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Date 6/4/87

ETHICAL REVIEW COMMITTEE, ICDDR,B.

Principal Investigator Dr. A.K. Azad Chowdhury Trainee Investigator (if any)

Application No. PCC/1/87

Supporting Agency (if Non-ICDDR,B)

Title of Study Anti-Shigella drugs from Garlic, Asok and Neem

Project status:

(☒) New Study

() Continuation with change

() No change (do not fill out rest of form)

Circle the appropriate answer to each of the following (If Not Applicable write NA).

1. Source of Population: NA

5. Will signed consent form be required: NA

(a) From subjects Yes No

(b) From parent or guardian (if subjects are minors) Yes No

2. Does the study involve: NA

6. Will precautions be taken to protect anonymity of subjects Yes No

7. Check documents being submitted herewith to Committee:

(a) Ill subjects Yes No

(b) Non-ill subjects Yes No

(c) Minors or persons under guardianship Yes No

(a) Physical risks to the subjects Yes No

(b) Social Risks Yes No

(c) Psychological risks to subjects Yes No

(d) Discomfort to subjects Yes No

(e) Invasion of privacy Yes No

(f) Disclosure of information damaging to subject or others Yes No

Does the study involve: NA

(a) Use of records, (hospital, medical, death, birth or other) Yes No

(b) Use of fetal tissue or abortus Yes No

(c) Use of organs or body fluids Yes No

Are subjects clearly informed about: NA

(a) Nature and purposes of study Yes No

(b) Procedures to be followed including alternatives used Yes No

(c) Physical risks Yes No

(d) Sensitive questions Yes No

(e) Benefits to be derived Yes No

(f) Right to refuse to participate or to withdraw from study Yes No

(g) Confidential handling of data Yes No

(h) Compensation &/or treatment where there are risks or privacy is involved in any particular procedure Yes No

Umbrella proposal - Initially submit an overview (all other requirements will be submitted with individual studies). Protocol (Required)

Abstract Summary (Required)

Statement given or read to subjects on nature of study, risks, types of questions to be asked, and right to refuse to participate or withdraw (Required)

Informed consent form for subjects

Informed consent form for parent or guardian

Procedure for maintaining confidentiality

Questionnaire or interview schedule *

* If the final instrument is not completed prior to review, the following information should be included in the abstract summary:

1. A description of the areas to be covered in the questionnaire or interview which could be considered either sensitive or which would constitute an invasion of privacy.
2. Examples of the type of specific questions to be asked in the sensitive areas.
3. An indication as to when the questionnaire will be presented to the Cttee. for review.

I agree to obtain approval of the Ethical Review Committee for any changes involving the rights and welfare of subjects before making such change.

(PTO)

Azad Chowdhury 6/4/87

Principal Investigator

Trainee



INTERNATIONAL CENTRE FOR
DIARRHOEAL DISEASE
RESEARCH, BANGLADESH

Phone: 600171-78
Telex: 65612 ICDD BJ
Cable: Cholera Dhaka
G.P.O. Box 128 Dhaka -2 Bangladesh.

February 17, 1987

Chairman
BMRC
Dhaka

Subject: Protocol entitled "Anti-Shigella drugs from Garlic, Asok
and Neem" by A.K. Azad Chowdhury and others.

BMRC-ICDDR,B COLLABORATIVE PROTOCOL PROGRAM

Dear Sir:

I discussed about my participation in this protocol with the
Head of my Scientific Division, Dr. David A. Sack, who gave
his verbal approval to my participation. Because he is out
of the country at the moment and because of the fact that there
is a time constraint in the processing of the application by
BMRC, I am sending this copy for initiation of processing.
A signed copy will be sent to you duly.

Thanking you,

Sincerely yours,

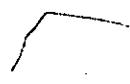
A handwritten signature in dark ink, reading 'Zia Uddin Ahmed', written over a horizontal line.

Zia Uddin Ahmed

Encl: Revised protocol to supersede the advance copy
filed earlier.

Copies of some papers cited in the text.

