



TOGETHER
for a sustainable future

OCCASION

This publication has been made available to the public on the occasion of the 50th anniversary of the United Nations Industrial Development Organisation.



TOGETHER
for a sustainable future

DISCLAIMER

This document has been produced without formal United Nations editing. The designations employed and the presentation of the material in this document do not imply the expression of any opinion whatsoever on the part of the Secretariat of the United Nations Industrial Development Organization (UNIDO) concerning the legal status of any country, territory, city or area or of its authorities, or concerning the delimitation of its frontiers or boundaries, or its economic system or degree of development. Designations such as “developed”, “industrialized” and “developing” are intended for statistical convenience and do not necessarily express a judgment about the stage reached by a particular country or area in the development process. Mention of firm names or commercial products does not constitute an endorsement by UNIDO.

FAIR USE POLICY

Any part of this publication may be quoted and referenced for educational and research purposes without additional permission from UNIDO. However, those who make use of quoting and referencing this publication are requested to follow the Fair Use Policy of giving due credit to UNIDO.

CONTACT

Please contact publications@unido.org for further information concerning UNIDO publications.

For more information about UNIDO, please visit us at www.unido.org

RESTRICTED

16898

DP/ID/SER.A/973
22 February 1988
ENGLISH

STRENGTHENING THE ROYAL DRUG RESEARCH LABORATORIES

DP/NEP/80/003/11-02

NEPAL

Technical report: Screening of plant compounds for biological activity:
findings and recommendations of the training mission

Prepared for the Government of Nepal
by the United Nations Industrial Development Organization
acting as executing agency for the United Nations Development Programme

Based on the work of R. C. Simal, pharmacologist/toxicologist

Backstopping officer: R.O.B. Wijesekera, Chemical Industries Branch

United Nations Industrial Development Organization

Vienna

ABSTRACT

As part of the project "Strengthening the Royal Drug Research Laboratories" (DP/NRP/80/003), for which the United Nations Industrial Development Organization (UNIDO) is the executing agency, an expert was sent on a two-month mission to Kathmandu, 18 March-15 May 1987, to train Royal Drug Research Laboratories staff in techniques that would allow screening plant extracts and compounds for biological activity.

For each of the several biological screens, the expert assessed the Laboratory's supply of animals, its facilities in terms of chemicals and equipment and the ability of its staff, after having received the training, to carry out the tests. He recommends in this report that the Laboratory's capabilities be strengthened by procuring the equipment needed for these routine screenings. He also recommends that a supply of basic surgical instruments and chemicals be provided, as well as subscriptions to pertinent journals.

The expert concludes that a much larger supply of animals is needed and that dogs should be obtained, if possible. He also concludes that deficiencies in equipment and chemicals must be remedied in order to achieve optimum output and that staff will be more confident in their work once this has been done.

CONTENTS

	<u>Page</u>
INTRODUCTION	4
RECOMMENDATIONS	5
FINDINGS	7
A. Animals	7
B. Equipment and chemicals	7
C. Staffing	8

Annexes

I. Equipment recommended for procurement	11
II. Essential chemicals and surgical instruments	12
III. Journals recommended for subscription by the library	13

INTRODUCTION

In the context of the overall aim of project "Strengthening the Royal Drug Research Laboratories" (DP/NEP/80/003), which is to strengthen the capabilities of the Royal Drug Research Laboratories (RDRL) at Kathmandu, the expert spent the period of his mission, from 18 March to 15 May 1987, training the staff of RDRL in techniques to screen plant extracts for biological activity. Since biological screening is a broad field, a few important screens had to be selected to suit both the facilities available at the Laboratory, animals as well as equipment, and the overall objectives of the project. The following areas were chosen for training staff after discussion with scientists at RDRL:

Hypotensive activity

Anti-inflammatory activity

Anti-gastric ulcer activity

Bronchodilator activity

Wound-healing properties

Analgesic activity

Studies carried out in isolated tissues.

RDRL had some equipment available for work in all these areas, and indeed testing was being carried out, but it also faced problems in all the areas.

