STUDY ON TRADITIONAL MEDICINE-MODERN DRUG INTERACTION AND ITS MOLECULAR MECHANISM ELUCIDATION IN RAT LIVER

by

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In the name of ALLAH
The most beneficent and merciful

Dedicated to my wife AWATEF
and my son FERAS
for their love, patience, devotion and understanding.

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ABSTRACT

Traditional medicines have been used for thousands of years in maintaining health as an alternative to or in conjunction with modern medicines. A majority of the world's population in developing countries take herbal medicine to meet their health needs following low cost traditional beliefs and multiple uses of practice adopted by their elders and ancestors. Most of traditional herbal preparations that we know of today usually comprise of one or more plants in different formulation. Despite their popularity, the active principles, mechanism of action and effectiveness of these preparations remain largely unknown. Nevertheless, they are used widely in Malaysia and other countries.

Many studies have been done to find out the efficacy and side effects of these preparations but most studies used crude extracts or a single purified compound. This does not give a clear picture of its activities as the herbal/traditional preparations are normally concoctions of more than one plant/plant products. It is possible that the effects of traditional preparations are a mixture of effects from all the plants used in the preparation. During the course of therapy, there is concern for some interaction between modern medicine and traditional herbal preparations.

The aim of this project is to study the effect of two herbal products sold in the Malaysian market under the name **AIR JAMU PAK TANI®** and **FAIZOL UBAT BATUK®** on the metabolism of aminopyrine in isolated rat hepatocytes. The influence of factors such as age, sex and diseases (diabetes and hypertension) on the effect of these herbal products on aminopyrine metabolism were also investigated. Diabetes, induced in normal rats using streptozotocin (60 mg/kg, i.v.) and spontaneously hypertensive rats (their blood pressure higher than 160 mmHg) were used to investigate the effect of diabetes and hypertension on aminopyrine metabolism under the influence of these herbal products. *In-vitro* study was carried out using $6x10^3$ hepatocytes, 250 mmol/L aminopyrine and incubation time of 18 minutes. AIR JAMU PAK TANI® or FAIZOL UBAT BATUK® dilutions were added to petri dishes containing aminopyrine, hepatocytes and incubation medium with final volume of 10 ml. In the case of *in-vivo* study (FAIZOL UBAT BATUK® only) rats were orally fed with several dilutions of FAIZOL UBAT BATUK® every 8 hours over a period of one day (acute *in-vivo* study) and over a period of seven days (sub-acute study) before sacrificed. Termination of the reaction was done by the addition of 25% $ZnSO_4$ solution followed by the addition of saturated $Ba(OH)_2$. The absorbance that was measured towards the complex 3,5-diacetyl-1,4-dihydrolutidine from the experiment was used to determine the concentration of formaldehyde formed (as metabolite of aminopyrine) during the experiment which can be used later to be determine the activity of the enzyme aminopyrine N-demethylase.

To study the molecular mechanism through which these herbal products may influence the metabolism of aminopyrine, certain cellular inhibitors/stimulants were pre-incubated with the hepatocytes for 15 minutes prior to the final incubation of aminopyrine with these herbal products FAIZOL UBAT BATUK® and AIR JAMU PAK TANI®.

In-vitro study of AIR JAMU PAK TANI® demonstrated that AIR JAMU PAK TANI® could enhance the N-demethylation of aminopyrine indicating that there is a direct effect of AIR JAMU PAK TANI® on liver aminopyrine metabolism. Effect of AIR JAMU PAK TANI® on the aminopyrine metabolism was found to be age, sex and disease dependent. Certain second messenger pathways (such as cAMP, cytochrome $P450_{1A2}$, phosphatase, G-proteins, Ca/CaM-dependent protein kinase and protein tyrosine kinase) were investigated to see the mechanism through which AIR JAMU PAK TANI® could enhance the aminopyrine metabolism.

Our data demonstrated that the molecular mechanism of AIR JAMU PAK TANI® involve various second messenger pathways. This is not surprising since AIR JAMU PAK TANI® consist of more than 10 plants and the effect of AIR JAMU PAK TANI® seen is most probably attributed to the summative effects of all the chemical components found in the whole preparation.

In the case of FAIZOL UBAT BATUK® *in-vitro* hepatic interaction with aminopyrine resulted in the increase in the metabolism of the latter (most probably ascribed to the total chemical components which consist of at least six plants or plant products). Sex, age and disease factors are found to influence the effect of FAIZOL UBAT BATUK® on aminopyrine metabolism.