FORMAT FOR PREPARATION OF BMRC & ICDDR,B COLLABORATIVE PROTOCOLSa. FACE SHEETb. SECTION-I : RESEARCH PROTOCOL

- (1) Title : Anti-shigella drugs from Garlic, 'Asok' and 'Neem'.
- (2) Principal Investigator(s) : Dr.A.K. Azad Chowdhury *Azad Chowdhury*
Professor in Pharmacy, D.U. 9.2.87
- (3) Co-Investigator(s), if any : i) Dr. Zia Uddin Ahmed,
Associate Scientist, ICDDR,B
- ii) Dr. Moniruddin Ahmed, *Moniruddin*
Associate Professor 9.2.87
Pharmacy Department, D.U.
- (4) Starting Date : 1st February, 1987
- (5) Completion Date : 30th January, 1988
- (6) Total Direct Cost : 
- (7) Scientific programme Head : Does not apply

This protocol has been approved by the _____
Working Group.

recommended

Chairman
9.2.87
Chairman
Department of Pharmacy
Dhaka University

Azad Chowdhury 9.2.87
Signature of the Principal Investigator

Countersigned:

Signature of the Scientific Programme Head

(Dr. David A Sack
Associate Director)

Date: _____

(8) Abstract Summary: (250 words or less)

Anti-shigella drugs from Garlic, 'Asok' and 'Neem'.

Herbal drugs such as garlic (Allium Sativum) extract, allicin (isolated from garlic), water soluble fraction of flowers and flowerbuds of Asok (Saraca indica) and Neem (Azadirachta indica) oil showed promising antibacterial activity against wide variety of pathogenic bacteria (some of which were clinically resistant). It is proposed to study the antibacterial and the antisecretory activities of these and related plant drugs on drug resistant *Shigella* strains in vitro and in rabbit model of shigellosis. In addition to this, acute and chronic toxicities of these drugs shall be studied in mice and rats. Isolation and characterization of the active constituents shall also be done by chemical and physical methods (I-R, U-V, N.M.R. spectroscopy).

(9) Reviews: (leave Blank)

(i) Ethical Review Committee: _____
(Approved/Not approved)

(ii) Research Review Committee: _____
(Approved/Not Approved)

(iii) Director's signature & remark, if any _____

C. SECTION-II : RESEARCH PLAN

A. INTRODUCTION

1. Objective

To develop a herbal drug for the treatment of Shigellosis, especially shigellosis caused by multiple drug resistance strains of *Shigella*.

2. Background

Diarrhoea is a major cause of morbidity and mortality, especially in children, in the developing countries¹. Child mortality due to diarrhoea in Bangladesh is also high²⁻⁴. The studies conducted in Bangladesh⁴ and elsewhere in the region^{5,6} suggest that major causes of diarrhoea are enterotoxigenic Escherichia coli (ETEC), rotavirus, shigella and protozoal infections. About 80-85% of cases of bacillary dysentery in Bangladesh is due to shigella infection⁷. Prior to 1970 the treatment of shigellosis was simple since most of the strains of shigella were susceptible to commonly available antibiotics such as tetracycline and chloramphenicol⁷. In latter years, owing to indiscriminate use of antibiotics, most of the strains of shigella developed resistance to most of the easily available antibiotics and antimicrobial drugs⁷⁻⁹ such as tetracycline, chloramphenicol, streptomycin, co-trimazole. Then the drugs of choice in the treatment of shigellosis caused by multiple drug resistant shigella strains were ampicillin and nalidixic acid^{8,10}. But resistance to these drugs has also been reported¹⁰⁻¹². This multiple drug resistance has created a serious problem in the treatment of shigellosis. Newly emerged drug resistant strains have been causing outbreak of shigellosis in recent years in Bangladesh^{10,13} and in other developing countries¹⁰. Hence the development of antimicrobial drugs those will be active against multiple drug resistant strains of shigella is a pressing need of the hour.

It is encouraging to note that some of the drugs of plant origin have shown promising antimicrobial activity against resistant strains of bacteria. The garlic extract exhibited anti-microbial activity against nine clinical resistant strains¹⁴ of staphylococcus, Escherichia, Proteus, Pseudomonas and other types of microorganisms including canadia¹⁵⁻¹⁹. The water soluble fraction of flower and flower bud of Saraca indica (Bengali: Aşok) revealed inhibitory effects²⁰ on E.coli, Shigella dysenteriae, Salmonella viballerup and V.cholerae 569-B,
Neem oil²¹ (Azadirachta indica) also showed promising antibacterial activity against both gram(+) and gram(-) organisms. Literature citation on antimicrobial activity of these drugs on multiple drug resistant strains of Shigella is limited, if any.

