Std. Doc.: NMHS/FG-FTR
National Mission on Himalayan Studies (NMHS) 2020

Template/Pro forma for Submission

NMHS-Himalayan Institutional Fellowship Grant

FINAL TECHNICAL REPORT (FTR)

NMHS Reference No.:	GBPNI/NMHS-2018-19/HSF23-01/152 dated 17.12.2018	**Date of Submission:**	2	9	1	2	2	0	2	2
			d	d	m	m	y	y	y	y

FELLOWSHIP TITLE (IN CAPITAL)

TO STUDY PROTECTIVE EFFECT OF MEDICINAL PLANTS GROWN IN SIKKIM HIMALAYA IN KIDNEY FUNCTION OF RATS WITH DIABETIC NEPHROPATHY

Sanctioned Fellowship Duration: *from* (**17.12.2018**) *to* (16.12.2021).

Extended Fellowship Duration (if applicable): *from* (17.12.2021) *to* (16.09.2022).

Submitted to:

Er. Kireet Kumar
Scientist 'G' and Nodal Officer, NMHS-PMU
National Mission on Himalayan Studies, GBP NIHE HQs
Ministry of Environment, Forest & Climate Change (MoEF&CC), New Delhi
E-mail: nmhspmu2016@gmail.com; kireet@gbpihed.nic.in; kodali.rk@gov.in

A Bag 29/12/21

Submitted by:
[Dr. Arundhati Bag]
[Sikkim Manipal Institute of Medical Sciences, Sikkim Manipal University]
[Contact No.:6297710494; 8900528657]
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GENERAL INSTRUCTIONS:

1. The Final Technical Report (FTR) has to be commenced from the date of start of the Institutional Fellowship (as per the Sanction Order issued at the start of the Fellowship) till its completion. Each detail has to comply with the NMHS Sanction Order.
2. The FTR should be neatly typed (in Arial with font size 11 with 1.5 spacing between the lines) with all details as per the enclosed format for direct reproduction by photo-offset process. Colored Photographs (4-5 good action photographs), tables and graphs should be accommodated within the report or should be annexed with captions. Sketches and diagrammatic illustrations may also be given giving step-by-step details about the methodology followed in technology development/modulation, transfer and training. Any correction or rewriting should be avoided. Please give information under each head in serial order.
3. Training/ Capacity Building Manuals (with detailed contents of training programme, technical details and techniques involved) or any such display material related to fellowship activities along with slides, charts, photographs should be sent at the NMHS-PMU, GBP NIHE HQs, Kosi-Katarmal, Almora 263643, Uttarakhand. In all Knowledge Products, the Grant/ Fund support of the NMHS should be duly acknowledged.
4. The FTR Format is in sync with many other essential requirements and norms desired by the Govt. of India time-to-time, so each section of the NMHS-FTR needs to duly filled by the Fellowship Coordinator/ PI and verified by the Head of the Implementing Institution/ University.
5. Five (5) bound hard copies of the NMHS-Institutional Fellowship Final Technical Report (FTR) and a soft copy should be submitted to the **Nodal Officer, NMHS-PMU, GBP NIHE HQs, Kosi-Katarmal, Almora, Uttarakhand** *via* e-mail nmhspmu2016@gmail.com.

The FTR is to be submitted into following two parts:

Part A – Cumulative Fellowship Summary Report

Part B – Comprehensive Report

Following Financial and other necessary documents/certificates need to be submitted duly signed and verified along with Final Technical Report (FTR):

Annexure I	Consolidated and Audited Utilization Certificate (UC) & Statement of Expenditure (SE), including interest earned for the last Fiscal year including the duly filled GFR-19A (with year-wise break-up)
Annexure II	Consolidated Interest Earned Certificate
Annexure III	Consolidated Manpower Certificate and Direct Benefit Transfer (DBT) Details showing the education background, i.e. NET/GATE etc. qualified or not, Date of joining and leaving, Salary paid per month and per annum (with break up as per the Sanction Order and year-wise).
Annexure IV	Details and Declaration of Refund of Any Unspent Balance as Real-Time Gross System (RTGS) in favor of NMHS GIA General
Annexure V	Details of Technology Transfer and Intellectual Property Rights developed.

DSL: *Date of Sanction Letter*

1	7	1	2	**2**	**0**	**1**	**8**
d	d	m	m	y	y	y	y

DFC: *Date of Fellowship Completion*

1	6	0	9	**2**	**0**	**2**	**2**
d	d	m	m	y	y	y	y

Part A: CUMULATIVE SUMMARY REPORT

(to be submitted by the Coordinating Institute/Coordinator)

1. Details Associateship/Fellowships

1.1 Contact Details of Institution/University

NMHS Fellowship Grant ID/ Ref. No.:	GBPNI/NMHS-2018-19/HSF23-01/152 dated 17.12.2018
Name of the Institution/ University:	Sikkim Manipal Institute of Medical Sciences, Sikkim Manipal University
Name of the Coordinating PI:	Dr. Arundhati Bag
Point of Contacts (Contact Details, Ph. No., E-mail):	Contact No.:6297710494; 8900528657 E mail: arundhati_bag@rediffmail.com