RECOMMENDATIONS

1. Animals are the essential ingredient of biological research. Even the limited objectives that RDRL is working towards will be difficult to achieve unless the supply of rats and guinea pigs is increased considerably. This will mean a corresponding increase in the staff at the animal house and in the amount of animal feed. The yearly requirements for different animals should be worked out on the basis of the number of plant samples to be tested, and an adequate supply of these animals should then be arranged for. Whenever possible, screening for cardiovascular activity should be carried out with dogs or with hypertensive rats. The latter alternative will necessitate procuring expensive equipment for measuring the blood pressure of conscious rats and either the production of hypertensive rats at RDRL or the procurement and maintenance of a colony of spontaneously hypertensive rats.
2. The pieces of equipment recommended for procurement for conducting the tests mentioned in the introduction are listed in annex I. If more tests are to be introduced later, as suggested in recommendation 4 below, some additional minor equipment will be needed.
3. The chemicals and surgical instruments required for routine screening are listed in annex II. It is very important that they be on hand when needed.
4. In addition to the tests that are already being conducted and which only need some streamlining, the following tests are also recommended. They can be performed without much additional cost, staff or equipment:
 - (a) Anticonvulsant activity. The supramaximal electroshock seizure test can be carried out in mice while doing behavioural tests, but only if a convulsimeter and a pair of ear electrodes are available.
 - (b) Diuretic activity. Diuretic activity can be tested in rats. The only additional equipment needed is some metabolic cages. The laboratory already has a flame photometer with which to measure sodium and potassium output in the urine.
 - (c) Local anaesthetic activity. Testing for infiltration anaesthetic activity can be done in guinea pigs if it is warranted by the results of isolated tissue experiments and cardiovascular testing.
5. A good library is essential to keep the staff abreast of new developments in the field. Unfortunately, financial constraints mean the library is no longer able to subscribe to the basic journals in pharmacology, physiology, toxicology or any other field. Some journals had been received until two or three years ago, but they had to be discontinued due to rising costs. The only journals now being subscribed to are Chemical Abstracts and Current Contents/Life Sciences. It is strongly recommended that at least some journals that deal with medicinal plants should be received regularly. Unless the scientific staff is exposed to new publications, they cannot undertake basic research, which is essential for their growth. Some of the more important books and proceedings of symposia should also be obtained for the library. A few suggested journals are listed in annex III.
6. It is understood that an expert toxicologist will be sent to RDRL to streamline the chronic toxicity studies. Although biochemistry and histology are equally important in chronic toxicity studies, it may not be easy to get a person who is expert in both disciplines. If such an expert is available, he/she would be the ideal choice for training the local staff; otherwise, two experts should be fielded for one month each. One should be a biochemist and

the other a pathologist with experience of histology, since that is still a weak point at the laboratory.

Some members of the technical staff should also be assigned to other laboratories where facilities are available for short-term (two or three months) training, so that when they come back they can do the work with greater confidence.

Overall, RDRL could become a first-class research facility; however, it needs continuous financial support and some technical support in selected areas.

FINDINGS

To carry out biological screening a laboratory must have a supply of experimental animals, proper equipment and chemicals and trained staff. Each of these requisites will be discussed separately.

A. Animals

RDRL has an animal house with a limited capacity for the breeding and maintenance of small animals. Larger animals such as cats and dogs are not available. Screening for hypotensive activity is best carried out in cats and dogs, but these are unobtainable owing to religious sentiments. However, it is expected that dogs may become available when the local municipality's programme of catching stray dogs becomes operational. Until that time, screening is to be continued in rats, as at present. Once an active plant has been identified, however, it will have to be tested in animals of higher species as well as in hypertensive rats. At that point, it may be a good idea to consider collaboration with a laboratory that has access to such animals.

Anti-inflammatory screening is carried out exclusively in rats, so a large and continuous supply of these animals is needed. The capacity of the animal house to supply rats is limited by the scarcity of trained staff and funds. For proper research to take place, this deficiency must be remedied. Screening for anti-ulcer activity is also conducted in rats. An effort was made to carry out the screening in mice, but several experiments failed to produce ulcers in the stomachs of mice, which makes this species unsuitable for such work.

