



REFLECTIONS OVER THREE DECADES OF JOURNEY FROM NEEM GUM TO HYPERTENSION

N. Mallikarjuna Rao

Department of Biochemistry,
Vishnu Dental College, Bhimavaram , India

Abstract:

My practice in biochemistry as researcher, teacher and author for the last 33 years contributed significantly for the advancement of biochemical knowledge. It may be interesting (for readers concerned) to know how work I started with bananas progressed to discovery of blood pressure lowering action. In this article what I learned, importance of collaboration, logical planning, pleasures and pains associated with this kind of practice are detailed. It indicates need for updating knowledge in chosen area of research, technical skills, non technical skills by new researcher for better career. Scientific achievements are deeply rooted to realization of culture and environment to some extent.

Keywords:

Neem Gum Proteases Blood Pressure Gestational Hypertension Cord Blood Bananas

Introduction :

Post graduate years

I was born in a village near by Tenali of Guntur district of Andhra Pradesh. I had my schooling in native place Kolakaluru. Buckingham canal of Krishna river provides food and water to my village. My interest to science was ignited by plant material collection for natural science classes in high school days and it was continued to inter and degree. My interest to study biochemistry was increased during my degree because I realized that it was youngest among biology and chemistry branches with unlimited scope for research. After completing my B.Sc degree in 1976 from Andhra University I was fortunate to get selected for M. Sc Biochemistry course at Kasturba Medical College, Manipal. I enjoyed learning human anatomy, physiology with biochemistry during first year. There were many good teachers in these subjects who inspired me to opt for teaching later. In biochemistry Dr. T.N. Pattabhi raman, Chief of the department taught enzymes kinetics and regulation by inhibition in a stimulating way.

After passing first year in 1977 I joined biochemistry department to complete second and third years experiments on enzymes like isolation, determination of K_m and K_i were carried out under supervision of chief during second year with much enthusiasm and I experienced pleasure of experimenting. So I was pleased when chief instructed me to assist one of his doctoral student B. R. Nayak working on neem gum during my final year. Initially I was assigned to study composition and separation of large glycol proteins of neem gum. It involved column chromatography, identification and estimation of carbohydrates, proteins, amino sugars, acid hydrolysis of glycoproteins and paper chromatography. Unfortunately it was abandoned due to inconsistent results. Neem gum the experimental material which was obtained from neem trees has to be brought from my native place as these plants were not grown around

REFLECTIONS OVER THREE DECADES OF JOURNEY FROM NEEM GUM TO HYPERTENSION

Manipal. I was bit proud of being researcher as my friends and parents in my village were very much discussing about neem gum collection by me for research work. Meanwhile proteolytic activity was detected by my supervisor in neem gum which was subsequently characterized as serine like protease (1). I acquired skills required for isolation and characterization of enzymes by the time I completed M.Sc.

Doctoral years

Though there were active researchers in several areas of biochemistry mostly led by our chief Dr.T. N. Pattabhiraman I preferred national laboratories for research after passing M. Sc in 1979 from Mysore University. I got a Junior Research Fellow (JRF) post at Post Graduate Institute of Medical Education and Research (PGI), Chandigarh to work on microbial lipids under Dr. G.K. Khuller. After few days I returned to native place as climate was not suited well. I was in search of a job in native state as my parents wants me nearby and get married. During that time Andhra sugars, Tanuku started a hospital under directorship of Dr. D.J. Reddy, Ex V.C.; S. V. University and they preferred biochemist from Manipal. I opted the post and planned for research also. While working at S. M. V. R. M. Hospital, Tanuku I married Naga Malleswari in 1980 who remains to be of immense strength for the last 33 years. Like now a days there were no kits and auto analyzers for measurement of blood constituents in those days. I developed manual methods for routine analysis. For serum electrolytes estimation flame photometer was only available choice and no ion selective electrodes those days. My attempt to engage in research was not fruitful. But a JRF offer from Manipal made me to resign hospital biochemist job at Tanuku and returned to my alma matter.