In-vivo study (acute and sub-acute) of FAIZOL UBAT BATUK® may suggest that a possible hepatic interaction with aminopyrine does occur inside the body resulting in increase or decrease in the metabolism of aminopyrine. This hepatic interaction was found to be age, sex and disease dependent. The effect of FAIZOL UBAT BATUK® on aminopyrine metabolism seen *in-vivo* but not *in-vitro* suggest that FAIZOL UBAT BATUK® does not have a direct effect on the liver aminopyrine N-demethylase activity. It's effect on the latter could be via activation or inhibition of other functioning physiological system. The induction of cytochrome P450 synthesis is possible but remain to be investigated.

Both acute and sub-acute *in-vivo* studies indicate that FAIZOL UBAT BATUK® is able to increase phase I aminopyrine metabolism in rat liver. The mechanism through which it is mediated may differ if it is orally administrated over a period of one day or over a period of seven days.

Kajian Interaksi Ubat-Ubatan Tradisional-Drug Moden Dan Pencirian Mekanisme Molekul Didalam Tikus

ABSTRAK

Ubatan tradisional telah digunakan beribu-ribu tahun untuk penjagaan kesihatan sebagai alternatif atau bersama-sama dengan ubat-ubatan moden. Sebahagian besar daripada penduduk dunia di negara-negara membangun menggunakan ubat-ubatan tradisional untuk menjaga keperluan kesihatan mereka kerana perbelanjaan kos yang rendah dan kegunaan nya yang pelbagai yang telah diamalkan oleh orang-orang tua dan keturunan mereka. Sebahagian besar daripada sediaan herba tradisonal yang kita ketahui mengandungi satu atau lebih tumbuh-tumbuhan didalam berbagai-bagai formulasi. Walaupun ia masyur, prinsipal aktif, mekanisme tindakan dan keberkesanan sediaan-sediaan tersebut masih belum diketahui.

Banyak kajian telah dijalankan untuk mengkaji keberkesanan dan kesan sampingan sediaan-sediaan di atas tetapi kebanyakkannya menggunakan ekstrak mentah atau bahan-bahan sebatian tulin. Ini tidak menggambarkan aktiviti sebenar kerana sediaan-sediaan herba/tradisional kebiasaannya adalah campuran lebih daripada satu tumbuhan/produk tumbuhan. Berkemungkinan besar kesan-kesan sediaan tradisional ini adalah hasil daripada kesan campuran kesemua tumbuhan-tumbuhan didalam sesuatu sediaan. Didalam sesuatu rawatan terdapat kebimbangan berlakunya interaksi diantara ubat moden dengan sediaan ubatan tradisional.

Tujuan projek ini ialah untuk mengkaji kesan dua produk herba yang dijual di pasaran dibawah nama AIR JAMU PAK TANI® dan FAIZOL UBAT BATUK® terhadap metabolisme aminopirin didalam hepatosit tikus terasing. Pengaruh beberapa faktor seperti umur, jantina dan penyakit (diabetes dan darah tinggi) keatas kesan produk herba tersebut terhadap metabolisme aminopirin juga telah diselidiki. Diabetes telah diaruh didalam tikus normal dengan menggunakan streptozotocin (60 mg/kg, i.v.) dan tikus SHR (tekanan darah sistolik melebihi 160 mmHg) telah digunakan untuk mengkaji kesan diabetes dan darah tinggi terhadap metabolisme aminopirin dibawah pengaruh produk herba tersebut. Kajian *in-vitro* telah dijalankan menggunakan 6 x 10^3 hepatosit, 250 mmol/L aminopirin dan waktu eraman selama 18 minit. Beberapa pencairan produk herba di atas telah ditambah kedalam piring petri yang mengandungi aminopirin, hepatosit dan medium eraman sehingga 10 ml isipadu akhir. Didalam kajian *in-vivo* (FAIZOL UBAT BATUK® sahaja) tikus telah diberi beberapa pencairan FAIZOL UBAT BATUK® secara oral setiap 8 jam selama satu hari (kajian *in-vivo* akut) dan selama 7 hari (kajian *in-vivo* subakut) sebelum dibunuh. Penamatan tindakbalas dilakukan dengan tambahan larutan 25% $ZnSO_4$ dan diikuti dengan tambahan $Ba(OH)_2$ tepu. Absorbans yang diukur untuk .kompleks 3,5-diasetil-1,4-dihidrolutidin daripada asai telah digunakan untuk menentukan kepekatan formaldehid yang terbentuk (sebagai metabolit aminopirin) sewaktu kajian yang boleh digunakan untuk menentukan aktiviti enzim aminopirin N-demetilase.