An abundant supply of guinea pigs is needed for the screening of plant extracts for bronchodilator activity. The guinea pigs that were available were used for a few experiments, but a larger supply is required for continuous work. Guinea pigs are also required for testing the ability of plant extracts to induce spasmolytic activity in isolated ilea. In the absence of guinea pigs, the isolated ilea of rats are being used, but this method is not as satisfactory.

The wound-healing properties of plant extracts can be tested in rabbits as well as in rats. A pilot experiment was thus set up using rabbits, but the test ointment was found to be inactive.

Analgesic activity can be tested in mice as well as rats, and mice have been successfully used at RDRL.

Conclusion

A much larger supply of rats, guinea pigs and mice is needed. Efforts should continue to get dogs whenever possible.

B. Equipment and chemicals

There are several deficiencies in the equipment at RDRL. Additional equipment is urgently required to carry out meaningful screening and to make optimum use of the existing equipment. A small respiration pump for rodents is needed for supporting respiration in rats and for setting up Konzott experiments that measure bronchial resistance in guinea pigs. The Laboratory has two large-capacity pumps suitable for cats and dogs, which are not obtainable, but no pumps for smaller animals such as rats. It also has a plethysmograph (Ugo Satile), but this instrument does not give a correct reading and needs

immediate replacement if anti-inflammatory screening is to be conducted properly. In addition, several other minor pieces of equipment must be provided to make the research work more efficient. A list of equipment suggested for procurement is presented as annex I.

Most of the chemicals that are used have to be imported, and a reliable supply is important for proper research. For instance, the Laboratory does not have Freund's adjuvant for inducing polyarthritis in rats, which is the most important model. Other chemicals are needed for routine work. A list of chemicals that should be on hand all the time is given in annex II.

Conclusion

Deficiencies in equipment and chemicals must be remedied to achieve optimum output.

C. Staffing

RDRL already has some trained staff to perform many experiments, but they are hampered in their work by the lack of some equipment and the inadequate number of animals.

Screening for hypotensive activity was already being conducted in rats. One problem was in the calibration of the polygraph pre-amplifier for recording blood pressure. The expert demonstrated the technique of calibration and explained the precautions that should be taken in recording blood pressure and heart rate, so now there is no problem with the use of the polygraph for these purposes. The problem of supporting respiration should it fail during the experiment can only be solved by acquiring a small respiration pump.

The screening of plants for anti-inflammatory activity cannot be carried out properly until a new plethysmograph becomes available. The subacute cotton pellet and granuloma pouch tests were demonstrated by the expert and can now be carried out by the local staff with confidence. The chronic adjuvant-induced polyarthritis test could not be demonstrated because no adjuvant was available, but the testing procedure was discussed and explained.

The tests for anti-gastric ulcer activity could not be demonstrated properly since too few rats were available and the test failed in mice despite several attempts. In one of the tests in rats, the pH of the gastric juice is monitored continuously. This requires a pH-meter with a sensitivity of up to three decimal places. Since no such instrument is available at the Laboratory at present, other methods are being considered. However, the tests described above have been discussed with the staff, who should be able to perform them when the equipment becomes available.

Detecting bronchodilator activity in guinea pigs requires a bronchospasm transducer. While the Laboratory had such a transducer, it did not have a small pump to deliver a fixed amount of air at every stroke. It also lacked a special glass cannula required for such experiments but since then has obtained one from the Central Drug Research Institute at Lucknow. Another problem with the use of the bronchospasm transducer was that it could not be satisfactorily connected to the Grass polygraph. The wires were opened and identified and a temporary connection made until a proper Cannon connector could be imported.

The wound-healing properties of plant extracts, applied locally or administered orally for a few days, can be tested in rabbits and rats. One experiment in rabbits was set up, but it could give only a qualitative idea of such activity. To get a quantitative idea, it is necessary to determine the breaking force of the healed skin, but the small pulleys needed for this are not available at RDRL. There are other methods of evaluating wound healing, but they are more sophisticated and require much more equipment.