As there were many active researchers in the department I had good colleagues to share views and over above my guide Dr. T.N. Pattabhiraman. Until then protease inhibitors were studied with purified bovine enzymes which may not reflect their effect on human enzymes and digestive juice contain mixture of enzymes. Hence my doctoral work involved study of inhibition of animal pancreatic proteases by tuber protease inhibitors. Human and bovine pancreas acetone powders were prepared and used (2). Arrow root tuber grown in western ghats, household gardens and arac nut plantations of west coast region showed entero kinase inhibition. Hence it was chosen for purification and characterization of a protease inhibitor (3). It was presented in Society of Biological Chemists of India Conference held at PGIMER and Punjab University, Chandigarh in 1982 (Fig.1).



Fig.1. Author (Extreme left) with colleagues Dr. Krishna Sharma and Dr. Chandrasekhar at Gandhi Bhavan of Punjab University in Chandigarh

Most of the protease inhibitor studies use synthetic substrates. But their effect on protease inhibition with natural substrates was difficult to assess. Therefore study of effect of tuber protease inhibitors on bovine enzymes with natural and synthetic substrates was also part of my doctoral work (4). I submitted thesis in 1986 and awarded Ph.D. in 1988 from Mangalore University.



Fig.2. Author (Extreme right) with Guide Dr.T. N.Pattabiraman (extreme left) and Dr. Shivraj , Dr. Gopalakrishna Bhat, Dr. Sudakar Prabhu, Dr.Sudakar nayak (From left to right) at manipal

Post Doctoral years

After completing doctoral work I wanted to do research independently. I tried to work on multi catalytic proteases, cathepsins and calpains which was hot field of research that time. There was no internet so getting information was time consuming due to non availability of journals. So starting work in new area was problematic. I tried to find calpain like activity in egg white of fertilized eggs after incubation but it was not successful. Then I turned to ripening bananas for calpains in which proteolysis occurs. Another reason for choosing bananas was its colorless extract which make suitable for colorimetric method. In contrast extracts of other fruits were colored. I could not detect calpain like activity in banana also. Absence of calpain like activity led me to think about existence of inhibitors. But pure calpains were not available. So I assayed for inhibitors of papain which is also a cysteine protease like calpain. To my surprise inhibitors to papain were detected in ripened bananas. Later I found papain inhibitors in un ripened bananas also.

Initially I found papain inhibition in both ripe and unripe bananas with casein as substrate. Before proceeding further I wanted to get inhibition confirmed by assaying with another method. So I assayed papain inhibition with synthetic substrate. I observed less color in tubes with banana extract and no color at high concentration of banana extract. Then I reviewed literature for any such observation with bananas. As there were no such report I studied further and reported (5). At the same time presence of cysteine protease inhibitors in potatoes which is also source of trypsin inhibitors was published (6). Based on this I searched for serine protease inhibitors in bananas and found trypsin / chymotrypsin inhibitors. Initially I searched in one variety of banana and extended to other types of bananas later. Those days role of H. Pylori in causing gastric/duodenal ulcers was not clear I proposed antibacterial role for banana cysteine protease inhibitors in curing stomach ulcers as reported in traditional Indian medicine (7).

My desire for doing research in area other than I pursued for doctoral work led to initiation of study of angiotensin converting enzyme (ACE) which is part of rennin - angiotensin system (RAS) involved in blood pressure regulation. Moreover treatment of hypertension a common modern days life style associated disease involve use of ACE inhibitors. Further ACE has several novel features like two active sites, acts on variety of substrates, releases dipeptides and influenced by chloride. How ACE inhibitors affect different organs functions other than blood pressure regulation by inhibiting ACE action was not clear. Now role of oxidative stress in cardiovascular and metabolic diseases is well recognized. Captopril an inhibitor of ACE contains sulfhydryl (-SH) group. Hence ACE action is affected by availability of sulfhydryl group which in turn related to oxidative stress. In human subjects these investigations are preferable. Due to problems related to involvement of human subjects in investigations I choose sheep as subject of study. A spectro photometric method was chosen to determine ACE activity and inhibition. ACE assay procedure was prepared. One of my P.G student Mr. Padmanabha Udupa joined me as doctoral student. Distribution of ACE in various sheep tissues, inhibition of different tissue ACE by its various inhibitors, influence of chloride and di amide were investigated (8-13).