3. Rationale

Considering the emergence of threat of drug resistant shigella strains to public health and the prospect of some of the herbal drugs as the antimicrobial agents, it is proposed to study the antibacterial and the anti-secretory property of these drugs on Shigella strains and in rabbit model of shigellosis respectively.

B. SPECIFIC AIMS

I. Phase I (1987)

- (i) Testing of the extracts of three medicinal plants (Garlic, 'Asok' and 'Neem') on Shigella strains to assess antibacterial activity.
- (ii) Testing two of the most potent components (from bacterial sensitivity data) in rabbit model using Shigella flexneri
- (iii) Isolation and characterization of active constituents from the plant drugs.
- (iv) Determination of acute and chronic toxicity of the plant extracts in animal models.

C. METHODS OF PROCEDURE

1. Preparation of the agents

- (i) Garlic extract shall prepared according to the literature method.¹⁷
- (ii) Allicin shall be isolated from garlic using literature method¹⁸.
- (iii) The water soluble fraction of flowers and flower buds shall be prepared by the method²⁰.
- (iv) The major component from the T.L.C. analysis of the extract of flowers and flowers buds shall be prepared by eluting the components with relevant solvent, (dried and later reconstituted).
- (v) Neem oil produced from the ripe seeds of Neem²¹ (Azadirachta indica)
- (vi) Isolation and characterization of two most potent antibacterial principles (assessed from in vitro antibacterial testing results) from these plant preparations by chromatographic techniques, physical (I-R, U-V, T.M.R., Mass spectroscopies) and chemical methods.

2. ANTIMICROBIAL AND ANTISECRETORY ACTIVITY STUDIES

- i. In vitro antimicrobial activity of the agents mentioned above shall be studied on multiple drug resistant shigella strains such as shigella dysenteriae type I, Shigella flexneri and Shigella sonnei (to be provided by ICDDR, Bangladesh) using standard methods¹⁵ of antibiotic/ antimicrobial assay.
- ii. In vivo antimicrobial and antisecretory activities shall be determined in the rabbit model of shigellosis²².

3. TOXICOLOGICAL STUDIES

- i. Acute toxicity of the agents shall be determined in mice and rats through determination of L.D.₅₀.
- ii. Chronic toxicity of the agents shall be studied in mice and rats. In the study, ^{the} effects of these drugs on vital organs and systems such as liver, kidney, heart and haematopoiesis shall be determined by different method²³ such as enzyme assays histopathology, physical and microscopic examinations.

4. ASSESSMENT OF THE THERAPEUTIC POTENTIAL OF THE AGENTS .

- i. Rabbit experimental infection studies shall be conducted to assess the therapeutic potentiality of the most active agents.

D. SIGNIFICANCE OF THE WORK

- i. The development of a drug(s) for the treatment of shigellosis (especially the shigellosis caused by multiple drug resistant shigella strains) from herbal source shall be of immense benefit to the public health problem of the country.

E. FACILITIES REQUIRED

(a) General facilities required for chemical studies involving isolation and characterization of the active principles and also the facilities to prepare the agents in appropriate pharmaceutical dosage forms for antimicrobial activity testing in vitro (disc diffusion method) and in vivo (rabbit model of shigellosis) shall be provided by the Department of Pharmacy, Dhaka University.

(b) The facilities necessary for initial antibacterial activity testing (disc diffusion method) using the extracts against standard bacteria shall be provided by the Department of Pharmacy, Dhaka University.

(c) The facilities necessary for toxicological studies except animals shall also be provided by the Department.

F. COLLABORATION ARRANGEMENTS

i) ICDDR,B Collaboration

ICDDR,B shall provide bacterial strains media, plates and other routine laboratory supplies for carrying out the in vitro work at the university. When a potent component is identified the therapeutic/prophylactic value of the component shall be assayed in rabbit test in ICDDR,B Animal Resources Branch. A technician has to be provided to do this work after learning the technique from the workers at Animal Resource Branch. So ICDDR,B collaboration will involve; a) disc diffusion assay of extracts and compounds for growth inhibitory activity on *Shigella*, (b) testing whether two most potent (antibacterial in vitro) extracts compounds can protect rabbit against a virulent challenge by *Shigella flexneri*.

1. Department of Pharmacy
Dhaka University, Dhaka

2. ICDDR,B, Dhaka.

d. REFERENCES

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M.Pharm. Thesis, Department of Pharmacy, University of Dhaka, 1985.