Untuk mengkaji mekanisme molekul produk herba-herba ini mempengaruhi metabolisme aminopirin, beberapa bahan perencat/perangsang sel telah dipre-eramkan dengan hepatosit selama 15 minit sebelum eraman terakhir aminopirin bersama AIR JAMU PAK TANI® atau FAIZOL UBAT BATUK®.

Kajian *in-vitro* AIR JAMU PAK TANI® menunjukkan bahawa AIR JAMU PAK TANI® berupaya meningkatkan N-demetilasi aminopirin mencadangkan kesan langsung AIR JAMU PAK TANI® terhadap metabolisme aminiopirin didalam hati. Kesan AIR JAMU PAK TANI® terhadap metabolisme aminopirin didapati bergantung kepada umur, jantina dan penyakit. Beberapa lintasan pengutus kedua (seperti cAMP, sitokrom P-450$_{IA2}$, fosfatase, G-protein, protein kinase bersandarkan Ca/Calm dan protein tirosin kinase) telah diselidiki untuk mengetahui mekanisme AIR JAMU PAK TANI® meningkatkan metabolisme aminopirin.

Data kami menunjukkan bahawa mekanisme molekul AIR JAMU PAK TANI® melibatkan beberapa lintasan pengutus kedua. Ini tidaklah memeranjatkan kerana AIR JAMU PAK TANI® mengandungi lebih daripada 10 tumbuhan dan kesan AIR JAMU PAK TANI® yang dilihat berkemungkinan besar adalah disebabkan oleh jumlah kesan kesemua komponen kimia yang wujud didalam sediaan tersebut.

Berhubung dengnan kajian *in-vitro* FAIZOL UBAT BATUK®, interaksi hepat bersama aminopirin berkeputusan dengan peningkatan metabolisme aminopirin (kemungkinan besar disebabkan oleh kesemua komponen kimia daripada sekurang-kurangnya enam tumbuhan atau produk tumbuhan). Faktor jantina, umur dan penyakit didapati mempengaruhi kesan FAIZOL UBAT BATUK® terhadap metabolisme aminopirin.

Kajian *in-vivo* (akut dan subakut) FAIZOL UBAT BATUK® mencadangkan kemungkinan berlakunya interaksi FAIZOL UBAT BATUK® dengan aminopirin didalam tubuh menghasilkan peningkatan/penurunan metabolisme aminopirin. Interaksi hepar ini didapati bergantung kepada umur, jantina dan penyakit. Kesan FAIZOL UBAT BATUK® terhadap metabolisme aminopirin dapat dilihat *in-vivo* tetapi tidak *in-vitro* mencadangkan FAIZOL UBAT BATUK® tiada kesan langsung terhadap aktiviti aminopirin N-demetilase hepar. Kesannya mungkin menerusi pengaktifan atau perencatan sistem fisiologi lain yang berfungsi didalam tubuh. Aruhan sintesis sitokrom P-450 mungkin berlaku tetapi perlu diselidiki.

Kedua-dua kajian akut dan subakut menunjukkan FAIZOL UBAT BATUK® berupaya meningkatkan metabolisme fasa 1 aminopirin didalam hati tikus. Mekanisme yang menentukan pengaruh ini berbeza jika FAIZOL UBAT BATUK® diberi secara oral selama satu hari atau tujuh hari.

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LIST OF ABBREVIATIONS

The following abbreviations have been used:-

£	United Kingdom pound
$\alpha\beta\gamma$	Alpha, beta and gamma G-proteins sub-units
ACTH	Adenocorticotrophic hormone
ADP	Adenosine-5'-diphosphate
Ad Libitum	To be taken as wanted
AJPT	Air Jamu Pak Tani®
Al^{3+}	Aluminium ion
ATP	Adenosine-5'-triphosphate
Ca/CaM	Calcium calmodulin complex
Ca^{2+}	Calcium ion
cAMP	cyclic Adenosine-3',5'-monophosphate
cCMP	cyclic Cytidine-3',5-monophosphate
cGMP	cyclic Guanosine-3',5'-monophosphate
cIMP	cyclic Inosine-3',5'-monophosphate
CNS	Central nervous system
CRM	Committee on Review of Medicines
cUMP	cyclic Uridine-3',5'-monophosphate

