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MODERN PHARMACEUTICAL FORMULATIONS BASED UPON THE THAI TRADITIONAL PHARMACOPOEIA
DP/THA/87/010

THAILAND

Technical Report: Findings and Observations*

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

Based on the work of Mr. Pitambar Somani, UNIDO Expert

Backstopping Officer: R.O.B. Wijesekera
Chemical Industries Branch

United Nations Industrial Development Organization

Vienna

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* This document has not been edited.

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The Thailand Institute of Scientific and Technological Research (TISTR)

November 1, 1989 to January 9. 1990

I. BACKGROUND

The background and major objectives of the program DP/THA/87/011/01 were explained in the interim report after the visit to TISTR in 1988. During the first mission, an intense and co-ordinated effort was devoted to develop various protocols for initial screening and identification of active plant extracts on the cardiovascular system. Several natural substances with potentially significant activity in the antihypertensive and cardiotonic screens were identified from the list of drugs with a long tradition of their successful use by the local practioner of Thai medicine in patients with these disorders. The infra structure was already established in the Pharmaceutical and Natural Products Division (PNPD) at TISTR, and external expertise to help the staff of PNPD in setting up detailed experimental protocols for screening and further evaluation of the plant extracts from the traditional sources was the main reason for appointing Dr. Somani as the UNIDO Technical Expert in Cardiovascular Pharmacology to help train the staff in this field.

II. OBJECTIVES

Based upon the recommendations in the interim project report and initial discussions with Mrs. Sasithorn Wasuwat, Director, PNPD, and her staff after arrival in Bangkok on November 1, 1989, the following objectives were agreed upon:

II.A. SETTING-UP OF THE CARDIOVASCULAR LABORATORY.

In the interim report, it was recommended that additional equipment be purchased immediately to be able to test in conscious rats the drugs already identified as active antihypertensives, and to expand the work on the isolated tissue (atria) preparation. The list of equipment was reviewed in details and the final list was prepared (see letter dated Nov 16, 1989, see Appendix I). All these items have been ordered, and it is hoped that the laboratory will be set up by March of 1990.

A detailed discussion was also held about the continuing rapid progress of the work with the lotus plant extracts (see below), and the need to set-up a full cardiovascular laboratory as soon as possible so that additional experiments to characterize the detailed cardiovascular pharmacology and mechanism of action of the active extracts necessary for planned clinical trials can be initiated without any undue interruptions. In the letter of Dec 13, 1989 (appendix II), it was recommended that funds be re-allocated as soon as possible to be able to purchase the necessary laboratory equipment for this laboratory, so that the proposed experiments can begin by summer of 1990

II.B. SCREENING OF THE THAI MEDICINAL PLANTS FOR CARDIOVASCULAR ACTIVITIES

The screening of the drugs from plants already identified to possess potential activities on the cardiovascular system, as discussed in my interim report of last year, should continue for the following activities:

1. Antihypertensive,
2. Cardiotonic,
3. Antianginal (antihypoxic)
4. Cholesterol lowering, and
5. Potassium supplement for heart diseases.

As reported earlier, the following plants have already been identified, and their extracts have undergone screening in the experimental models set up last year in PNPD:

1. Zingiber cassumunar (rhizome)
2. Nelumbo nucifera (lotus embryo)
3. Tinospora crispa L.(Borapet)
4. Cyperus rotundus Linn (Ya-Haw-Moo)
5. Allium sativum (garlic natura)
6. Zingiber officinale

II.C. FURTHER LABORATORY INVESTIGATIONS WITH NELUMBO NUCIFERA

Last year, the initial screen had provided us with a very interesting lead when it was found that the crude extract of lotus embryo had a short duration of antihypertensive and cardiotonic activity. Further work with subfractions of the embryo extracts was planned during the rest of 1989. It was also decided to test the crude extract of the lotus leaves, since it was hypothesized that the embryo would be in short supply and it may be possible that the leaves also have cardiovascular activities. The crude extract of the lotus leaves was tested during 1989 for antihypertensive and cardiotonic activity, and both activities were discovered. The objective during this mission was to further characterize the cardiovascular profile of the lotus embryo and leaf extracts, and to attempt to obtain pure fractions for both pharmacological workup and chemical identification.

