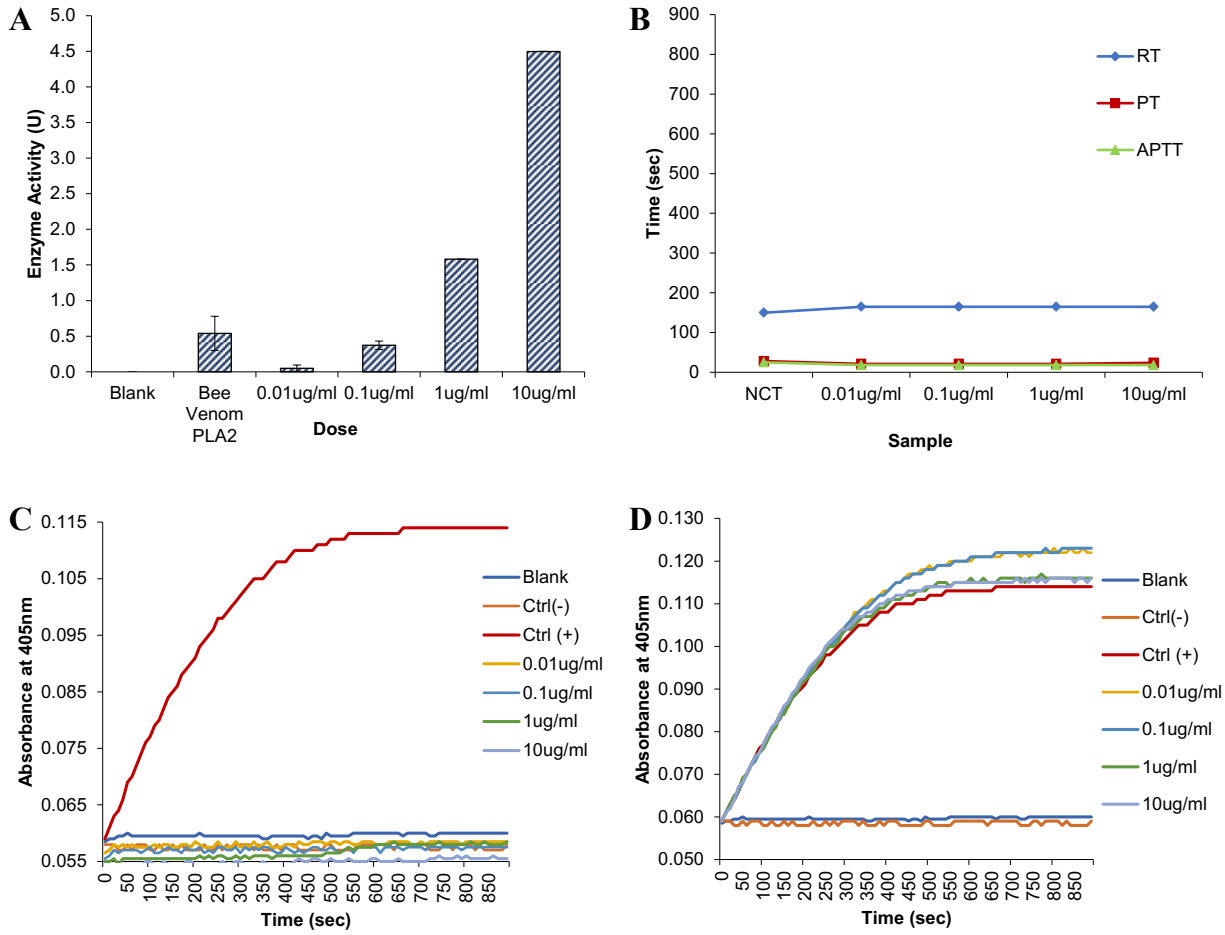


Figure 7: Dose dependent effect of P29 on (A) sPLA2 activity, (B) Coagulation Assays RT- Recalcification time, PT- Prothrombin time, APTT- Activated partial thromboplastin time, (C) Thrombin like activity (D) Thrombin inhibition.