DCA	Drug Control Authority
DG	Diacylglycerol
DMSO	Dimethylsulphoxide
et al	Else where or and others
FAD	Flavin adenine dinucleotide
FMN	Flavin mononucleotide
FSH	Follicle stimulating hormone
FUB	Faizol Ubat Batuk®
g	Gram
G_{α}	Alpha sub-unit of guanine nucleotide regulatory protein
$G_{\alpha\gamma}$	Gamma, alpha sub-units of guanine nucleotide regulatory protein.
G-protein	A guanine nucleotide regulatory protein
G_i	inhibitory guanine nucleotide regulatory protein
$G_{i\alpha}$	Inhibitor alpha sub-unit of G-protein
GTP	Guanosine triphosphate
H	Hormone
HBSS	Hank's Balanced Salt Solution
IBMX	3-isobutyl-1-methylxanthine
$In(1,3,4,5)P_4$	Inositol 1,3,4,5-tetraphosphate
IP_3	Inositol-1,4,5-triphosphate
K^+	Potassium ion

K_m	Substrate concentration producing half maximum
KT5720	cAMP dependent protein kinase inhibitor
KT5823	cGMP dependent protein kinase inhibitor
MCA	Medicines Control Agency
Mg^{2+}	Magnesium ion
ml	Milliliter
mmol/L	millimol per liter
NADPH	Nicotinamide adenine dinucleotide phosphate (reduced)
OK	Okadaic acid
OTC	Over-the-counter drug
PDE	Phosphodiesterase enzyme
PI	Phosphatidyl inositol
PK_A	Protein kinase A
PK_C	Protein kinase C
PK_G	Protein kinase G
PLC	Phospholipase C
PMA	4β-phorbol-12β-myristate-13α--acetate
PPA2	Phosphatase A2
PPi	Inorganic phosphate
PPI	Phosphatase I
R	Receptor
S.E.M	Standard error of mean

S.D	Standard deviation
STZ	Streptozotocin
TCM	Traditional Chinese Medicine
UK	United Kingdom
US$	United State dollar
USA	United State of America
V_{max}	Maximal velocity
WHO	World Health Organization

CHAPTER ONE / INTRODUCTION

1.0 INTRODUCTION

1.1 Introduction To Traditional Medicine and it's Definition

Much of the ancient literatures on traditional medicine and medicinal plants use certain technical terms of botany, pharmacology and medicine which may not be familiar to the reader. Some confusion has developed over the years in the use and misuse of terms describing the practitioners of traditional medicine and the various specialists of this art. For example, even as recently as 1976, it was not possible at original committee meeting of the African region of the world health to get the agreed definition of practitioner. Opinion was divided as to whether the practitioners of this type of medicine should be called "traditional healers, or traditional medical practitioners".

A traditional healer can be described as a person who is recognized by the community in which he lives as competent to provide health care by using vegetable, animal, and mineral substances and certain other methods. These methods are based on social, cultural, and religious background as well as on the knowledge, attitudes, and beliefs that are prevalent in the community regarding physical, mental, and social well being and the causes of disease and disability. There are certain common factors in the principles and practices of traditional medicine throughout the world, similarities evolved across oceans and mountains before the present era of international

communications. Study and knowledge of these common denominators is important, not only as to the discovery of their basis and origins, but also in developing traditional medicine as an independent discipline in medicine, and ultimately to integration and advancement of the science of healing. While there are major fundamental differences, for example between the Chinese, Arab, Indian, African, and Red Indian system of traditional medicine, these differences are often reflection of the sociocultural and physical environments in which they have evolved; however, the common threads are of greater significance. Health is generally considered as a state of harmony between various forces within and outside the body, and disease as an imbalance of these forces, personal efforts are required to stay healthy and prophylactic measures are used. Role of diet and maintenance of social laws and taboos are stressed. Non-physical causes of disease are freely acknowledged. Conceptually, ultimate causation and remedies are thought to be due to higher intangible forces who have ultimate control in health and healing and maintenance of harmony and equilibrium. The above definition is all-embracing and incorporates all the facets of traditional healer. However, some authorities do not agree with certain aspects of this omnibus definition. For example, the fact that a true healer has to be recognized by the community to which he belongs has been debated. Yet, in most developing countries it is generally true that a traditional practitioner is recognized by the community in which he lives as someone to whom the people can turn for help on health matters. Other authorities oppose the above definition as it would include witch-doctors, diviners, seers, or spiritualists, a situation which would make research into the field of traditional medicine almost impossible (Lewis,1981). Traditional medicine can be described as the total

combination of knowledge and practice, whether explicable or not, used in diagnosing, preventing, or eliminating a physical, mental, or social disease and which may rely exclusively on past experience and observation handed down from generation to generation, verbally or in writing (Sofowara, 1982). Plants have been used as sources of medicine for centuries and currently, it is estimated that some 20,000 species are used medicinally throughout the world. Every country and region has its own system of traditional medicine in which plants feature to a great extent. In the majority of countries, particularly in the rural areas of developing countries, plants continue to play a major role in primary health care (Phillipson,1993).