II.D. STEPS NECESSARY TO DEVELOP A DRUG FROM INITIAL SCREENING TO THE FDA APPROVAL.

Since a new drug development requires extensive pharmacological and toxicological testing, followed by clinical trials, a well planned approach is necessary to bring these drugs into market. Adequate plans should be drawn for each group of drugs, including the relative position with respect to currently available drugs, as well as the use of adequate control experiments in their pharmacological testing, and clinical pharmacological evaluation. Although the Thai traditional drugs have been used extensively for many years, if not many centuries, their active constituents still must receive the same degree of extensive preclinical pharmacological and toxicological workup as any new synthetic drug from modern medicine.

III. REPORT

CARDIOVASCULAR ACTIVITIES

The following experimental protocols for screening the cardiovascular activities of the Thai medicinal plants were established to continue necessary work with these drugs during the next 12 months. As the expert technical mission was split into two parts and the preliminary report from the first phase has already been submitted, the work that was accomplished while during this second phase of the visit is being described in details, and additional work for the next 12 months which will continue to be carried out at PNPD is being reported here along with recommendations.

III.A. ANTIHYPERTENSIVE ACTIVITY.

(A). Anesthetized rat experimental preparation. This screening model was set up last year and will be continued this year also for direct measurement of blood pressure. However, there are several major limitations of this protocol, such as only one drug may be tested per animal; only intravenous activity can be tested; the duration of action may not be very long; we would not know if the drug is orally active (as is necessary for patients). However, the major advantage of this screen is that a direct measurement of blood pressure identifies drug activity and one does not have to worry whether or not the drug is absorbed when given by mouth. In this method, male adult wistar rats weighing 275-300 gm were anesthetized with 40 mg/kg i.p. pentobarbital sodium, supplemented with urethane (.875 mg/kg i.p.). Additional urethane was given as necessary. The femoral vein was cannulated with a fine polyethylene tube for injection of drugs. The carotid artery was cannulated for recording of the systolic and diastolic blood pressure via a strain gauge transducer. Heart rate was counted from the blood pressure tracings. The arterial cannula was filled with heparin (50 units/ml) saline solution.

Protocol. In each experiment, the drug was prepared in an appropriate solvent, and the effect of 3-4 doses of each drug was investigated in each animal. The response with a standard drug, i.e.,epinephrine (adrenaline, 1-2.5 ug/kg) was first obtained to establish that the animal was showing normal responsiveness to the drugs.The effect of the solvent was also tested, followed by the administration of the experimental preparation. No drug was given at less than 10 min interval between each dose.

Observations. The following six drugs were tested in the anesthetized animals, and the results with each Thai plant extract were described in the expert's report last year and are summarized below, along with new data obtained during the last 2 months:

1. Nelumbo nucifera (Lotus). The crude extract obtained from the embryo of the lotus seed, which has been identified to contain several active principles, including neferine and isoliensinine, was injected intravenously in the dose range of 3.2 to 12.8 mg/kg, and was found to produce a dose-dependent decrease in both systolic and diastolic blood pressure. These preliminary results clearly showed that the extract of this Thai plant has potential antihypertensive activity, and definitive experiments need to be carried out to extend these observations, including the mechanism of action, oral activity, role of its active ingredients etc.

Since hypertension is a very common clinical disease, during the second phase of the mission, efforts were concentrated to establish the antihypertensive profile in order to decide whether this drug is worth pursuing in view of the availability of many other imported synthetic drugs to treat hypertension. As the antihypertensive action of the lotus embryo crude extract was of short duration (appr. 5 min after intravenous injection), and the source of the material is very limited,the problem was tackled in two different directions. First, in order to increase the potency, and to attempt further purification of the lotus embryo, methanol, hexane, chloroform and ethyl acetate extracts of the embryo were prepared and except for the ethyl acetate extract, all three subfractions showed powerful cardiotonic activity. Their antihypertensive potencies still need to be established.