1.2 Research Title and Area Details

i.	Institutional Fellowship Title:	Himalayan Fellowship 2018-19; Title: "To study protective effect of medicinal plants grown in Sikkim Himalaya in kidney function of rats with diabetic nephropathy"					
ii.	IHR State(s) in which Fellowship was implemented:	NA					
iv.	Scale of Fellowship Operation	Local:		Regional:		Pan-Himalayan:	
iii.	Study Sites covered (*site/location maps to be attached*)	East, West and South districts of Sikkim					
v.	Total Budget Outlay (Crore) :	INR 16,06,968/- (Rupees: Sixteen Lakh Six Thousand Nine Hundred Sixty Eight only)					

1.3 Details Himalayan Research /Project Associates/Fellows inducted

Type of Fellowship	Nos.	Work Duration	
		From	To
Research Associates			

Sr. Research Fellow			
Jr. Research Fellows			
Project Fellows	01	03.05.2019	16.09.2022

2. Research Outcomes

2.1. Abstract (not more than 1000 words) (it should include background of the study, aim, objectives, methodology, approach, results, conclusion and recommendations based on the institutional fellowship proposal sanctioned under the NMHS).

Background: Diabetes is a current global epidemic, which is associated with diabetic nephropathy (DN) that can lead to kidney failure. DN is resulted from changes in kidney due to disturbed glucose homeostasis. Present day management of DN includes intensive control of blood glucose, blood pressure and lipid but does not directly target kidney. Podocyte injury in kidney is an early feature of DN. A significant number of diabetic patients develop nephropathy irrespective of glycemic control thus therapeutic strategies that can directly counteract and/or reverse the progression of DN are needed to be developed. Sikkim records highest percentage of diabetics in India. In Sikkim local people are largely dependent on medicinal plants for the treatment of diabetes. Drugs from natural origin are expected to have minimum side effects, more readily available and cheaper than synthetic drugs. In view of this, anti- diabetic properties and kidney protective effect of plants grown in Sikkim, and used by people of this state for diabetes control were evaluated in this study.

Methodologies: Plants with hypoglycemic effects were identified and documented by field survey in east, west and south districts of Sikkim. Plant extracts were screened *in vitro* for their hypoglycemic activities by two *in vitro* techniques i) yeast glucose uptake and ii) α- amylase inhibition assay. Two plants with highest hypoglycemic effects were selected for animal studies. Diabetes was induced in the rats, and they were treated with plant extract.

Results:

i) Altogether 30 villages were covered during this ethno-medicinal survey and interviewed 110 people

ii) All together 205 medicinal plants were used to treat various diseases, out of which 52 plants are used by the folk healers to control diabetes.

iii) *In vitro* experiments showed that the aqueous extracts of *Catharanthus roseus* and *Nyctanthes arbor-tristis* displayed considerably greater activity than other plant extracts

iv) *Catharanthus roseus* was selected for animal study on the basis of the results from *in vitro* analyses and survey- based findings

Animal study:

i) The acute toxicity study observed no mortality or any toxic reactions within 24 h and there after 2 days by oral administration of even at the highest dose (2 g/kg)

ii) The extracts have shown blood- sugar reducing properties in diabetic rats.

iii) Other biochemical parameters also showed improvement in treated animals in dose-dependent manner.

iv) Histopathological studies showed improved morphology of the glomerulus in kidneys of treated diabetic rats.

v) Study on the effect of plant extracts on expression of two podocyte- specific proteins podocin and nephrin, are under progress in rats with DN.

Conclusion: This study will contribute in developing new and efficient drugs of natural origin for DN. In future the study can be extended towards identification of active compounds responsible for amelioration of diabetes and nephropathy. This will also encourage the local people to cultivate these plants, to enrich and protect their knowledge on these plants.

Objectives/ Aim:

- Isolation and identification of plants used for diabetes treatment in the Sikkim
- Identification of ITK based formulations used by local tribes against diabetes

- To study protective effect of plant extracts in kidney damage due to diabetes
- To study protein expression two podocyte- specific proteins podocin and nephrin in the presence of plant extracts
-

Methodology(ies):

ected through face-to-face interviews, using a semi-structured proforma. Key informants were elder people of villages and local folk-healers who were familiar with the herbal treatment of diabetes. Some local weekly markets were also visited for the uses and availability of the medicinal plants.

Collection and Identification of Plant Samples

The plant samples were collected after survey work with the help of local communities, as per the Research Permit, granted by "Government of Sikkim, Department of Forest and Environment Office of PCCF/PRIN. Secretary Cum Chief Wildlife Warden Deorali, Gangtok 737102 (F. No: 78/GOS/ FEWMD/BDR/PCCF/Secy/R&E/45)" The plants were preliminarily identified with the help of the local informants and then taxonomically identified and voucher samples was deposited in Department of Botany, Sikkim University. Sikkim Govt. database are also used for verification of correct and accepted name of recorded plants to avoid repetitive enlistment of the same plant with synonyms. *Approach:* …………… ……… …… … … …………………… ………… …………… ……………… … … ……………………………………..

Results: …………………………..… Conclusion: ……..……………….………………………………………
……………………………..………………..……………...……………..