Potato a well known source of serine protease inhibitors contain carboxy peptidase inhibitors

REFLECTIONS OVER THREE DECADES OF JOURNEY FROM NEM GUM TO HYPERTENSION

(14). Further ACE inhibitors are designed based on the active site of carboxy peptidase. Since ACE is carboxy peptidase an attempt with bananas for the presence of ACE inhibitors was planned. Literature survey revealed use of bananas in the Indian traditional medicine for treatment of hypertension (15). Further antihypertensive action of bananas in experimentally induced hypertensive rats is reported (16). But spectrophotometric ACE assay was not suited for banana inhibition detection due to use of phosphate buffer and it was a time consuming procedure. Hence a colorimetric assay for detection of ACE inhibitors in bananas where inhibition may be visualized as decrease in color intensity is needed. Literature survey resulted in getting a colorimetric ACE assay method using cyanuric chloride and borate buffer (17). Based on this an ACE assay procedure was prepared and cyanuric chloride was imported. Initially poovan bananas were investigated for ACE inhibition with Dr. K.V.S. R. G. Prasad and Dr. Ranganada Pai of pharmacology department. Like banana cysteine protease inhibition here also inhibition of ACE was visualized as decrease in color in addition to measurement with colorimeter. Later other banana cultivars were also investigated. This work was communicated to current science for publication. A study on effect of bananas consumption on cold stress induced hypertension in human volunteers was started with Dr. K. L. Bairy and Dr. Chayana Sarkar of pharmacology department due to the presence of ACE inhibitors in bananas. The results showed fall in blood pressure and serum ACE levels in test subjects compared to controls. Dr. C. Sarkar presented these findings in hypertension conference held at Central Drug Research Institute (CDRI), Lucknow (18). The paper sent to current science came for revision with reviewer comment on number of bananas can be consumed safely per day due to the presence of ACE inhibitors. While submitting revised manuscript I included findings on human volunteers as reply to reviewer comment in the discussion section. Moreover I expected that our findings create news. Excellent editorial with title 'Bananas and Blood pressure' in current science issue based on our paper published was reported World wide in print and electronic media as blood pressure lowering action of bananas (19-24). When I was in the department that day (29-1-99) I received a call from BBC New Delhi Science Correspondent. I gave brief description of our work and answered some of queries posed.



Fig.3. Author in biochemistry research lab at KMC, Manipal

Later Dr. K.L. Bairy submitted work containing clinical data for publication as they were not included in current science paper (25). To explore other roles of ACE it was purified and characterized from ram epididymis (26). Though blood pressure lowering action of bananas was sensational for several years there were no such reports. Recently it was reported that consumption of one banana per day for a week lowered blood pressure in women with cold stress induced hypertension (27).

My interest turned to gestational hypertension which is responsible for maternal and fetal morbidity in pregnant women worldwide. Abnormal lipid profiles are associated with hypertension (28). But studies related to lipid profiles in gestational hypertension are few and involvement of any enzyme in its pathogenesis is yet to be known. Adenosine deaminase a zinc metallo protease like ACE levels and lipid profiles in serum of gestational hypertension women were studied to know their role in causing disease (29).

Other activities

Cord blood research is one of the prime area in medicine. Developing cardiovascular disease in adult life may originate during neonatal life. Lipid profile abnormalities are common in cardiovascular diseases (28). But little is known about cord blood lipid profiles of newborns. So cord blood lipid profiles of

REFLECTIONS OVER THREE DECADES OF JOURNEY FROM NEEEM GUM TO HYPERTENSION

pre and full term newborns were studied (30). Another emerging medical research area is finding of caspase inhibitors which can be used as drugs for the treatment of autoimmune and infectious disease. Hence I delivered an invited lecture on inhibitors of caspases as new therapeutic agents at a international conference (31). To know the effect of magnesium deficiency on maternal and fetal health in pregnant women serum magnesium levels were measured (32).