The second approach was to test whether the cardiotonic and antihypertensive activities could also be found in the lotus leaf, since this may provide an abundant source of active drugs if indeed this were the case. The crude extract of the lotus leaf exerted both cardiotonic and antihypertensive actions. The four subfractions of the crude leaf extract were prepared in order to further characterize the activity of the lotus leaf. The results were even more interesting than originally expected, and are summarized as follows:

PREPARATION	CARDIOTONIC activity	ANTIHYPERTENSIVE activity
Crude extract	yes	yes
Methanol "	yes	yes
Hexane "	no*	yes
Chloroform	negative	---
Ethyl acetate	no	---

* it has only negative chronotropic action

Clearly then, the above results with these extracts suggest that the crude extract must have at least three different, if not more, active chemical constituents, and it may be possible to separate the antihypertensive from cardiotonic action (Hexane extract). As mentioned earlier, a new antihypertensive drug from the Thai traditional plant would be very desirable, and the above described ***observations are indeed original, since there are no reports in the literature describing antihypertensive activity in the lotus leaf extracts. It is quite possible that others may not have discovered this very important activity either because they have not tested the lotus leaf or because the crude extract has only transient activity which may have been masked by the cardiotonic component, and therefore failed to interest other investigators.***

Several series of additional experiments were carried out with the hexane extract, and the results have been exceedingly exciting. The dose-response activity showed that a decrease in blood pressure was seen in a dose dependent manner from 3.1 to 50 mg kg IV. The duration of action was at least 2 hr (and may be even longer than 3 hours) as the diastolic blood pressure still is lower than control at this time after a single 12.5 or 25 mg/kg IV dose. Oral anti-hypertensive activity may be even more prolonged, which would be possible to test as soon as we receive the equipment for measurement of blood pressure in conscious rats. It was also observed that

the prolonged decrease in blood pressure was more of the diastolic than of the systolic pressure, which would be extremely desirable in clinical practice. There was no increase in heart rate at the time blood pressure was lowered, again a very desirable feature of this drug for possible use in patients. Further experiments showed that the antihypertensive action of the hexane extract is not due to some of the common mechanisms by which other drugs are known to lower blood pressure (histamine release; alpha or beta adrenergic blocking action; cholinergic action). All these experiments have been performed in anesthetized rats, and at this time no additional work on the detailed cardiovascular actions in larger animals or on the other mechanisms of blood pressure lowering activity is possible without a complete cardiovascular laboratory set up of the type recommended in the letter of November 16, 1989 as well as in the preliminary report from last year's visit.

During the last four weeks, Miss Wilaiporn Chamchaang, Ph.D., who recently returned from Cincinnati, Ohio, has attempted to prepare chemically pure fractions of the hexane extract using a preparatory column. From the limited amount of the crude starting material available, she was able to obtain two fairly pure substances for testing in the rat experiments. Fraction A had no antihypertensive action, but Fraction B which was tested in one animal (due to very small amount of the drug) appeared to exert prolonged antihypertensive action similar to that of the crude hexane extract. These preliminary observations are highly exciting and, if confirmed in further experiments, these results will allow further work with a chemically pure drug for additional pharmacological and structure-activity (medicinal chemistry) studies.

2. Tinospora crispa L. This is a newly identified drug in Thai traditional medicine, and at present no special clinical use has been ascribed to this drug. The extract of this plant was tested in the rat model, and a very gradual but striking decrease in both systolic and diastolic blood pressure was observed. Further experiments were carried out in two control and two drug-treated rats where a single dose of the extract was injected to determine the extent and duration of the antihypertensive action. It was found that unlike the effect of the solvent in control experiments, the extract of Tinospora produced a slow decrease in blood pressure which was maximum in 10-20 min with 20-30 min duration.

Further work with this plant was carried out with various chemical subfractions, but it appears that additional work will be necessary since unlike the crude extract, the subfractions (methanol, hexane, chloroform or ethyl acetate) did not produce any antihypertensive action in the anesthetized rat model. This should be the next drug (after lotus) to study in further details in the next phase of this project.

3. Ginger Oleoresin. This extract from the rhizome of the plant Zingiber officinale produced a moderate increase in both systolic and diastolic blood pressure in a dose dependent manner. The maximum increase in blood pressure was 20-30 mm/Hg, and the duration of action was from 5 to 10 min. There were no changes in heart rate in the doses tested.