Recommendations: ……...…………...…………...

2.2. Objective-wise Major Achievements

S. No.	Cumulative Objectives	Major achievements (in bullets points)
1.	• Isolation and identification of plants used for diabetes treatment in the Sikkim • Identification of ITK based formulations used by local tribes against diabetes • To study protective effect of plant extracts in kidney damage due to diabetes • To study protein expression two podocyte- specific proteins podocin and nephrin in the	• Altogether 30 villages were covered during this ethno-medicinal survey and interviewed 110 people • All together 205 medicinal plants were used to treat various diseases, out of which 52 plants are used by the folk healers to control diabetes. • *In vitro* experiments showed that the aqueous extracts of *Catharanthus roseus* and *Nyctanthes arbor-tristis* displayed considerably greater activity than other plant extracts • *Catharanthus roseus* was selected for animal study on the basis of the results from *in vitro* analyses and survey- based findings • The acute toxicity study observed no mortality or any toxic reactions within 24 h and there after 2 days by oral administration of even at the highest dose (2 g/kg) • The extracts have shown blood- sugar reducing properties in diabetic rats. • Other biochemical parameters also showed improvement in treated animals in dose-dependent manner. • Histopathological studies showed improved morphology of the glomerulus in kidneys of treated diabetic rats.

2.3. Outputs in terms of Quantifiable Deliverables*

S. No.	Quantifiable Deliverables*	Monitoring Indicators*	Quantified Output/ Outcome achieved	Deviations made, if any, and Reason thereof:
	As mentioned in 2.2		As mentioned in 2.2	Two *in vitro* studies have been added to screen the hypoglycaemic activity of large number of plants collected from survey which were not included in original proposal.

(*) As stated in the Sanction Letter issued by the NMHS-PMU.

2.4. Strategic Steps with respect to Outcomes (in bullets)

S. No.	Particulars	Number/ Brief Details	Remarks/ Enclosures
1.	New Methodology developed:		
2.	New Models/ Process/ Strategy developed:		
3.	New Species identified:		
4.	New Database established:	Hypoglycaemic activities of ten plants used by Sikkim's folk healers are experimentally studied	
5.	New Patent, if any:		
	I. Filed (Indian/ International)		
	II. Granted (Indian/ International)		
	III. Technology Transfer (if any)		
6.	Others, if any:		

3. Technological Intervention : Nil

S. No.	Type of Intervention	Brief Narration on the interventions	Unit Details (No. of villagers benefited / Area Developed)
1.	Development and deployment of indigenous technology		
2.	Diffusion of High-end Technology in the region		
3.	Induction of New Technology in the region		
4.	Publication of Technological / Process Manuals		
	Others (if any)		

4. New Data Generated over the Baseline Data: Nil

5. Linkages with Regional & National Priorities (SDGs, INDC, etc.)/ Collaborations: NA

6. Financial Summary (Cumulative)*

*Please attach the **consolidated and audited Utilization Certificate (UC) and Consolidated and *Year-wise* Statement of Expenditure (SE)** separately, *ref*. **Annexure I. Enclosed**

7. Quantification of Overall Research Progress

S. No.	Parameters	Total (Numeric)	Attachments* with remarks
1.	IHR State(s) Covered:	NA	
2.	Fellowship Site/ LTEM Plots developed:	NA	
3.	New Methods/ Model Developed:	Nil	
4.	New Database generated:	Nil	
5.	Types of Database generated:	Nil	
6.	No. of Species Collected:	*52*	
7.	New Species identified:	Nil	
8.	Scientific Manpower Developed (PhDs awarded/ JRFs/ SRFs/ RAs):	SPF pursuing PhD from SMU	
9.	No. of SC Himalayan Researchers benefited:	Nil	
10.	No. of ST Himalayan Researchers benefited:	Nil	
11.	No. of Women Himalayan Researchers empowered:	Nil	
12.	No. of Knowledge Products developed:	Nil	
13.	No. of Workshops and training participated:	32	
14.	No. of Trainings participated:		
15.	Technical/ Training Manuals prepared:	Nil	
	Others (if any):		

***** Please attach the soft copies of supporting documents word files and data files in excel.

8. Knowledge Products and Publications*

S. No.	Publication/ Knowledge Products	*Number*		*Total Impact Factor*	*Remarks/* Enclosures**
		National	International		
1.	Journal Research Articles/ Special Issue (Peer-reviewed/ Google Scholar)	**Publications**: 02 published in pubmed/ scopus indexed journals; three submitted		1.5	
2.	Book Chapter(s)/ Books:	01 Chapter			
3.	Technical Reports/ Popular Articles	01 Chapter			
4.	Training Manual (Skill Development/ Capacity Building)	Nil			

S. No.	Publication/ Knowledge Products	Number		Total Impact Factor	Remarks/ Enclosures**
		National	International		
4.	Training Manual (Skill Development/ Capacity Building)	Nil			
5.	Papers presented in Conferences/ Seminars	**Oral presentation**: four **Poster presentation**: 01 (2nd prize)			
6.	Policy Drafts (if any)	Nil			
7.	Others (specify)	Nil			

*Please append the list of KPs/ publications (with impact factor and URL link details) with due Acknowledgement to NMHS.