A Medicinal plant, is any plant which in one or more of its organs, contains substances that can be used for therapeutic purposes or which are precursors for the synthesis of useful drugs. **Herbalist,** a term which describes a traditional healer whose specialization lies in the use of herbs to treat various ailments. He is expected to be highly knowledgeable in the efficacy, toxicity, dosage, and compounding of herbs. **Bone setter,** is a specialist in one aspect of traditional healing, being skilled in the ability to treat fractures. He performs his skills without the aid of X-rays and sometimes also undertakes manipulation of joints and massaging. The term "crude drugs of natural or biological origin" is used by pharmacists and pharmacologists to describe whole plants or parts of plants which have medicinal properties. Medicinal plants include the following: plants or plant parts used medicinally in glacial preparation (e.g. decoctions), plants used for extraction of pure substances either for direct medicinal use or for the semi-synthesis of medicinal compounds (e.g. semi-synthesis of sex hormones from diosgenin), food, spice, and perfumery plants used medicinally,

microscopic plants (e.g. fungi, actinomycetes, used for isolation of drugs especially antibiotics), fiber plants (e.g. cotton, flax, jute, used for the preparation of surgical dressing). It has been recommended by a WHO consultative group that the term "vegetable drug" be applied to that part of medicinal plant (leaf, bark,..etc.) used for therapeutic purposes. Such material, which possesses a cellular structure, is referred to in pharmacy as an "organized drug", while medicinal agents such as gums, balsams, which have no cellular structure, are called "un-organized drug" (Sofowara, 1982). However, any manufacturer wishing to introduce a new herbal product into the market as a medicine must apply for a license from the Medicines Control Agency (MCA) or in Malaysia, the Drug Control Authority (DCA) and must submit detailed documentation on the quality, safety, and efficacy of their product. Furthermore, full details of manufacturing protocols are required and the products and their production processes are subjected to inspection procedures. Subsequently, all medicinal products, including herbal products, have been subjected to a review procedure conducted by the Committee on Review of Medicines (CRM). Approximately 1000 herbal medicinal products containing some 550 herbs have been approved by the CRM (United Kingdom) and they now have full product licenses (Phillipson, 1993).

1.2 The use of traditional medicine in the world

In reviewing the current states of traditional medicine, the regional zones designated by the World Health Organization (WHO) will be used. According to the WHO and other reports, the position up to 1980 was as follows: **IN THE AFRICA** region traditional medicine has become a part of the people's culture even though this form of medicine is not as well organized as for example in India and China. Many countries in Africa now have a division, department, or task-force on traditional medicine, usually attached to their ministries of health. Most African countries now have at least a research group investigating medicinal plants. It is noteworthy that a number of research institutions on traditional medicine in Africa now have herbalists on their staff. **IN THE AMERICAN** region, more is heard of traditional medicine and its practitioners in South America and amongst the American Indians than in North America and Canada. The latter two countries however, use many drugs of plant origin (Sofowara, 1982). In the USA, approximately 50% of laxative preparation contain plant ingredients and this market was valued at US$ 331 million during 1980. Health food shops in the USA sold herbs worth US$ 360 million during 1981 (Phillipson, 1993). In several countries, traditional midwives are given basic training and are partly or wholly incorporated into the health system. **IN THE EUROPEAN** region of the world, apparently there is little use for traditional medicine. Many countries disapprove of the WHO's promotion of traditional medicine, perhaps because these countries have much highly developed health care for their people. And also, middle-level health personnel had replaced the traditional healers of this region of the world. For example in Britain,