All these years I am carrying out research after fulfilling my teaching assignments. From 1982 onwards I have been involved in teaching to medical, dental, nursing, pharmacy, biomedical engineering, medical laboratory technology, physiotherapy etc. under and post graduate students. More than 20 different text books written by authors like A.. Lehninger, Stryer, L, Devlin .T. , Voet and Voet , Harper, Bhagavan, N.V, West and Todd, Tietz, Henry, Varley, Kaplan etc. were referred to teach classes after making notes as per the syllabus prescribed by respective councils and universities. Due to lack of subject matter in one book to present it in a more logical way many books were referred. As the years passed I felt need to write textbook based on notes prepared in which subject matter may be presented in coherent way. To suit needs of larger audience advances in frontier areas of biochemistry, cell biology and molecular biology were included (33). After publishing Medical Biochemistry in 2006 to meet under graduate laboratory needs I published Medical Biochemistry Laboratory Manual in 2008(34).

I liked books with color graphics like Lehninger, Stryer, Devlin etc. from post graduate days. In class room teaching also I used color chalks to describe metabolic pathways, functional groups, ring structures, graphs, translation, transcription, replication, PCR etc. for quick learning of concepts by students. Though medical biochemistry is best seller color graphics were not used. My desire to publish textbook with multi color led to publication of Biochemistry : Principles and Practicals (35). In this book six color combinations are used for easy understanding of metabolic path ways, translation etc. All these years I noticed a section of students are unable to write answers to questions given in exams. So to help them in clearing exams I published Biochemistry : Questions and Answers (36).

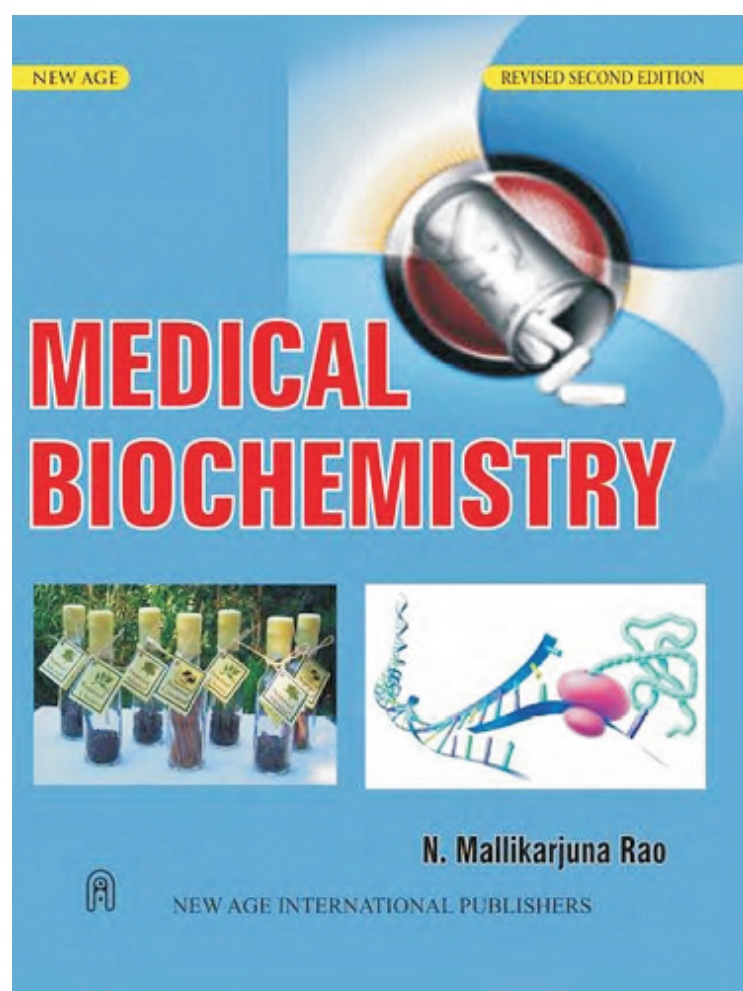


Fig.4. Cover page of authors book Medical Biochemistry

REFLECTIONS OVER THREE DECADES OF JOURNEY FROM NEEM GUM TO HYPERTENSION

One's life time will not be sufficient to visit and appreciate various places and cultures of our country as it is so vast and diverse. From my school days to till I went Manipal I stayed at my village. It is illuminated by paddy, banana, turmeric, black gram and green gram fields in rainy and winter seasons respectively. In hot summer I watched moon light lit sandy banks of nearby canal on full moon days. Till I reached Manipal I had no idea about its climate or surroundings and no Google earth those days to know. But during my first journey to Manipal I watched nature in its finest form with greened sloppy hills, coconut plantations, paddy fields and lakes in between from Palghat to Netrvati river at Mangalore. Manipal is located on hill top and surrounded by green scenario like Kerala as Kasargod is close. Nearby Western Ghats with Agumbi make Manipal much close to nature. Arabian sea can be seen from Manipal and Mmalpe port with beach is hardly few kilometers away.