**Please provide supporting copies of the published documents.

9. **Recommendation on Utility of Research Findings, Replicability and Exit Strategy: NA**

29/12/22

(NMHS FELLOWSHIP COORDINATOR)

(Signed and Stamped)

(HEAD OF THE INSTITUTION)

Dr. Muralidhar Nepal
DEAN
Sikkim Manipal Institute of Medical Sciences
5th Mile Tadong, Gangtok-737102
Sikkim (India)

(Signed and Stamped)

Place: .....

Date: ...../...../.....

PART B: COMPREHENSIVE REPORT (including all sanctioned positions of Researchers)

Based on the Fellowship Proposal submitted/approved at the time of sanction, the co-ordinating Principal Investigator shall submit a comprehensive report including report of all individual researchers.

The comprehensive report shall include an **Executive Summary** and it should have separate chapters on (1) **Introduction** (2) **Methodologies, Strategy and Approach** (3) **Key Findings and Results** (4) **Overall Achievements** (5) **Impacts of Fellowship in IHR** (6) **Exit Strategy and Sustainability** (7) **References/ Bibliography** and (8) **Acknowledgements** (It should have a mention of financial grant from the NMHS, MoEF&CC).

Further, description of Technical Activities, List of Trainings/ Workshops/ Seminars with details of trained resources, list of New Products developed under the fellowship, Manual of Standard Operating Procedures (SOPs) developed, Technology developed/Transferred etc should be enclosed as Appendix.

Report (hard copy) should be submitted to:

Er. Kireet Kumar
Scientist 'G' and Nodal Officer, NMHS-PMU
National Mission on Himalayan Studies (NMHS)
G.B. Pant National Institute of Himalayan Environment (GBP NIHE)
Kosi-Katarmal, Almora 263643, Uttarakhand

Report (soft copy) should be submitted at:

E-mail: nmhspmu2016@gmail.com; kireet@gbpihed.nic.in; kodali.rk@gov.in

PART B: COMPREHENSIVE REPORT

EXECUTIVE SUMMARY :

The Executive Summary of the fellowship should not be more than 3–5 pages, covering all essential features in precise and concise manner as stated in Part A (Cumulative Fellowship Summary Report) and Part B (Comprehensive Report).

Fellowship Report No.: ***n of N*** *(n = Sequential number; N= Total no. of fellowships granted to the Institute/ University)*

Researchers Details

Type of Fellowship (HRA/HJRF/HJPF)	Name of Himalayan Researcher	Date of Joining	Date of Resignation**	Research Title	Name of the PI & Designation
(*in case of continuation of fellowship*)	Mr. Abhishek Byahut	03.05.2019	NA	To study protective effect of medicinal plants grown in Sikkim Himalaya in kidney function of rats with diabetic nephropathy	Dr. Arundhati Bag

*If the appointed researcher resigned in the mid of the fellowship duration, then also mention the name of the Himalayan researcher who carried forward the fellowship.

1 INTRODUCTION

Diabetes is a current global epidemic, which is associated with diabetic nephropathy (DN) that can lead to kidney failure. DN is resulted from changes in kidney due to disturbed glucose homeostasis. Present day management of DN includes intensive control of blood glucose, blood pressure and lipid but does not directly target kidney. Podocyte injury in kidney is an early feature of DN. A significant number of diabetic patients develop nephropathy irrespective of glycemic control thus therapeutic strategies that can directly counteract and/or reverse the progression of DN are needed to be developed.

Sikkim records highest percentage of diabetics in India. In Sikkim local people are largely dependent on medicinal plants for the treatment of diabetes. Drugs from natural origin are expected to have minimum side effects, more readily available and cheaper than synthetic drugs. In view of this, anti- diabetic properties and kidney protective effect of plants grown in Sikkim, and used by people of this state for diabetes control were evaluated in this study.

Plants with hypoglycemic effects were identified and documented by field survey in all districts of Sikkim. Plant extracts were screened *in vitro* for their hypoglycemic activities. Two plants with highest hypoglycemic effects were selected for animal studies.

Diabetes was induced in the rats, and they were treated with plant extract. The extracts have shown blood- sugar reducing properties in diabetic rats as well. Other biochemical parameters also showed improvement in treated animals in dose- dependent manner. Histopathological studies showed improved morphology of the glomerulus in kidneys of treated diabetic rats.

Effect of plant extracts on expression of two podocyte- specific proteins podocin and nephrin, will be further studied in rats with DN. This study will contribute in developing new and efficient drugs of natural origin for DN. In future the study can be extended towards identification of active compounds responsible for amelioration of diabetes and nephropathy. This will also encourage the local people to cultivate these plants, to enrich and protect their knowledge on these plants.

2 METHODOLOGIES, STARTEGY AND APPROACH

Study Area

Whole Sikkim, it is a very small hilly state with four districts (East, West, North and South) of total geographical area of 7096 sq. kms, altitude ranging from 300m to 8540m. Data were collected through face-to-face interviews, using a semi-structured proforma. Key informants were elder people of villages and local folk-healers who were familiar with the herbal treatment of diabetes. Some local weekly markets were also visited for the uses and availability of the medicinal plants.