SECTION III-BUDGET

A. BUDGET FOR A YEAR

1. PERSONNEL SERVICES

<u>NAME</u>	<u>POSITION</u>	<u>%TIME REQUIRED</u>	<u>Tk. (P.M)</u>	<u>TK. (P.A)</u>
Dr.A.K.Azad Chowdhury	Professor Department of Pharmacy D.U.	25%	2500x12=	30,000
Dr.Zia Uddin Ahmed	Associate Scientist, ICDDR, B	5%	-	-
Dr.Moniruddin Ahmed	Associate Professor Department of Pharmacy D.U.	10%	1000x12=	12000
A Research Fellow	(to be appointed)	100%	Tk.3000x12=	36000
A Lab. Assistant	(Mr.Momtazuddin)	20%	Tk.600x12 =	7200

SUPPLIES

Bacteriologic media, Disposable petridishes & Equipments:	10,000
Animals Rabbit (Tk.250x3)	7500
Rat (Tk.25x40)	1000
Mice (Tk.12 x 100)	1200
Supplies & chemicals (TL.C. Column Chromatography purchase of drugs Silicagel, Alumina etc Solvents, other chemicals)	20,000
Conveyance & Transport	5,000
Contingency and overhead	5,000
Grant Total Tk. 1,34,900	

CURRICULA VITAE

PRINCIPAL INVESTIGATOR Dr. A.K. Azad Chowdhury

1. Surname/Family Name: Azad Chowdhury

First name/other names Abul Klam

2. Date of birth: 21st October 1946

Place of Birth: Feni, Bangladesh

Nationality: Bangladeshi

3. Degrees

<u>Degree</u>	<u>Year</u>	<u>Institution</u>	<u>Disciplines</u>
B.Sc. (Hons.)	1967	Dhaka University	Biochemistry
M.Sc. (thesis)	1968	Dhaka University	Biochemistry & Nutrition
Ph.D	1976	Manchester University	Pharmacy
Post doctoral fellow 1976-1978 Nottingham University			

4. Academic Distinctions:

	<u>Degree</u>	<u>Year</u>
First class	B.Sc	1967
First class	M.Sc.	1968

5. Present post (Title, Institution, Dates)

Title: Professor

Institution Department of Pharmacy
University of Dhaka, Dhaka-2, Bangladesh.

Dates: From October, 1985.

CURRICULA VITAE

6. Previous posts (Title, Institution Dates)

Title: a. Associate Professor & Chairman
b. Assistant Professor
c. Lecturer

Institution:

a. Department of Pharmacy, Dhaka University
b. 1 -do-
c. -do-

Dates:

a. 1980 to 1985 (study leave for post doctoral research
1984-1985)
b. 1978 to 1980
c. 1969 to 1978 (study leave for doing Ph.D & Post
doctoral research 1973 to 1978).

7. Academic & Research Awards, Consultant & other posts

- a) Awarded Rab Chowdhury Gold Medal by Dhaka University for contribution to Science
- b) Awarded Commonwealth Academic Staff Fellowship by Commonwealth Commission U.K. 1984-1985.
- c) Awarded DAAD Fellowship by W.German Govt. 1984.

8. Other University & Institutional Posts

- a) Post doctoral fellow- Nottingham University U.K. 1976-1978
- b) Visiting fellow, Braunschweig Technical University, West Germany 1984.
- c) Commonwealth Academic Staff Fellow, Nottingham University 1984-1985.

9. Current Research Interests including details of Projects of which Applicant is Principal Investigator. (Separate sheets attached)

10. Publications & Communications (Separate sheets attached)

Current Research Interest.

1. Antifertility drug from plant source (Marsdenia tinctoria) (sponsored by the University Grants Commission)
2. Antifertility drug from plant source (Hibiscus-rosa-s nensis) (sponsored by Bangladesh Medical Research Council)
3. Areca catechu (Betel nut) in the treatment of cerebro vascular diseases- jointly conducted by Prof. A. Mannan, IPGMR and the author.
4. Moringa oleifera in the treatment of hypertension - jointly conducted by Prof. Serajul Hoque, IPGMR. A. Rashid, Muniruddin Ahmed and the author from the Department of Pharmacy.
5. Antibacterial Principles isolation from plant sources their characterization and biological testing.
6. Role of vitamin A deficiency in infectious, parasitic diseases and immune response.