Although these preliminary results show biological activity with ginger oleoresin on the cardiovascular system, with the limited time and resources available at present, further work with this drug was at a lower priority, and no additional work was performed during 1989.

4. Terpinen-4-ol. This drug derived from Zingiber cassumunar rhizome when given to the anesthetized rats produced a dose dependent decrease in both systolic and diastolic blood pressure but no further work was carried out with this drug during 1989 (low priority).

5. Cyperus rotundus. Eating the bulb of this plant is believed to keep a person very healthy and looking very young despite increasing age. The extract of the bulb of Cyperus rotundus was injected intravenously in two rats, and although a slight increase in both systolic and diastolic blood pressure was observed, there was no dose response relationship. Although it may be possible that further experiments in the models for hypertension or in the model to study its effects on myocardial hypoxia may show some cardiovascular activity, at present its effect on blood pressure appear to be minimum and no further experiments were performed in 1989.

6. Garlic natura. This water soluble extract of garlic bulb is quite different from the traditional garlic oil, since many of the essential oils are removed in the spray-drying process of its preparation. Intravenous injection of this preparation did not result in any acute change in blood pressure, but a careful analysis of the records suggested that with each dose, both systolic and diastolic blood pressure were slightly lower at about 5 min after the injection as compared to the pre-drug pressures. Further experiments were then carried out in two additional rats where only a single large dose (12.8 mg/kg) of Garlic natura was injected. As compared to the solvent controls, it appeared that Garlic natura was able to induce a slow and gradual decline in both systolic and diastolic blood pressure which was maximum between 20-30 min. Although further work was recommended with Garlic natura as well as with its odorless spray dried preparation, no additional experiments were carried out in 1989.

(B). Conscious rat experiments. The PNPD staff is in the process of setting up the screening technique where conscious normotensive as well as hypertensive rats will be utilized to study the effect of previously identified active drugs. The methodology has been discussed with the staff, and they are now awaiting the arrival of the instruments ordered in December of 1989. The following models of hypertension, similar to those in man, may be utilized for further work with active drugs (e.g. lotus extracts; tinospora extracts):

Spontaneously hypertensive rats (SHR)
DOCA-induced hypertension
Renal hypertensive rats.
Salt-sensitive rats (work may be carried out in the cardiovascular expert's lab)

The staff is not ready as yet to work with these models, but the SHR and DOCA rat models should be the easiest to work with, and such rats can be ordered from commercial sources as soon as the technique is operational. ***As explained in the letter of Dec 8, 1989, if needed, the Cardiovascular expert may be invited back for a short visit in 1990.*** In all the above models of hypertension, the dose-response and duration of action will be established for the active drugs (as compared with the solvent placebo) derived from the Thai traditional plants. Other Thai traditional plant extracts also will be screened in these conscious rats.

III.B. CARDIOTONIC ACTIVITY

Experimental preparation. Guinea pig atrial preparation. The standard technique for the isolated guinea pig atrial preparation was set up and utilized in the present project. After removing the heart quickly after stunning the guinea pig, the atria were removed and placed in an oxygenated buffer solution (Kreb-Hensleit) at 36 C. The atria were attached to the force displacement transducer with a fine silk thread to record the rate or force of contraction.

Protocol. Right atria. The right atria has the sinus pacemaker activity which allows it to beat spontaneously at a rate of between 150 and 180 min. Thus, the effect of a drug under study on heart rate can be investigated in this preparation. In order to compare the effect of an unknown drug from the plant source, it also is important to show that the preparation has a normal response to a standard drug. In each experiment, after the atrium has been allowed to stabilize, response to isoproterenol (5X10-8) is first obtained, the peak changes normally occurring in 1-2 min. After several washes, the test drug is added to the tissue bath at 5 min intervals, and the effects of increasing drug concentrations are tested in the same preparation to obtain data for plotting the dose-response relationship on heart rate.