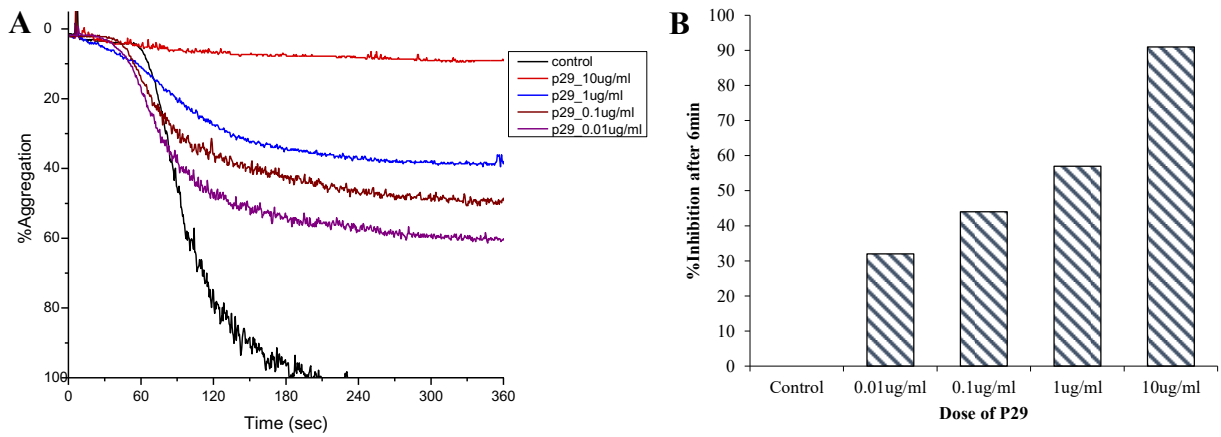


Figure 8: Dose dependent effect of crude P29 on platelet aggregation induced by collagen (A) Progressive curve (B) Bar diagram

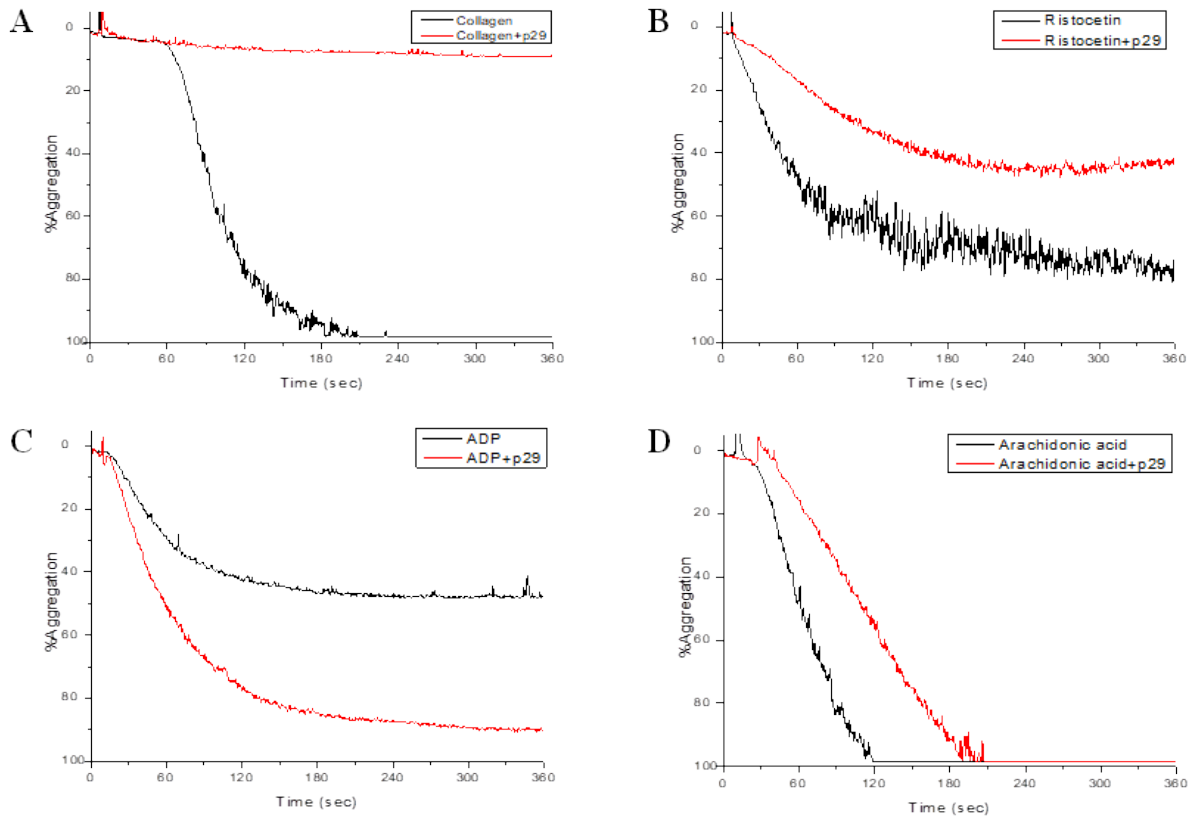


Figure 9: Progressive curve of effect of 1ug of P29 on platelet aggregation induced by various agonists (A) Collagen, (B) Ristocetin, (C) ADP, (D) Arachidonic acid.

Other objectives (Tezpur University):

1: Purification anti-thrombin peptides from venom using chromatographic approach

Purification of the anti-thrombin peptide could not be carried out due to lockdown

2: Biochemical and biophysical characterization of anti-thrombin peptides.

Characterization of the anti-thrombin peptide could not be carried out due to lockdown

Objectives of Jamia Milia Islamia University:

3: Evaluation of antithrombotic potential of purified peptides using in vitro and in vivo animal model systems.

4: Platelet proteome analysis to elucidate the signalling cascade induced by anti-platelet and anti-thrombin peptides.

Final Remarks

Due to lockdown, purification of anti-platelet and anti-thrombin peptide could not be carried at Tezpur University. Therefore objective 3 & 4 could be carried out due to lack of samples. Moreover due to non-release of remaining funds further experiments could be conducted. Base on the results obtained from the experiments following papers was published.