As oldest civilization our country is enriched by marvelous stone carved temples with presiding gods. The Krishna temple and matt at Udipi are places of spiritual importance for Maipalits. As devoted Indian (hindu) I visited Sringeri, Dharmastala, Kolluru, Beluru, Helabedu, Chamundeswari temple, Brindavan garden and maharaja palace at Mysore, Bangalore lalbagh, iscon temple and jog falls. When I was in Nellore I went to banks of Penna river frequently where Tikkana wrote Mahabharatam in telugu and to have darshan of lord at Ranganadha at temple. I toured Shirdi, Puttaparti, Mantralayam for the blessing of our saints. I also visited Tirupati, Srisailam, Kanipakam, Hyderabad, Vijayawada frequently as they are close to my village.



Fig.5. Author with wife at Ello ra caves

When I was in Rajahmundry road cum rail bridge on mighty Godavari and iscon temple are spots of my frequent visits. Papi kondalu tour on river Godavari by AP tourism boat from Pattisam was most wonderful and unforgettable. Konaseema of Andhra Pradesh is Andhra Kerala with paddy, coconut, cocoa, banana plantations. Without touch of music and literature an Indian life is not that worthy. I like our indian music and literature of any language. Those days radios and libraries were only source of them. With change in technology one can download MP3 stores on mobiles. Internet provides everything including advances in sciences with a click on mouse or browser.

References

1. B.R.Nayak, N.Mallikarjuna Rao and T.N.Pattabhiraman Proteases in Neem gum, J.Biosci. (1979) 1, 393-400.
2. N.Mallikarjuna Rao and T.N.Pattabhiraman. Comparison of the action of tuber protease inhibitors on nine animal pancreatic systems. Indian J. Biochem. Biophys. (1985) 22, 373-375.
3. N.Mallikarjuna Rao, H.N.Rao and T.N.Pattabhiraman. Isolation and Characterization of a Protease inhibitor from arrow root tuber. J. Biosci. (1983) 5, 21-34.
4. N.Mallikarjuna Rao and T.N.Pattabhiraman. Effect of tuber protease inhibitors on bovine enzymes with protein and synthetic substrates. J. Food Sci. Tech (1988) 25, 296-299.
5. N.Mallikarjuna Rao. Cysteine protease inhibitors from banana. Curr. Sci. (1989) 58, 1320-1322.
6. Brizn j; Popovic, T; Drobnickosok, M; Kotnik, M. and Turk. V. Inhibitors of cysteine proteases from potato. Hoppe-Seyler's Biol. Chem.(1988) 369S,233-238.