Left atria. Since there is no spontaneous pacemaker activity, the left atria is electrically stimulated at a fixed rate of 120 beats/min, and in this preparation the effect of the given drug on the force of contraction of the heart (cardiotonic activity) is investigated. Here again, the response to the control drug, isoproterenol is first obtained, followed by 2-3 washes, and then the effect of the drug under study is determined at 5 min intervals in increasing concentrations, unless the duration of action of the drug is long, in which case we must wait for an adequate time until the original force of contraction has returned to the baseline. The main advantage of using the paced atria is that the indirect effect of a change in heart rate on cardiac contractility is eliminated. Thus, each drug must be independently examined on the rate (right atrium) and force (left atrium) of contraction.

Observations. In view of the limited time available, it was decided to first establish the technique and then if time permits, to test the effect of one or more of the Thai plant extracts described above. In very preliminary experiments, it was only possible to test a very limited dose range of the extracts.

(1). Nelumbo nucifera. Some very interesting results were obtained with the extract of Nelumbo nucifera (lotus), which produced an increase in the force of contraction but no change in the heart rate. The positive inotropic action was seen with concentrations as low as 0.5 ug/ml. This was the most exciting observation, since to our knowledge, no report in the literature has described the selective positive inotropic action of Nelumbo extract on the heart. During the last two months, this observation was confirmed and extended further, and the effects of various concentrations of several different subfractions were examined in this laboratory. Preliminary studies on possible mechanisms of action were also investigated during this phase of the expert mission. However, which of the known active principle has the cardiotonic activity still needs to be established. The chemical structure of the active principle also needs to be determined. As mentioned earlier (p 5), the hexane extract is devoid of the cardiotonic activity, and has only a

negative chronotropic (i.e. decrease in heart rate) action. This work will begin in 1990, but it should continue in the phase III (renewal) of this project.

(2). Other drugs. In the preliminary report, it was reported that 5 other Thai natural products were screened in this preparation, but the results were not very exciting, except for the negative effects of Garlic natura and Cyperus rotundus, and no further experiments were carried out in 1989. These two drugs may have some unique antihypoxic effects during myocardial ischemia, but this needs to be established in future utilizing the experimental model described below.

III.C. SCREENING FOR ANTIANGINAL/ANTIHYPOXIC ACTION

Experimental preparation. The isolated guinea pig left atrium paced at a constant rate of 120 beat/min as described above was utilized in this experimental model to test the potential antianginal/antihypoxic activity of the Thai natural products. Details of the technique, which was demonstrated to the staff, and its justification, were provided in my preliminary report from last year. The effect of various extracts of the Thai natural products, including the lotus, tinospora, Cyperus rotundus and garlic natura, need to be investigated in this preparation, but time was too short to carry out any of these experiments in 1989. This work may be possible during the phase III continuation of this project.

III.D. ANTIARRHYTHMIC ACTIVITY

Although time did not permit to actually demonstrate the technique for screening of the antiarrhythmic activity of the Thai traditional drugs, especially in view of a lack of sophisticated instruments, e.g., even a simple two channel stimulator, details of the screening procedures for future use were discussed. A very simple screening test is to study their action on the relative and effective refractory periods in the isolated guinea pig atria, a technique already established here. The other screening method is ventricular fibrillation induced by barium chloride in mice. Both these screening methods may be demonstrated during the next phase of the expert mission.

III.E. SCREENING FOR CHOLESTEROL LOWERING ACTIVITY

Since there is such a world wide interest in the cholesterol lowering activity of natural products, including oat bran, garlic, guar gum, chitosan etc., and the cholesterol lowering activity of Garlic Natura has been confirmed both in the experimental animals (by Dr. S. Nitya Anand at CDRI, Lucknow, India) and in the human volunteers in Thailand, much discussion was held to develop an experimental model. The planned screening experiments will be carried out in male Sprague-Dawley rats divided into groups of 10 rats each fed high cholesterol diet (Jennings et al. A comparison of the lipid lowering and intestinal morphological effects of cholestyramine, chitosan and oat gum in rats. Proc. Soc. Exper. Biol. & Med. 189: 13-20, 1988). The major emphasis will be to compare the effects of garlic oil, Garlic Natura, and the new odorless Garlic Natura preparation developed at TISTR. If the new odorless Garlic Natura is as effective as Garlic Natura in lowering serum cholesterol and increasing HDL cholesterol then its world wide acceptance will be much better than other garlic preparations. Other natural products (e.g. Nilumbo nucifera extracts, tinospora) also will be screened in the same experimental models.