Collection and Identification of Plant Samples

The plant samples were collected after survey work with the help of local communities, as per the Research Permit, granted by "Government of Sikkim, Department of Forest and Environment Office of PCCF/PRIN. Secretary Cum Chief Wildlife Warden Deorali, Gangtok 737102 (F. No: 78/GOS/ FEWMD/BDR/PCCF/Secy/R&E/45)" The plants were preliminarily identified with the help of the local informants and then taxonomically identified and voucher samples was deposited in Department of Botany, Sikkim University. Sikkim Govt. database are also used for verification of correct and accepted name of recorded plants to avoid repetitive enlistment of the same plant with synonyms. Samples were thoroughly washed, air-dried, ground, and kept at 2-4°C in the refrigerator. The details of plants with herbarium accession number are as follow (table no.1).

Preparation of Plant Extracts

Traditional herbalist primarily prepares their formulations using aqueous methods. Water is a safe and ecofriendly substance that has no negative effects on the environment during its production, usage, or disposal. Peptides, proteins, and glycans with α-amylase inhibitory activity are also likely to be extracted in an aqueous system rather than organic solvent or high temperature extraction, which would otherwise denature them(Mihaylova and Lante, 2019; P et al., 2011).

To produce crude extract, 10g of dry plants powder were macerated in 100ml of distilled water for 72 hours and the mixture was kept on rotatory shaker for 3 hours. After that, it was filtered using Whatman filter paper #1 to get rid of solid particles and the supernatant was collected and the samples were lyophilized after freezing over night by using freeze dryer method. Stored at −20 °C until further use. For the inhibition assay, stock solutions were made by dissolved in 10% DMSO of each extract and diluting it appropriately before use.

In Vitro Assays

Glucose uptake by yeast cells: Commercial baker's yeast were washed by repeated centrifugation (3000 × g, 5 min) in distilled water until the supernatant fluids were clear and a 10%v/v suspension were prepared in distilled water. Various concentration of extract (0.5-2.5 mg/ml) were added to 1 ml of glucose solution (5, 10 and 25 mM) and further incubated for 10 min at 37 ˚C. Reaction were started by adding 100 µl of yeast suspension, votex and further incubate for 60 min. After 60 min, the tubes are centrifuged (2500 × g, 5 min) and glucose were estimated in the supernatant (Vijayalakshmi. K, 2014).

The following formula was used to determine the percentage increase in glucose absorption by yeast cells:

$$\text{Increase in Glucose uptake (\%)} = \frac{Abs\ (Sample) - Abs\ (Control)}{\text{Abs}\ (Sample)} \times 100$$

where Abs control denotes the absorbance of the control reaction (which contains all reagents except the test sample) and Abs sample denotes the absorbance of the test sample.

α- amylase inhibition activity: The α-amylase inhibitory assay for various aqueous extract plants was performed using a previously described method by Wickramaratne, M. N., with minor modification. In brief, stirring soluble starch (1g) in 0.02 M sodium phosphate buffer with 0.006 M NaCl (pH 6.9; 100 mL) and bringing to a gentle boil in order to dissolve a starch completely (1 percent w/v). 500 µl of extract of different concentrations (50 to 250 µg/ml) was mixed with 500 µl of α-amylase solution (0.5 mg/ml) which was prepared in 0.02 M sodium phosphate buffer. The mixture was incubated at 37°C temperature for 10 min and 500 µl of starch solution was added and after 10 min incubated at 37°C temperature, the reaction was stopped with 1ml of dinitrosalicylic acid (DNSA) colour reagent, the test tubes were placed in a boiling water bath for 5 min and cooled until they reached room temperature. After that, the mixture was diluted with 10 ml distilled water and the absorbance was measured at 540 nm. By replacing plant extract with 500 µl of buffer, a blank with 100% enzyme activity was created. In the absence of the enzyme solution, a blank reaction was prepared using the plant extract at each concentration. Acarbose was used as a positive control sample, and the reaction was carried out in the same way as the plant extract reaction (Wickramaratne, M. N., 2016). The α-amylase inhibitory activity was determined using the equation below and ex pressed as a percent inhibition:

$$\%\ \alpha\text{-amylase inhibition} = \frac{Abs\ (Control) - Abs\ (Sample)}{\text{Abs}\ (\text{Control})} \times 100$$

The IC50 values were calculated by plotting the percent α-amylase inhibition against the extract concentration.

To study protective effect of plant extracts on expression of podocyte – specific proteins, namely podocin and nephrin, in diabetic kidney damage

Animals

Wistar rats, 7-8 weeks old of either sex weighing 130–200g were used. The animals were kept in controlled condition: temperature 25–26^{0}C, relative humidity 60–70% and 12/12 hr light/dark cycle at the Animal house facility, Sikkim Manipal University, Gangtok, Sikkim and provided with standard pellet diet and water adlibitum. This study was approved by Institute's Animal Ethics Committee vide

Acute toxicity study

Acute toxicity testing was performed for plant extracts following the fixed dose procedure of Organization for Economic Cooperation and Development guideline (OECD). The healthy rats were observed after administration of a single oral dose of 0.25, 0.50, 0.75, 1.00, 1.25, 2.00 g/kg extract for any adverse signs and symptoms for the next 24 h and thereafter for 2 days.