1. Rafika Yasmin, Shankar Chanchal, Mohammad Zahid Ashraf, Robin Doley. Daboxin P, a phospholipase A2 of Indian Daboia russelii venom, modulates thrombin-mediated platelet aggregation. J Biochem Mol Toxicol. 2023 Nov;37(11):e23476. doi: 10.1002/jbt.23476. Epub 2023 Jul 19.

2. Susmita Thakur, Rafika Yasmin, Anita Malhotra, Hmar Tlawmte Lalremsanga, Vishal Santra, Surajit Giri, Robin Doley. Isolation and Functional Characterization of Erythrofibrase: An Alfa-Fibrinogenase Enzyme from Trimeresurus erythrurus Venom of North-East India. Toxins (Basel) 2024 Apr 22;16(4):201.. doi: 10.3390/toxins16040201.

Instrument procured

Name of the instrument: 700-2 dual channel optical aggregometer
Sanction amount: 15.00 lakhs
Actual cost: 13.13707 lakhs
Purchase order: TU/11-12/Pur/MBBT/2018-19/5359 dt. 30/03/2019
Invoice No. WI-CHE-CAP-0045 Dt. 3/05/2019
Date of Installation: 23/05/2019

Photograph of the Instrument installed in the laboratory

(Prof. Robin Doley)
Principal Investigator
Tezpur University

(Prof. Mohammad Zahid Ashraf)
Co-Investigator
Jamia Milia Islamia University

Statement of Expenditure referred to in para 9 of the Utilization Certificate

Showing grants received from the Department of Biotechnology and the expenditure incurred during the period from **1/04/2021** to **27/09/2022** (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)	Remark
1	2	3	4	5	6	7	8
A. Non –Recurring							
Equipment	1.86293	0		1.86293	0	1.86293	
B. Recurring							
Human Resource	0.03334	0		0.03334	0	0.03334	Fellowship paid up to Dec 2019
Consumables	0.08251	0		0.08251	0	0.08251	
Travel	0.16430	0		0.16430	0	0.16430	
Contingency	0.19421	0		0.19421	0.04720	0.14701	
Overheads (if applicable)	0.05570	0		0.05570	0	0.05570	
Interest earned	0	0	0.05864	0.05864	0	0	Deposited to Bharatkosh
Total	**2.39299**	**0**	**0.05864**	**2.45163**	**0.04720**	**2.34579**	

(PROJECT INVESTIGATOR)
(Signed and stamped)

Dr. Robin Doley
Professor
Dept. of Molecular Biology and Biotechnology
Tezpur University
(a Central University)
Napaam, Tezpur- 784 028
Sonitpur, Assam (India)

(HEAD OF THE INSTITUTE)
(Signed and stamped)

Registrar
Tezpur University

(FINANCE OFFICER)
(Signed and stamped)

प्रभारी वित्त अधिकारी
तेजपुर विश्वविद्यालय
Finance Officer I/c
Tezpur University

Assets acquired wholly or substantially out of Government grants
Register to be maintained by Grantee Institution

Name of the Sanctioning Authority: **Government of India, Ministry of Science and Technology, Department of Biotechnology (NER-BPMC)**

1. Sl. No. **327**
2. Name of Grantee Institution : **Tezpur University**
3. No. & Date of sanction order : **BT/PR24531/NER/95/755/2017 Date: 28/09/2018**
4. Amount of the sanctioned grant : **15.0 lakhs**
5. Brief purpose of the grant : **Biotechnology research and development**
6. Whether any condition regarding the right of ownership of Govt. in the property or other assets acquired out of the grant was incorporated in the grant –in-aid sanction order. :**DBT**
7. Particulars of assets actually credited: **700-2 dual channel optical aggregometer** or acquired.
8. Value of the assets as on : **13.13707 lakhs**
9. Purpose for which utilised at present: **will be for Research and Development___**
10. Encumbered or not **__Not___**
11. Reasons, if encumbered **__NA__**
12. Disposed of or not **__NA_**
13. Reasons and authority, if any, for disposal**__NA__**
14. Amount realised on disposal **__NA__**
15. Remarks: **Instrument sanction under the project is procured and under use.**

(PROJECT INVESTIGATOR)
(Signed and stamped)
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Professor
Dept. of Molecular Biology and Biotechnology
Tezpur University
(a Central University)
Napaam, Tezpur- 784 028
Sonitpur Assam (India)

(FINANCE OFFICER)
(Signed and stamped)
Finance Officer
Tezpur University

(HEAD OF THE INSTITUTE)
(Signed and stamped)
Registrar
Tezpur University

List of Assets

S. No.	Name of equipment	Quantity	Sanctioned Cost	Actual Purchased cost	Purchase details
1	**700-2 dual channel optical aggregometer**	01	15.00 lakhs	13.13707 lakhs	Purchase order: TU/11-12/Pur/MBBT/2018-19/5359 dt. 30/03/2019 Invoice No. WI-CHE-CAP-0045 Dt. 3/05/2019 Date of installation: 23/05/2019

(PROJECT INVESTIGATOR)
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