The experimental models for screening for cholesterol-lowering activity were discussed in details, and the protocols have been agreed upon. One such model will include testing the effect of drugs given to rats fed a high cholesterol diet for 2-3 weeks, and then dividing the animals into placebo and drug treatment groups. In another model, Triton will be used to elicit an increase serum cholesterol. Appropriate control experiments with known cholesterol lowering drugs already in the market will also be carried out. This project is expected to begin in January 1990 as soon as the animals arrive, and the high cholesterol diet with vitamin-mineral additives is received.

III.F. POTASSIUM SUPPLEMENT FROM NATURAL SOURCES

Most patients with hypertension and heart failure receive potassium supplements by mouth, since they are either on low sodium diets or are given diuretics which cause loss of potassium from the body. Most potassium supplements are inorganic compounds with very poor patient compliance because of unacceptable taste. It would be desirable to develop an alternate source of potassium supplement which is wholly derived from the natural source. Musa Sapientum (banana) is readily available in Thailand, and is known to have high potassium content; however, it also has very high amount of carbohydrates which cause patients to gain weight. Attempts will be made to compare the mineral contents of bananas with three grades of ripeness: raw, intermediate and very ripe, and to explore the possibility of retaining the high fiber content but eliminating digestible carbohydrates.

III.G. STEPS NECESSARY TO DEVELOP A DRUG FROM INITIAL SCREENING TO THE FDA APPROVAL

Several sessions were held with the staff of PNPD to review the general requirements of the FDA on the pharmacology and toxicology of a new drug, including possible mechanism(s) of action, before clinical trials are permitted. Brief details of the process necessary for the approval of the Institutional Review Board (IRB) before starting the clinical trials were discussed. As pointed out in letters dated Nov 16, 1989 and Dec 13, 1989, the necessary experimental work required and the time frame from initial discovery of a potentially new drug in the primary screen to the final approval of the drug by the FDA was discussed with the PNPD staff. This usually takes any where from 5-7 years with extensive support.

IV. PROPOSED ACTIVITY FOR THE NEXT 12 MONTHS

IV.A. DETAILED CARDIOVASCULAR WORKUP OF THE LOTUS EXTRACTS

The most exciting finding of the project thus far has been the discovery of the antihypertensive and cardiotonic activity of the lotus extracts. As reported on page 4, detailed investigations with the subfractions of the lotus leaf and embryo extracts showed that more than one chemical may be involved with different pharmacological activities, and that at least the hexane extract has the best potential for yielding a very useful antihypertensive drug. During the next year, the crude extract as well as the chemically pure fraction (Fraction B; see page 6) needs additional studies to establish the pharmacological profile of the drug which is necessary before the drug can be tested in man. Some of the proposed experiments outlined below can be started with the crude extract, but it will be necessary to perform similar experiments with the pure chemical (Fraction B) whose structure should be established as quickly as possible.

(1). Mechanism of antihypertensive action. Preliminary experiments have already shown that the antihypertensive action is not via alpha or beta adrenergic, cholinergic or histaminergic mechanisms. Further experiments in isolated tissue (aortic strips) as well as whole animals (cat and dog) will be required to determine whether it acts as a direct vasodilator or by some other known mechanism (central nervous system action; renin-angiotensin mechanism; Ca channel blockade; ganglionic blockade etc.).

(2). Detailed antihypertensive actions. Since the initial screening was performed in normotensive rats only by intravenous route only, it will be necessary to establish whether this drug will be able to lower blood pressure in several different models of hypertension, and that the drug is active when given by mouth. Duration of action is also very important, and this should also be established in the conscious rat models of hypertension (see page 7).

(3). Detailed cardiovascular actions. The direct effects of the hexane extract or pure Fraction B on the cardiovascular function need to be established as compared to some of the known antihypertensive drugs (e.g. propranolol, verapamil) on cardiac output, cardiac contractile force, heart rate, and blood flow to the coronary, renal and other vascular beds. Such a workup is necessary before the doctors will be willing to try this drug in man, and in the US, this type of detailed data are included in the IND application before clinical trials can be started.