Induction of diabetes nephropathy

Intraperitoneal administration of freshly prepared solution of streptozotocin (STZ) (65 mg/kg; i.p.) in citrate buffer was used for induction of diabetic nephropathy in rats, 15 min after nicotinamide (NAD) administration (110 mg/kg; i.p.). After 72 h of STZ administration, fasting blood glucose (FBG) level were determined to confirm the development of diabetes. The inclusion criterion for rats in the study was FBG levels >200 mg/dl.

Animal grouping and treatment

To investigate the effects of *C. roseus*, the animals were divided into six groups and each group consisted of six rats:

Group 1: Untreated normal rats

Group 2: Untreated diabetic rats

Group 3: Diabetic rats treated with 200 mg *C. roseus* /kg b.w.

Group 4: Diabetic rats treated with 400 mg *C. roseus* /kg b.w.

Group 5: Diabetic rats treated with 600 mg *C. roseus* /kg b.w.

Group 6: Diabetic rats treated with 5mg Glibenaclamide/kg b.w.

Plant extracts were administered orally daily using an intragastric tube. The normal control (NC) and diabetic control (DC) groups will be treated by the same volume of distilled water.

Monitoring of body weight and blood sugar level during treatment

Fasting blood glucose (FBG) level were determined using glucometer by tail bleeding after fasting the rats for overnight and body weight were determined using standard analytical weighing balance at the end of every week of treatments.

Blood Biochemical Analysis

At the end of the experiment (on 8th week), anaesthesia (diethyl ether) was given to the rats and blood samples were collected from each rat through orbital venous plexus and cardiac puncture. Biochemical parameters such as glycosylated haemoglobin, protein profile (total protein, albumin, globulin), lipid profile (cholesterol, triglycerides, high-density lipoprotein cholesterol and low-density lipoprotein cholesterol), were determined by following standard procedures using commercially available kits.

Tissue collection

After blood collection, the kidneys were removed and then a part of renal cortical section were collected for Western blot analysis and other part of kidney samples will be fixed in 4% paraformaldehyde for histological examination.

Histological Assessment of the Kidney Using Haematoxylin and Eosin Stain

After sacrificing, the one of the kidneys of all rats were fixed in 10% (v/v) neutral buffered formalin and dehydrated by passing through increasing concentrations of alcohol, cleared and embedded in paraffin blocks. Blocks were cut to produce 5-µm-thick sections that were mounted on a slide. The paraffin in tissue sections were removed by passing them through xylene, decreasing concentrations of alcohol and finally in water. The sections were then stained in haematoxylin and eosin stain.

Western blot assay

Western blot analysis from renal cortical sections for two podocyte-specific proteins podocin and nephrin are under progress following previously described protocol (Mohmood T, 2012).

3 KEY FINDINGS AND RESULTS

Survey

Regular field trips were conducted in different villages between August 2019 to April 2021 and data were collected through face-to-face interviews, using a semi-structured proforma seeking demographic information of the informants, local names of plants, plant parts used as medicines, method of medicine preparation and administration etc. Interviews were conducted in local language by the researcher. The key informants were traditional healers (Baidyas, Bongthing, Jhankries or professional herbal medicine practitioner), some were farmers and elder people of the villages having knowledge on medicinal plants for the treatment of diabetes.

Altogether 30 villages were covered during this ethno-medicinal survey. Collection and preliminary identification of plant materials, local name and their usage were recorded with the help of the informants. Photographs were also taken (Fig.1). Multiple synonyms are used for medicinal plants in different literature. Therefore, we listed our most frequently used plants (Table 1) according to the accepted scientific species names from 'The Plant List' database (http://www.theplantlist.org) accessed in December 2021. Plant specimens were also identified in the Department of Botany, Sikkim University.

In the present study we interviewed 110 people. Most of the respondents were traditional healers, farmers and older people of the villages especially who had the knowledge on remedies and medicinal plants. In total 52 species of medicinal plants belonging to 36 families were found to be used to control diabetes in Sikkim, from which 10 most frequently used plants were selected for *in-vitro* studies.

Glucose uptake by yeast cells

At all glucose concentrations, the aqueous extracts of *Catharanthus roseus* and *Nyctanthes arbor-tristis* displayed considerably greater activity than other plant extracts and Standard drug, with the greatest increase in 25 mM Glucose concentration (80.20% ± 3.01 and 78.83% ± 2.66 respectively) increase at 2.5 mg/mL of plant extracts, followed by *Phlogacanthus pubinervius* (72.80% ± 1.27) *Azadirachta indica* (69.13% ± 1.56).

α-amylase inhibition assay

Catharanthus roseus and *Nyctanthes arbor-tristis* extract showed maximum inhibitory activity among the plants with 72.29% ±0.58 inhibition (IC_{50} 154.30µg/mL) and 69.98% ±1.16 inhibition (IC_{50} 150.48 µg/mL) respectively as compared to the standard drug (acarbose) with 80.44% ±0.73 inhibition (IC_{50} 72.38µg/mL) at 250 µg/mL concentration of extracts, for their α-amylase inhibitory activity.