the days of using a concoction of some 20 plants to treat dropsy have long gone, and now the active component, digitalis leaf is used in standardized tablet form. Official and professional attitudes in Europe towards an extensive use of traditional practitioners is to say the least, cautious. It is considered that traditional practitioners and their successors should receive training from the biological and scientific point of view. There is no evidence, however, that this is happening any where in Europe. However, herbalists and herbal associations do exist in Britain and Sweden (Sofowara, 1982). The European market for herbal products is annual growing at rapid pace and in 1990 it was valued at £1,446.7 million with an average growth rate for all member states of 13%. The growth rate for over-the-counter products across Europe is 5.7% and the value for pharmaceuticals in the European market is £69,652 million. Although the herbal product market is only 3.5% of pharmaceutical market it is expanding more rapidly. In the UK during 1989, the expenditure on garlic pearls was £4 million and on ginseng preparation was £3 million. The herbal teas market in the UK has increased steadily since 1984 and the predicted value for 1992 was £10 million. It is salutary to realize that herbal teas were only introduced into the UK around 1978. The number of specialist health food shops increased by 19% between 1987 and 1992 when it was an estimated 2,020. In Europe, Germany is the major importer of medicinal products, and in 1979 some 28,000 tons of plant material were imported at a value of around US$ 56 million. In the UK there is a growing interest in Traditional Chinese Medicine (TCM) and numerous shops and TCM clinics have opened up businesses in recent years (Phillipson, 1993). **IN THE EASTERN MEDITERRANEAN** region, several countries have developed training programs for traditional birth attendants, thus

enabling them to play more effective role in the maternal and child health services. In several countries, activities have been focused on research on medicinal plants and the production of pharmaceuticals from locally available herbs. There is no official framework in any country for traditional and modern medicine to collaborate in the interest of the patient. **IN THE SOUTH EAST ASIAN** region, traditional medicine has been integrated into the official health care system and formal as well as informal training of practitioners exists. Formalized system of indigenous medicine include ayurveda, yoga, unani-tibbi, modified Chinese, and the Amchi system (a tibetan system of medicine). Integrated systems go a step beyond the inclusive category, in which the modern and traditional are still formally, if not wholly functionally, separate. The policy of the WHO is generally to encourage some type of integration to optimize health care coverage (David, 1990). Dhungel & Dias, 1988 note both official encouragement for integration and fairly frequent co-use of modern and traditional facilities. It seems, too, that Korea has, since 1980, enacted legislation to combine traditional therapeutic methods with modern diagnosis, including research and training in traditional medicine. Overall, however, integration is unlikely to be easily achieved (Bibeau, 1985). Non-formalized training of practitioners of indigenous medicine also exists in this region including that of herbalists, bone setters, practitioners of thaad (element system), home remedists, and spiritualists. In India alone there are 500,000 indigenous medical practitioners and 115 recognized institutions of indigenous medicine of which 98 colleges train exclusively in ayurveda. University departments of traditional medicine existed for many years in India, and institutes carrying out multidisciplinary research on medicinal plants abound through this region. Medicinal

products compounded from herbs are common sight in the drug stores. **IN THE WESTERN PACIFIC** region, the traditional medicine and the use of medicinal plants thrives. Traditional Chinese medicine has had several thousands of years of practical experience as well as its own theory. Schools and research institutes for traditional medicine have long been established. China offers an enviable example of integration of traditional medicine and modern medicine and delegations from various countries of the world (Nigeria, U.S.A,...etc) have visited China to study the successful marriage of traditional and modern medicine. Many methods have been developed that are economical, and easy to apply. Research on medicinal plants and production of phytopharmaceuticals is being undertaken in several countries in this region, and attempts have been made to compile a regional pharmacopoeia of crude drugs (Sofowara, 1982). Present world economic conditions do not permit pharmaceutical companies in their obligation for the extremely expensive research on brand new drugs. The companies rather would combine the available brands to obtain "new" ones with "better and improved" effects. Traditional medicine all over the world might well provide us with vast resources in the search of medicine for "brand new drugs" and "brand-new ways of therapies" as well (Negara, 1984). Thousands of years ago, Chinese prehistoric ancestors have developed and compiled a healing art by the use of needles. Based on their logic and their ancient experience, this practice is magical cure of so many diseases. Since the discovery of acupuncture anesthesia, about half an era ago, for even major surgery, acupuncture has drawn the interest of the west. What really does acupuncture do ? Medical science attempts to solve the riddle in the laboratories and in the clinics, emphasizing on the effect in the central nervous system,

especially the neurotransmitters. The results are encouraging, though the final word is still unsaid (Negara, 1984). Health food shops are now starting to lose some of their business to pharmacies and pharmacy sales of "healthy type" products has increased from 17 % of the market to 20 % (Phillipson, 1993).

