Attachment 1. (FACE SHEET)

Library

ETHICAL REVIEW COMMITTEE, ICDDR,B.

Date 10/12/96

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Principal Investigator G. H. RABBANI Trainee Investigator (if any) _____

Application No. 96-025 Supporting Agency (if Non-ICDDR,B) _____

Title of Study Evaluation of Two Plant Extracts in Expt. Shigellosis in Rabbits.

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 (Animal study)
   (a) Ill subjects Yes No
   (b) Non-ill subjects Yes No
   (c) Minors or persons under guardianship Yes No
2. Does the study involve: NA
   (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
3. 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
4. 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
5. Will signed consent form be required: NA
   (a) From subjects Yes No
   (b) From parent or guardian (if subjects are minors) Yes No
6. Will precautions be taken to protect anonymity of subjects Yes No NA
7. Check documents being submitted herewith to Committee:
   - ✓ 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.

G. H. Rabbani
Principal Investigator

Trainee

ENTERED 2 1 JUN 1996

CHECK-LIST FOR SUBMISSION OF PROPOSALS
TO THE RESEARCH REVIEW COMMITTEE (RRC)
[Please tick (✓) the appropriate box]

1. Has the proposal been reviewed, discussed and cleared at the Division level ?

   Yes ☑

   No ☐

   If 'No', please clarify the reasons: _____

   _____

   _____

2. Has the proposal been peer-reviewed externally ?

   Yes ☐

   No ☑

   If the answer is 'NO', please explain the reasons: Small animal Study.

   _____

   _____

3. Has the proposal scope to address gender issues ?

   Yes ☐

   No ☑

   If the answer is 'YES', have these been adequately incorporated in the proposal. Please indicate: _____

   _____

   _____

4. Has a funding source been identified ?

   Yes ☐

   No ☑

   If the answer is 'YES', please indicate the name of the donor: _____

   _____

   _____

5. Whether the proposal is a collaborative one ?

Yes [✓]

No [ ]

If the answer is 'YES', the type of collaboration, name and address of the institution and name of the collaborating investigator be indicated:

Prof. Kamaluddin Ahmad
Bangladesh Herbal Med. Institute.
Dhaka,

6. Has the budget been cleared by Finance Division ? NA

Yes [ ]

No [ ]

If the answer is 'NO', reasons thereof be indicated: _____

_____

_____

7. Does the study involve any procedure employing hazardous materials, or equipments ?

Yes [ ]

No [✓]

If 'YES', fill the necessary form.

10/12/96
Date

S. N. Zaidi
Signature of the
Principal Investigator

RESEARCH PROTOCOL (SMALL STUDY)

Title:	Evaluation of Two Plant Extracts in the Treatment of Experimental Shigellosis in Rabbits
Principal Investigator:	G H Rabbani, MD, PhD, FACG
Co-Investigators:	M. John Albert, PhD and Prof. Kamaluddin Ahmad, PhD
Starting Date:	January 1997
Completion Date:	June 1997
Total Cost:	US$ 7, 515
Donor:	Open

This protocol has been approved as a small project.

Signature of Division ~~Director~~, Laboratory Sciences Division

11/12/96
Date Approved

Abstract Summary

The objective of this small protocol is to make a preclinical assessment of two plant extracts (*Euphorbia hirta and Nigella sativa*) in the treatment of experimental shigellosis in rabbits. Extracts from the dried leaves of these two plants have been shown to possess in-vitro antimicrobial activities against different serotypes of shigellae. These extracts may be useful in the treatment of human shigellsois. However, no information is available regarding their safety and efficacy in man. Thus, preclinical studies in a suitable animal model would be required before considering human trial. Therefore, we are proposing a small study to determine the safety and efficacy of these plant extracts in a rabbit model of shigellosis. In this study, adult rabbits will be infected with *S. flexneri* 2a and then treated with the plant extracts and placebo preparation. The therapeutic efficacy will be evaluated by changes in clinical, bacteriologic, and histopathologic characteristics.

OBJECTIVE

The objective of this study is to make a preclinical evaluation of two plant extracts (*E. hirta* and *N. sativa*) known to possess antimicrobial activities in the treatment of experimental shigellosis in rabbits.

Background:

Shigellosis is an important cause of childhood morbidity and mortality, particularly in the developing countries associated with poverty and poor sanitation. Although, antimicrobial agents have been shown to be useful in the treatment of shigellosis, they often pose problems such as drug-resistance, non-availability, and high cost. Development of simple, safe, and effective therapeutic agents against shigellosis would thus be a priority option in the child survival strategies in the third world. Today's therapeutic approaches relies heavily on the use of antibiotics, discouraging research and development of herbal medicines, the therapeutic potential of which have been recognised since the dawn of human civilization.

Before the advent of modern medicine, there had been widespread use of many herbs, particularly in the Vedic and Unani system of medicine in India. In modern China, the traditional medicine based on the use of herbs is practised widely in their health care system. *Bengal Plants (Prain, London,1908)* in two volumes list some 2,824 plants and herbs, many of them are used for healing different illnesses. The vast literature on the subject is based on the wisdom of the ancient healers, and a great deal of their knowledge, was in fact, empirical. Therefore, there is wide scope of exploring the world of herbal medicine using today's advanced technology.