Acute toxicity study of *Catharanthus roseus* leaves extracts

The acute toxicity study observed no mortality or any toxic reactions within 24 h and there after 2 days by oral administration of even at the highest dose (2 g/kg).

Body weight changes

The relative body weights of the animals. The diabetic control group had the highest weight loss of about 34.69 %. The treatment groups however, showed improvement in body weight at the end of the experiment compared to the diabetic control group. However, the CR (200 mg/kg) supplemented diabetic groups have 17.49% weight loss while CR (400 mg/kg) supplemented group has 21.48 % weight loss and CR (600 mg/kg) supplemented has 18.60% weight loss.

Blood glucose levels changes

Blood glucose levels of all groups were calculated to be from 69.33 ±2.08 mg/dL to 76.67 ± 9.07 mg/dL at the beginning of the experiment. At 72 h following STZ + NAD injection, blood glucose levels were significantly increased compared with the control group. At the end of day 60, mean blood glucose concentrations were significantly decreased in all treatment groups compared with the diabetic control group.

Effect of aqueous extract of *C. roseus* on lipid profile and total proteins

Serum concentration of Cholesterol, Triglyceride and LDL was found to be significantly elevated while HDL concentration was found to be significantly decreased in Diabetic control rats when compared to normal control group. Treatment with aqueous extracts of *C. roseus* (200, 400 and 600 mg/kg) for 60 days were dose dependently ameliorated the level of aforementioned lipoproteins as compared to DN control rats

Histopathology

Histological changes in kidney tissue from normal control, diabetic control and different treatment groups are shown in 1. Normal control rats showed normal architecture, glomerular size, normal intrarenal arterial walls and basement membrane thickness (Fig.1b). diabetic control rat's kidney showing increased bowman's space, glomerular congestion, atrophy of glomerulus seen with dilated glomerular space and dilated & degenerated tubules (Fig.1a & 1d), However, morphology of the glomerulus was improved by CR treated group viz., reduction in mesangial expansion, membrane thickness and atrophy with different doses (Fig.1e,f,h). Compared to matched renal arterial lumen diameter of control group (Fig.1g), the increase of thickness in the intrarenal arterial walls has been observed in all diabetic rats (Fig.1c),

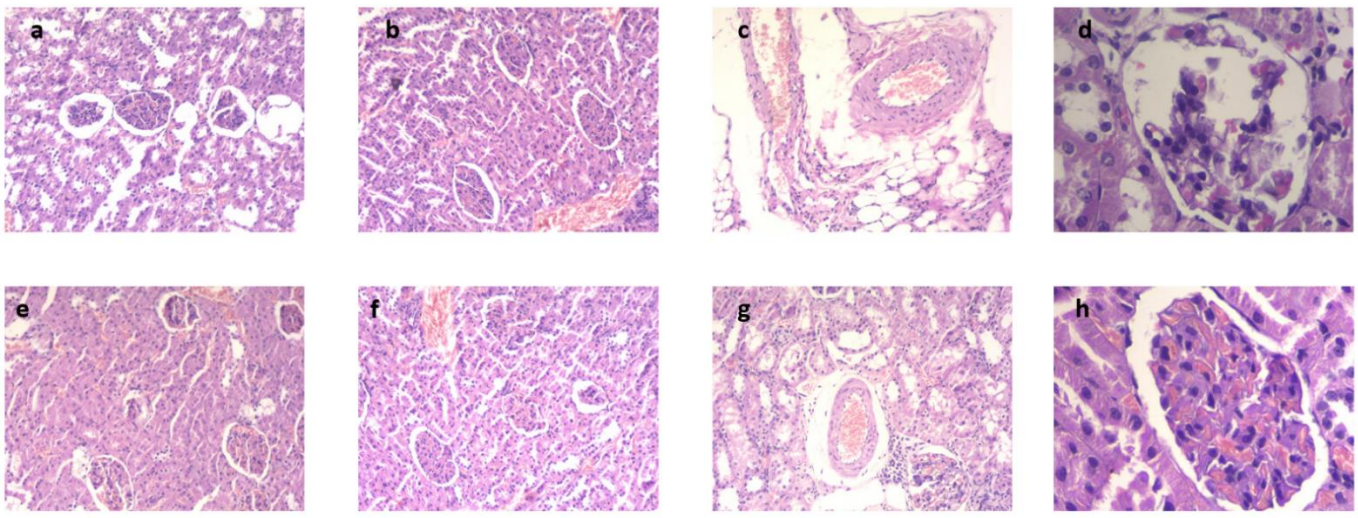

Fig 1:-

Histopathological changes in Kidney of normal control, diabetic control, treated groups (CR 200, 400, 600 mg/kg) of Rats. H&E stained kidney tissue sections from diabetic control (a),(c)&(d) normal control (b) &

(g), CR 200 mg/kg treated diabetic (e), CR 400 mg/kg treated diabetic (f), CR 600 mg/kg treated diabetic (h).