1.3 The use of plants in treatment of disease

It is reported that some 80% of the world's inhabitants rely chiefly on traditional medicines, mainly plant based, for their primary health care needs (Akerele, 1993). Some 21,000 species of medicinal plant have been listed for medicinal use (Penso, 1983) although it has been argued that this is a most conservative estimate and that the number of medicinally-used species is probably between 35,000 to 70,000 (Farnsworth and Soejarto, 1991). The continued use of herbal medicines has been stressed more recently by Dr. X. Zhang, medical officer, traditional medicine, WHO (Zhang,1996), who has pointed out that because there is a shortage of medical doctors and pharmaceutical products, the majority of the population in developing countries still rely mainly on traditional practitioners and local medicinal plants to satisfy their primary health care needs. The popularity of complementary therapies, including the use of herbal remedies, is widespread in North America, Europe and Australia. Extrapolation of statistics obtained via a questionnaire in the USA indicates that possibly some 42.5 million visits were made to herbalists in 1990 (Eisenberg *et al*, 1993). Furthermore the same survey indicated that 425 million visits would have been made to unconventional therapists contrasting with the 388 million visits actually made

to primary health care physicians. OTC sales of herbal medicines in the USA and Canada reached US $ 860 million in 1990 with an annual growth rate of 15 % (Zhang, 1996).

One European company specializing in the extraction of medicinal plant (Indena, Milan) extracts some 12,000 metric tones of plant material per annum and additionally prepares pure medicinal drugs by direct isolation from plants and by semisynthesis. Some 1139 plant extraction are produced commercially as ethical drugs, OTC or nutritional supplements for use in Europe, Japan, South Korea and USA (Bombardelli,1996). Among the active ingredients isolated are some 202 pure compounds which are used almost entirely as medicinal drugs. In recent years, natural products from higher plants have been highlighted as potential sources of new drugs; two notably examples being the antimalarial artemisinin from *Artemisia annua* and the anticancer drug taxol from *Taxus brevifolia* (Phillipson & Bodeker, 1996).

1.4 Practice of traditional medicine in Malaysia

Malaysia is a country that treasures its natural forests. These forests are the largest source of natural products which can be used for medicinal purpose. Since the early years, the Malays have been using plant products such as the leaves, roots, stems, and fruits as ingredients in the preparation of traditional medicine. Plants were used primarily for treatment rather than cure. This was because the practitioners of traditional medicine diagnosed disease in a very general way, also every tribe and race has its own methods or ways of curing the affliction of disease. Traditional medicine

has been used since time immemorial and until today, it remains a popular method of treatment. In the earlier centuries, the local community held traditional medicine in very high regard. This is obvious from the respect accorded by the community to the ***Bomoh*** (medicine-man), the ***Mak bidan*** (midwife) and also the ***Mudim*** (religious man who performs circumcision on boys). These practitioners were revered and were completely entrusted with the task of dispensing medical care. Malaysia is rich in natural resources basic to traditional medicine.

There are over six thousand species of tropical plants all over the country and in Malaysian Peninsula, there are 550 genera containing 1,300 species (Zakaria & Mohd, 1993). Most of these are medicinal plants good for the human use. Malaysia, as an agriculture-based country surely has the potential for increasing its produce of medicinal and herbal plants. Even the World Health Organization (WHO) has proposed that by the year 2000, the world population should have learnt to adopt all forms of medicine, be they traditional or modern, to eliminate their health problems. Recently, there has been a resurgence of awareness among Malaysians to revive traditional medicine as part of the Malaysian culture. Academicians are now beginning to study this particular area more deeply. In this country, Malay traditional medicine has been influenced by various elements from foreign medicine. This has been brought about by the inter-racial interaction since the days of the Malay Malacca Sultanate in the fourteenth century. Chinese and Indian immigrants, too, brought with them various medicinal plants which grew well in this country. The local Malay traditional medicine is actually based on old Indonesian and Arab traditional medicine, which has been modified to suit the local and current needs. Malaysia therefore presents a very