(4). Toxicity and other pharmacological actions. Drug toxicity (LD50, acute and chronic toxicity) as well as a general profile of its actions on various systems in the body, including CNS function, as part of the IND work up will be required in future. It also will be necessary to study many of the biochemical actions of this drug in general and on carbohydrate and fat metabolism in particular.

(5). Structure-activity relationship. Once the active principle in the hexane fraction has been characterized, it may be necessary to decide on synthesis of various analogs of this drug, and to study the structure-activity relationship. Such studies are necessary to prepare a whole series of potentially useful drugs, and to choose the best possible drug (based on potency, safety and duration of action) from this group of compound for future studies. This type of work is also necessary for ***patent application.***

IV.B. CONTINUING SCREENING FOR CARDIOVASCULAR ACTIVITY

Experiments will be continued in anesthetized Wistar rats as well as in conscious genetically hypertensive rats (SHR) for screening of the antihypertensive activity as described above. As before, in the anesthetized rats, at least three to five doses of each new plant extract will be injected intravenously at 10 min intervals after an initial response to a control dose of adrenaline shows that the preparation is stable. Screening for the cardiotonic action will also continue in the isolated guinea pig atria as described above. In conscious rats, a single dose of the plant extract will be given by mouth (oral feeding) and the blood pressure will be recorded from the tail artery by the inflatable cuff and pressure transducer.

If any plant extract shows an antihypertensive action, an appropriate dose of this drug will be selected for additional experiments to determine the maximum response as well as the time course and duration of

antihypertensive action. Similarly, further experiments will be performed for drugs with cardiotonic, antianginal or antiarrhyt mic activities. The following plants have been recommended for these experiments, the priority of their testing to be determined by their availability and literature review:

*1. ACANTHUS ILICIFOLIUS (stem and leaf)

*2. ALPINIA CHONCHIGERA

*3. ALPINIA GALANGA

*4. ANDROGRAPHIS PANICULATA

5. CEREBERA MANGHAS

6. C. OLOLLAM

7. DRYOBALENOPS AROMATICA

8. ERIOBOTRYA BENGALENSIS

@9. HYOROPHYTUM FARNICARIUM

10. MANSONIA GAGEI

11. MUSA SAPIENTUM

#12. NELUMBO NUCIFERA

13. OCHROCAPUS SIAMENSIS

*14. PANADUS ODORUS

15. RHEMANIA GLUTINOSA

*16. SALIX TETRASPERMA

17. STERCULIA LYCHNOFORA

#18. TINOSPORA CRISPA (L.)

@19. UNCARIA FERREA

plants already screened and show activity
* plants that will be screened in 1990
@ plants which may be difficult to procure

It may be pointed out that the selection of these plants is recommended (please see the Midterm Technical Report by Dr.Nitya Anand, Dec 9-19,1988) after a careful review of the literature as well as consultation with the Ayurved Vidhayalai, Bangkok. However, it may also be emphasized that many of these plants were not easily available in 1989, and therefore the screening may be possible only for those plant extracts which can be located and procured in 1990.

IV.C. DETAILED ANTIHYPERTENSIVE ACTIONS

Detailed antihypertensive activity of the following drugs which were identified to lower blood pressure in preliminary screening (see above) will be performed to confirm and extend the dose response relationship, duration of action as compared to the vehicle control:

Tinospora
Garlic natura (to be tested at CDRI, Lucknow)

Oral activity of these drugs also needs to be established in further experiments. After performing these experiments, appropriate statistics will be applied to the results.

In case of Tinospora, attempts should be made to perform chemical identification of the active principle(s), since the extract is known to be composed of many alkaloids, and other substances. The antihypertensive activity of the pure chemical components of Nelumbo nucifera still needs to be tested.

After these experiments have been completed, detailed studies on the mechanism of antihypertensive action of these drugs will be necessary, and these may be similar to those with the lotus extracts.

IV.D. DETAILED CARDIOTONIC ACTIONS

Preliminary results with the crude and methanol extract of Nelumbo nucifera suggest that it has a cardiotonic action. These observations need to be extended further in the following series of experiments:

a. Detailed concentration-response relationship study,
b. Effect of prolonged exposure on the cardiotonic action,
c. Effect of different concentrations on heart rate,
d. Possible mechanism of action,
e. Chemical identification; possible separation of anti-hypertensive from cardiotonic action.