Plan of the present study: To assess the therapeutic potential of some plants, we have studied extracts from roots, barks, and leaves of *Euphorbia* species. This small, annual herb grows widely in tropical and subtropical areas of the world. Crude extracts from the roots, barks, and leaves of this plant have been used for the treatment of pulmonary disorders in Australia and Africa (Wong-Ting Fook 1080). The plant extract has also been used to treat diarrhoea and dysentery in East Africa and Southeast Asia. Other medicinal usage of the plant includes conditions such as conjunctivitis, helminthiasis, and cattle diseases (Sofowara 1982. Dalziel 1956. Oliver 1956, Kerharo 1974, Ayensu 1978).

The extracts of *E. hirta* inhibit the growth of *E. histolytica* (Dhar 1968) and has been useful in the treatment of amoebiasis (Ridet 1964). *E. hirta* extract is a powerful antimicrobial agent in-vivo against a number of pathogenic and non-pathogenic bacteria (Pousset 1981), and has cytotoxic effects against cancer cells (Belin 1952)

Nigella sativa, synonym, *kala jira* or black cumin is a common annual herb which grows abundantly in the Indian subcontinent, Africa, and Europe. It produces edible black seeds which are used as spices in the Indian cuisine. The seeds of *N. sativa* have been used to treat various ailments by the traditional healers of the Indian subcontinent (Chopra et al, 1982). The seeds have also been used in the treatment of infectious diseases, to stimulate lactation in rural mothers (Chopra 1982), and in the treatment of menstrual disorders.

Recently, *N. sativa* seeds have been investigated at the Bangladesh Herbal Institute and ICDDR,B laboratories to assess their antimicrobial activities. The material was prepared by expressing oil from the seeds and extracting the oil with n-hexane after steam distillation. The seeds yielded about 0.5% volatile oil after evaporation. This oil was sterilized by passing through millipore filter and then tested for antibacterial activity against shigellae. Disc-diffusion method in nutrient agar plates and tube dilution method

were used. The extract was able to inhibit the growth of all serotypes of shigellae (minimum zone of inhibition, 12 mm) and the MIC values were found to be 150-250 ug/mL. An alcoholic extract and a n-hexane extract of the seed oil obtained by column chromatography were also found to possess similar antimicrobial properties against shigelle. In the present study, we are proposing to test this material in an in-vivo model of rabbit shigellosis.

Assessment of Antimicrobial Activity

The plant preparation was an alcoholic extract of the dried powder from the leaves of *E. hirta* (called Hirtacin) from which the solvent has been removed by evaporation in vacuum. The extracts were dissolved in different solvents (polar and non-polar) and the active fractions separated using high performance liquid chromatography (HPLC) and thin layer chromatography (TLC). The extracts were tested for their antimicrobial activities against a variety of pathogenic microorganisms, including shigellae. Antimicrobial activity was assessed by Kirby-Bauer method of zone inhibition in nutrient agar media. The zone of inhibition was measured after overnight incubation at 37°C and was used as an index of activity. Most shigellae isolated from patients at the ICDDR,B hospital, including those which were resistant to many antimicrobial agents, were susceptible to the plant extracts.

Growth of many enteric pathogens in-vitro, other than *Shigella* were also inhibited by the plant extracts. The extract from one plant designated as Plant H inhibited the growth of the following pathogenic bacteria:

Shigella dysenteriae type 1
Shigella flexneri
Shigella boydii
Shigella sonnei
Staphylococcus aureas
Streptococcus pyogenes
Enterotoxigenic Escherichia coli (ETEC)
Salmonella typhi
Salmonella paratyphi A
Bacillus subtilis
Vibrio cholerae O1

The alcoholic extracts and the acetic acid fraction of the HPLC were found to possess antimicrobial activity against different serotypes of shigellae that were resistant to multiple antibiotics including ampicillin, nalidixic acid, tetracycline, gentimicin, mecillinum. The effective zone of inhibition ranged from 12 mm to 50 mm. The minimal inhibitory concentration (MIC) against *S. dysenteriae* type 1 was found to be 2 ug/mL.

The strains of *Shigella* and *V. cholera* isolated from patients in the Rwandan refugee camps were also found susceptible to *E. hirta* extract.

The active ingredients responsible for antimicrobial activity have not yet been identified. However, preliminary studies have indicated that there are more than one antibacterial components; some of them are phenolic in nature and others non-phenolic. At least there are two active non-phenolic components. The chemistry and biological actions of the individual constituents are being studied at the moment.

Rationale

Sufficient in-vitro data indicate that the extracts from *E. hirta* leaves have significant antimicrobial activity, specially against shigellae. This indicates that the plant extracts may be

useful in the treatment of shigellosis. However, its effects in an animal model of shigellosis is not known. Thus, the proposed in-vivo study in adult rabbit with shigellosis would be very important to assess the potential benefits and risks (if any) of this preparation before considering any human clinical trial.