4 OVERALL ACHIEVEMENTS

- Altogether 30 villages were covered during this ethno-medicinal survey and interviewed 110 people
- All together 205 medicinal plants were used to treat various diseases, out of which 52 plants are used by the folk healers to control diabetes.
- *In vitro* experiments showed that the aqueous extracts of *Catharanthus roseus* and *Nyctanthes arbor-tristis* displayed considerably greater activity than other plant extracts
- *Catharanthus roseus* was selected for animal study on the basis of the results from *in vitro* analyses and survey- based findings
- The acute toxicity study observed no mortality or any toxic reactions within 24 h and there after 2 days by oral administration of even at the highest dose (2 g/kg)
- The extracts have shown blood- sugar reducing properties in diabetic rats.
- Other biochemical parameters also showed improvement in treated animals in dose- dependent manner.
- Histopathological studies showed improved morphology of the glomerulus in kidneys of treated diabetic rats.

5 IMPACTS OF FELLOWSHIP IN IHR: NA

5.1 Socio-Economic Development (max. 500 words, in bullet points)
5.2 Scientific Management of Natural Resources In IHR (max. 500 words, in bullet points)
5.3 Conservation of Biodiversity in IHR (max. 500 words, in bullet points)
5.4 Protection of Environment (max. 500 words, in bullet points)
5.5 Developing Mountain Infrastructures (max. 500 words, in bullet points)
5.6 Strengthening Networking in IHR (max. 700 words, in bullet points)

6 EXIT STRATEGY AND SUSTAINABILITY: NA

6.1 How effectively the fellowship findings could be utilized for the sustainable development of IHR (max. 1000 words)
6.2 Efficient ways to replicate the outcomes of the fellowship in other parts of IHR (max. 1000 words)
6.3 Identify other important areas not covered under this study, but needs further attention (max. 1000 words)
6.4 Major recommendations for sustaining the outcomes of the fellowship in future (500 words in bullets)

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8 ACKNOWLEDGEMENTS

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APPENDICES

Appendix 1 – Details of Technical Activities
Appendix 2 – Copies of Publications duly Acknowledging the Grant/ Fund Support of NMHS
Appendix 3 – List of Trainings/ Workshops/ Seminars with details of trained resources and dissemination material and Proceedings
Appendix 4 – List of New Products (utilizing the local produce like NTFPs, wild edibles, bamboo, etc.)
Appendix 5 – Copies of the Manual of Standard Operating Procedures (SOPs) developed
Appendix 6 – Details of Technology Developed/ Patents filed
Appendix 7 – Any other (specify)

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(Signature of HRA/HJRF/HPF)

Abhishek Byahut
29/12/22

29/12/22
(NMHS FELLOWSHIP COORDINATOR)
(Signed and Stamped)

(HEAD OF THE INSTITUTION)
(Signed and Stamped)
DEAN
Sikkim Manipal Institute of Medical Sciences
5th Mile Tadong, Gangtok-737102
Sikkim (India)

Place: .....
Date: ...../...../.....

Annexure-III

National Mission on Himalayan Studies (NMHS)

DIRECT BENEFIT TRANSFER (DBT) DETAILS

(To be Updated to finance.nmhspmu2017@gmail.com; nmhspmu2016@gmail.com on 5th of every month)

Scheme Name:	**National Mission on Himalayan Studies (NMHS)**
Scheme Type:	**Central Sector (CS) Grant-in-Aid Scheme**
Scheme Code:	**NMHS**
Category:	**Fellowship Grant**
Month-Year:	**August 2022**

Pro Forma for DBT Details

University/Institution Name: Sikkim Manipal University

S. No.	Position (H-RA, H-JRF/ H-JPF)	Name	DoB*	DoJ*	PI	Research title	Objectives	Study Area, IHR State	Contact details (Complete corresponding address), Mobile No., E-mail ID	Bank details (Account number, IFSC Code)	Emoluments /Fellowship	Aadhaar No.
1.	H-SPF	Abhishek Byahut	05.05.1993	03.05.2019	Dr. Arundhati Bag	To study protective effect of medicinal plants grown in Sikkim Himalaya in kidney function of rats with diabetic nephropathy	Isolation and identification of plants used for diabetes treatment in the Sikkim Identification of ITK based formulations used by local tribes against diabetes To study protective effect of plant extracts in kidney damage due to diabetes To study protein expression two podocyte- specific proteins podocin and nephrin in the presence of plant extracts	Sikkim	7001883401 abhishekbyahut93@gmail.com	Bank of India, A/c: 507910410000021 IFSC Code: BKID 0005079	Rs. 24840	4495-8338-2426

Note: For each month, the DBT Details Pro forma dully filled and signed for each Himalayan Fellowship Grant under NMHS must be submitted at finance.nmhspmu2017@gmail.com; nmhspmu2016@gmail.com.
*DoB (Date of Birth); DoJ (Date of Joining).

(Authorized Signatory)

Latest Updated List of Himalayan Researchers or Fellows *(working in the current month)*

(To be Updated to finance.nmhspmu2017@gmail.com; nmhspmu2016@gmail.com **on 5th of every month)**

S. No.	Name	*Fellowship (RA/JRF/JPF)
1.	Abhishek Byahut	Rs. 24840
2.		

*The availing fellowship is as per the new guidelines of MoEF&CC