IV.E. CHEMICAL PURIFICATION AND IDENTIFICATION

As with the lotus extracts, it would also be necessary to carry out chemical subfractionation and characterization of the active principle(s) in tinospora and cyperus rotundas materials.

V. CRITIQUE AND RECOMMENDATIONS

As described in the preliminary report last year as well as continued personal observations during this second mission, it is clear that a very impressive array of important contributions have been made by the staff of PNPD under the able leadership of Mrs. Sasithorn Wasuwat. ***Since there is so much renewed world wide interest in the natural products, every effort should be made to continue this very important work.*** As we all know very well from previous experiences with modern drug discovery that an immense amount of concerted effort must be maintained for many years before a drug can be brought to the market for general use. It takes many painstaking years to assemble a very well trained team of scientists in several fields to work together on drug development, and now that this team is in place in PNPD at

TISTR, their combined efforts will bear fruit when new products from the Thai traditional medicine are ready for clinical testing in treatment of cardiovascular diseases. ***The current status of the project, as can be seen from this progress report and planned activity in 1990 and beyond, clearly shows that an excellent opportunity exists for the discovery of a unique new drug which may soon be ready for clinical trials if continuing support is provided in terms of both manpower and resources.*** This project should therefore be renewed for three additional years.

The present technical staff in pharmacology is well trained in general pharmacology, and are very competent and hard working. Several members have also received specialized training abroad to enhance their competence in various aspect of drug development and this must be continued. In addition, the present team should be designated for "cardiovascular drug development" since they have now learned special skills in the last two months and there are at least two exciting drugs that need to be thoroughly investigated. The present three members of the team (Mrs. Pattama, Miss Chuleratana, and Miss Tuanta) will be very busy in detailed cardiovascular work up of the two promising leads (Nelumbo extract, and Tinospora extract) and they may find it difficult to continue screening new drugs in their laboratory. Screening of the Thai plants identified above may be assigned to two new technical staff members who should be hired as soon as possible, and their full responsibility would be to continue screening for the cardiovascular activity of new drugs.

Facilities. Facilities for screening cardiovascular drugs are at a bare minimum, and unlike other similar institutions in other developing countries such as CDRI, Lucknow, India and most drug companies, only one drug in one animal can be screened per day. There is also no provision to test the oral activity of any drug in unanesthetized animals. Once the screening phase is over, a lot more sophisticated cardiovascular work up would be necessary to provide detailed information on the activity of the selected compound in a variety of experimental animal preparations. For example, once a drug is found to lower blood pressure in normal rat screen, further experiments will be necessary to test its activity in various experimental models of hypertension. Plans should be made now to prepare for this expanded work load in the very near future, since it often takes 1-2 years before the instruments arrive and the personnel can be fully trained. Recommendations to upgrade the equipment have been made in the preliminary report of 1988 as well as in letters of Nov 16, and December 13, 1989.

VI. THAILAND INSTITUTE OF SCIENTIFIC AND TECHNOLOGICAL RESEARCH

Many meetings and discussions were held with the following:

Dr. Smith Kampempool, Governor
Mrs. Sasithorn Wasuwat, Director, PNPD
Dr. Sunthorn Tandhanand
Mr. Taweesak Suntorn
Mrs. Pattama Soontornsaratune
Mrs. Siripen Jarikasem
Miss Archaraporn Punruckvong
Dr. Montree Attatippaholkun
Mr. Jakkarapong Limpanussorn
Mr. Pongpreeda Pramoj
Mrs. Pattra Ahmadi P.

Miss Natthamas Phootsree
Miss Puttarin Wannissorn
Miss Chuleratana Banchonglikitkul
Miss Banjongjit Mahintratep
Miss Anong Chaichumroen
Miss Tuanta Sematong
Miss Patchree Samanasena
Miss Sirinan Jantorn
Miss Chularatana Chanchana
Miss Saipin Sanghirun
Mrs. Chantara Phoonsiri
Dr. Wilaiporn Chamchaang
Miss Penjai Sematong

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