Clinical Studies

We have evaluated the therapeutic efficacy and safety of hirtacin in a small, uncontrolled clinical trial in shigellosis. At the hospital of the Bangladesh Institute of Herbal Medicine, 32 patients, including 4 children and 28 adults with acute shigellosis were treated with hirtacin (*E. hirta*) and their clinical and bacteriological courses were followed. Each patient received hirtacin orally, 4-6 times a day depending on the age and severity of illness. Each dose had active ingredients extracted from about 100 g of fresh *E. hirta* leaves.

The results indicate that the patients improved clinically as assessed by improvements in stool quality (blood and mucus) and stool frequency. Symptomatic improvements (pain, tenesmus, fever) also were observed. The patients tolerated the drug well and there were no adverse effects as assessed by changes in serum SGOT, SGPT, bilirubin, alkaline phosphatase, electrolytes, creatinine, and hematologic parameters. These uncontrolled observations, when assessed in comparison to clinical experience or historical control, indicate that hirtacin may be useful and safe in the treatment of shigellosis. A preliminary report of this trial was published in the Bangladesh Journal of Biological Sciences, 1985-88; vol. 14-16, 45-56.

MATERIALS AND METHODS

Plant extracts: Extracts from two different plants will be evalauted, one will be designated compound EH (*E. hirta*) and the other, compound NS (*N. sativa*). Active ingradients will be extracted from the leaves of the plants by alcohol extraction and then evaporation. The material will be lyophilised and stored in sealed container.

Antimicrobial activity: The extract will be dissolved in aqueous solution and will be tested for antibacterial activity using Kirby-Bauer (nutrient agar) and tube dilution technics at the ICDDR,B Laboratory Sciences Division. Zone of inhibition and minimum inhibitory concentration (MIC) will be determined against different serotypes of shigellae, including *S. flexneri*, *S. dysenterae* type 1, *S. sonnei*, and *S. boydii*.

Clinical evaluation in rabbit shigellosis: The therapeutic effects of the plant extracts will be evaluated in a rabbit model of shigellosis recently developed at ICDDR,B (Rabbani et al, Infect Immun 1995). Briefly, rabbits will be infected with *S. flexneri* 2a through a in situ catheter implanted in the proximal colon. Once active colitis is established (24h), rabbits will be treated with compound EH (extracts equivalent to 25 g of dried, powdered leaves of *E. hirta* daily, ie, one-fourth of the adult human dose) given by per oral route. The dose of compound NS will be based on MIC values, ie, 250 mg of oil extract/kg per day in 4-6 divided doses. A dose given on the basis of per kg body weight is being worked out. Infected rabbits will also be treated with pivamdinocillin (Mecilinum) as positive control and placebo as negative control.

The following treatment groups will be used:

Treatment Groups

Compound EH (N=10 rabbits)
Compound NS (N=10 rabbits)
Pivamdinocillin (N=10 Rabbits)
Placebo (N=10 rabbits)

Assessment of treatment effects: The effects of treatment will be evaluated by clinical, bacterilogical, and histopathological examinations.

Clinical parameters will include: evaluation of body temperature, blood and mucus in stool, and leucocytosis. Hematological evaluation will be done by complete blood count and differential counts. Biochemical tests will include SGOT, SGPT, serum bilirubin, creatinine, and urinary protein.

Bacteriological assessment will be done by daily culture and bacterial counts in the colonic contents of the rabbits.

Histopathological evaluation will be done after autopsy after 72 h of starting treatment. Specimens of tissues from colon and terminal ileum will be examined.

BUDGET FOR THE SMALL STUDY

Dr. Rabbani's time	No Cost
Dr. Albert's time	No Cost
Bacteriology tests (Culture and Sensitivity), 100 tests @ $15	$ 1,500
Rabbits, 50 rabbits @ $20	$ 1,000
Animal Surgery (Personnel + Supplies)	$ 1,000
Drugs (pivamdinocillin)	$ 15
Histopathology tests, 100 specimens @ $15	$ 1,500
Animal attendent, formalin, anesthetics etc.	$ 500
Toxicity studies (Liver function/ Renal function tests/ SGOT, SGPT, Alk Phos, ALT, Creatinine etc.), 100 tests @ $20	$ 2,000
Total Cost	$ 7,515.00

Refefences

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4. Dhar ML, Dhar MM, Dhawan BM, Roy C (1968). Screening of Indian Plants for biological activity: Part 1. Ind J Exp Biol, 6:232-47.
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6. Belin M, Fitzerald D, Cogan GW (1952). Tumour damaging capacity of plant materials: plants used as cathertic. J Cancer 13:139-55.
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8. Prain D (1903). Vol. 1 and Vol. 2. Messers West Neuman and Co, London, 1903.
9. Rabbani GH, Albert MJ, Rahman H, Islam M, Mahalanbis D. Development of an improved animal model of shigellosis in adult rabbit by colonic infection with shigella flexneri 2a. Infect and Immunity 1995, 63:4350-4357.