REFLECTIONS OVER THREE DECADES OF JOURNEY FROM NEEM GUM TO HYPERTENSION

- 7.N.Mallikarjuna Rao. Protease inhibitors from ripened and un ripened bananas. *Biochem. Int.* (1991) 24, 13-22.
- 8.E.G.P. Udupa and N.Mallikarjuna Rao. Distribution and some properties of sheep angiotensin converting enzyme. *Biochem. Mol. Biol. Int.* (1993) 31, 851-860.
- 9.E.G.P. Udupa and N.Mallikarjuna Rao. Sheep testicular and epididymal angiotensin converting enzyme: Inhibitions by captopril, lisinopril and enalapril. *Biochem. Mol. Biol. Int.* (1997) 43, 1063-1070.
- 10.E.G.P. Udupa and N.Mallikarjuna Rao. Inhibition of angiotensin converting enzymes from sheep tissues by captopril, lisinopril and enalapril. *Indian J. Biochem. Biophys.* (1997) 34, 524-528.
- 11.E.G.P. Udupa and N.Mallikarjuna Rao. Effect of chloride and diamide on angiotensin converting enzymes from sheep testis and epididymis. *Indian J. Exp. Biol.* (1998) 36,43-45.
- 12.Rao, N.M. and Udupa, E.G.P. Sheep angiotensin converting enzyme from mammary, lingual and other tissues. *Indian J. Exp. Biol.* (2007) 45, 1003-1006.
- 13.Rao, N.M. and Udupa, E.G.P. Effect of chloride and diamide on angiotensin converting enzyme from sheep kidney, lung and serum. *Indian J. Clin. Biochem.* (2008) 23(1) 53-56
- 14.Ryan, C,A; Hass, G.M and Kuhn, R.W. Purification and properties of carboxypeptidase inhibitor from potato. *J. Biol. Chem.* (1974) 249, 5495-99
- 15.Ambasti, S.P.(ed.). *The useful plants of india.*, CSIR, NewDelhi,1986, p.386.
- 16.Perfumi, M; Massi, M and Decaro,G. Effect of banana feeding on deoxy corticosterone induced hypertension in rats. *Int. J. Pharmacognosy*, (1994),32,115-125
- 17.Schnaith ,E; Beyrau ,R; Buckner, B; Klein,R.M. and Rick, W. Optimized determination of angiotensin-1 converting enzyme activity with hippuryl -L- histidy l-L- leucine as substrate. *Clin. Chim. Acta.* (1994), 227,145-158.
- 18.Sarkar,C; Bairy; K.L and Rao, N.M. Abstract No. OCL. I-4, The 31 st Annual conference of Indian Pharmacological Society held from 18-20th Dec.1998 at Central Drug Research Institute, Lucknow , India,p-89.
- 19.N.Mallikarjuna Rao, K.V.S.R.G.Prasad and K.S.R .Pai. Angiotensin converting enzyme inhibitors from ripened and un ripened bananas. *Curr. Sci.* (1999) 76, 86-88.
- 20.Two bananas a day keep blood pressure at bay, BBC on line network Health News,28-1-1999
- 21.Bananas bring down blood pressure, Nature India News, April 1999
- 22.Bananas can help lower hypertension, The Hindu,29-1-99
23. Eat bananas to avoid blood pressure, Times of India , 29-1-99
- 24.Going bananas, India Today , Feb.15,1999
- 25.Sarkar, C. Bairy , K. L. Rao, N.M. and Udupa, E.G.P. Effect of banana on cold response test, peak expiratory flow rate in healthy volunteer Indian. *J. Med. Res.* (1999) 110, 26-29.
- 26.N.Mallikarjunarao and E. G.P. Udupa. Purification and characterization of ram epididymal angiotensin converting enzyme. *J. Medical Sci. and Technol.* (2012)1(2),32-42.
- 27.Jo,S and Megawati, R. Effect of pisang ambon (*Musa Accumata Colla*) on adult female blood pressure on cold stress test. *J. Medica Planta*, (2010), 1 ,21-25.
28. Halperin, O. R; Sesso, H. D; Ma, J, Buring, J. E; Stampfer, M. J; Gaziano, M. Dyslipidemia and risk of incident hypertension in men. *Hypertension*.2006;47:45-50
- 29.N.Mallikarjuna rao and Sheeba . M. Serum lipid profiles in gestational hypertension. *Rev. Progr.* (2013) 1 (6), 1-7.
- 30.Durga, P. Cord blood lipid profiles in pre and full term new borns, ICMR Project, STS-2010
- 31.Rao, N.M. Inhibitors of Caspases as new therapeutic agents. *Pharma . Anal. Acta* (2010) S 1,20
- 32.Amar, N.K. Serum magnesium levels in pregnant women , M.Sc, Thessis. 2009
- 33.N.Mallikarjunarao, *Medical Biochemistry*,2nd edition, New age international publishers, New Delhi,2006, 824 pp. ISBN 9788122418231
- 34.N.Mallikarjunarao, *Medical Biochemistry Laboratory Mauval*, 1 st edition, New age international publishers, New Delhi,2008, 136 pp. ISBN 9788122421811
- 35.N.Mallikarjunarao, *Biochemistry: Principles and Practicals*, Seekay publishers, Bangalore, 2013, 274 pp, ISBN 9788192416922
- 36.N.Mallikarjunarao, *Biochemistry: Questions and Answers*, Seekay publishers, Bangalore, 2013, 244 pp, ISBN9788192416939