The analgesic activity of aspirin-type compounds can be determined by a writhing test carried out in mice. Phenylquinone is a better chemical for inducing writhing than acetic acid, but since only 20 per cent of the animals respond to it, a large number of mice must first be screened for susceptibility. Thus, until animals become available in greater numbers, acetic acid has to be used to induce writhing, and this has been done successfully at the Laboratory. The analgesic activity of morphine-type compounds can be tested using an Eddy hot plate, which RDRL has. The Haffner tail clip method is much simpler, but proper clips must first be procured. In any event, the manpower for conducting these tests is available.

Work in isolated tissues has been going on at RDRL for quite some time. When the animal supply situation improves, it would be better to use guinea pig ileum for screening rather than rat ileum, which is being used at present. There were some problems in getting the contractions of isolated rat vas deferens. These problems have been identified and tackled, and the method is working now. In addition, mouse vas deferens were used for the first time. To get a good response to nerve stimulation, proper electrodes will have to be procured.

The testing of plant extracts for anti-fertility activity in females is proceeding in rats, but a really active plant has yet to be discovered. Similarly, anti-tapeworm screening is being conducted in mice. Screening for plants that are effective against other helminth infections like hookworm and roundworm should also be started, and suitable infected animals will be needed for this. If problems arise once the tests get going, then either an expert should be brought to RDRL or a staff member sent for training.

Conclusion

Some trained people are available at RDRL for setting up experiments. They will be more confident in their work when the proper tools become available and when they are further trained in a laboratory having all these facilities.

Annex I

EQUIPMENT RECOMMENDED FOR PROCUREMENT

Plethysmograph	1
Miniature respiration pump for rodents	1
Cook & Wideley pole-climbing apparatus	1
Electroconvulsimeter	1
Histometer	1
Perfusion pump with different settings	1
pH-meter	1
Electrical hair clipper with spare heads	1
Balance for weighing small animals up to 2 kg	1
Analytical balance, single-pan	1
Metabolic cages, small	25
Isolated tissue bath	1

Annex II

ESSENTIAL CHEMICALS AND SURGICAL INSTRUMENTS

<u>Chemical/instrument</u>	<u>Quantity</u>
Noradrenaline bitartrate	3 g
Adrenaline	3 g
Histamine diphosphate	4 g
Apomorphine hydrochloride	0.5 g
Amphetamine sulphate	25 g
Indomethacin	5 g
Urethane	1 kg
Pentobarbitone sodium	50 g
Phenylquinone	5 g
Trypan blue	25 g
Cyproheptadine	1 g
Ergometrine maleate	0.1 g
Isoproterenol	5 g
Methysergide	0.5 g
Papaverine hydrochloride	5 g
Propranolol hydrochloride	5 g
Yohimbine hydrochloride	5 g
Egg albumin	5 g
Adjuvant (Difco)	20 ampoules
Acetylcholine	10 g
Mepyramine maleate	5 g
Cimetidine	5 g
Chromoglycate sodium	5 g
Thick rubber tubing of different sizes (5 to 25 mm i.d.)	25 m of each
Polyethylene tubing of different sizes (1 to 5 mm i.d.)	25 m of each
Tracheal cannula for rat/guinea pig	2
Two- and three-way stopcocks (disposable)	50 each
Surgical instruments such as artery forceps, scissors (large and small), blunt dissectors, surgical blades and handle, forceps (small and large) etc.	
Carrageenan, lambda form, type 4	25 g

Annex III

JOURNALS RECOMMENDED FOR SUBSCRIPTION BY THE LIBRARY

Journal of Ethnopharmacology

Journal of Pharmacy and Pharmacology

Planta Medica

International Journal of Crude Drug Research

Indian Journal of Pharmacology

Indian Journal of Physiology and Pharmacology

Indian Drugs

British Journal of Pharmacology