interesting case study of medical pluralism in an ethnically diverse and rapidly modernizing society. It embraces two of the three great systems of traditional medicine (Arabic, Hindu (Ayurvedic) and Chinese), as well as an extensive network of varied types of native medical systems (Kuang, 1983). Indeed, as many as 20 000 *bomohs* minister to the health of Malaysians compared with fewer than 2500 physicians (Vuori, 1982), as many traditional practitioners are readily accessible. In the Malay villages, one can easily find a number of traditional medicine practitioners such as ***Nujum*** (clairvoyant), ***Bomoh*** or ***Dukun, Tok mudim*** and ***Mak bidan.*** Each has, his or her respective role and is essential to the life and harmony of the village. For example, ***Mak bidan*** plays an important role in treating women as well as in advising young ladies. She is usually uneducated and not formally trained. She acquires her skills from experienced practitioners and through her own experience. In Malay medicine, the ***Mak bidan*** performs almost similar functions as a midwife. She carries out important nursing tasks, such as delivering baby, advising pregnant mothers before and after delivery, advising young ladies on health care and health problems. In Malay traditional medicine, various preparation and methods are used in preparing the medicine. These medicine are usually chanted over to ensure their potency, and to avoid toxicity (Zakaria & Mohd, 1993).

1.5 Liver and drug metabolism

1.5.1 Introduction:

Liver plays a crucial role in drug metabolism. Blood flow through liver, and its biochemical functions vary from person to person. Variation of function of the liver would therefore tend to vary drug metabolism (Lahiri, 1989). Cytochrome P-450 enzymes are important in the biotransformation of compounds that are normally involved in intermediary metabolism (i.e. steroids, vitamins and fatty acids) and xenobiotic substances that may be intentionally or inadvertently ingested by the host (i.e drugs, environmental pollutants, industrial chemicals and natural products). P-450s are found in prokaryotes and eukaryotes, in plants as well as animals. Most animal tissues contain some P-450, with the exception of striated muscle and erythrocytes, and the enzyme appear to be present in several subcellular organnelles. By the mid-1970, several P-450 forms had been isolated and substrate specificities were determined (Imai & Sato, 1974; Ryan *et al*, 1975; Haugen *et al.*, 1975; Guengerich,1977). Since then, the primary sequences of some P-450s and their genes have been determined (Black & Coon, 1986). First of all, the substrates for P-450s can be divided into two classes: compounds endogenous to mammals and exogenous chemicals which are ingested advertently or inadvertently (xenobiotics). Microsomal P-450s oxidize a number of endogenous substrates, and some of these reactions are recognized as being important to the well-being of the organism. Two isolated types of P-450s enzymes

have important roles in the formation of eicosanoids with potent biological activities: prostacyclin synthase (aorta) and thromboxane synthase (platelets) (Graf *et al.*, 1983; Guengerich,1987). Many chemicals require oxidation to exert biological activity. In this regard, electrophilic compounds are sometimes generated which can bind irreversibly to cellular macromolecules such as proteins and DNA. What is the real function of the P-450s ? Clearly the roles of most of the mitochondrial and microsomal P-450s in steroidogenic tissues are in the hydroxylation of steroids. Roles for the liver microsomal P-450s have been a matter of speculation. However, most of the major P-450 forms which have been characterized don't have catalytic activities of obvious physiological importance. Do changes in the composition of P-450 forms and in their catalytic activities have any physiological implications? Early studies showed that administration of certain chemicals such as phenobarbital, 3-methyl-cholanthrene, or pregnenolone 16α-carbonitrile to animals resulted in increases in level of P-450 (Conney *et al.*, 1956; Guengerich,1987; Remmer & Merker,1963; Solymoss *et al.*,1971). Gross toxicities of other chemicals could also be altered by pre-administration of such inducing agents, and correlations between P-450 levels and toxicity were made (Boyd,1980; Guengerich,1987). Unfortunately, the chemicals used to induce the P-450s have a number of other physiological effects as well, and these phenomena cannot always be sorted out. In individuals with impaired rates of oxidation, an increased of drug action may be seen e.g., increased heart rate due to nifedipine (Kleinbloesem *et al.*,1984) or adverse side effects may result e.g., phenacetin methaemoglobinaemia, captopril induced agranulocytosis (Oates *et al.*,1982; Cooper *et al* .,1984). These oxidation have all been shown to involve P-450s

(Guengerich *et al.*, 1985; Distlerath & Guengerich, 1987), although the exact basis of the deficiency in catalytic activity has not been ascertained. The effects of changes in P-450 composition upon normal physiological function are not well understood, except in congenital diseases related to anomalies of steroid metabolism such as adrenal c - 21 hydroxylase deficiency (White *et al.*,1984; Carroll *et al.*,1985). In humans, very little is known about the importance of the liver microsomal P-450s on normal physiological function. P-450_{Nf} appears to be responsible for much of the testosterone and oestradiol hydroxylation in human liver (Guengerich *et al.*,1986). The same human enzyme, P-450_{Nf}, is responsible for the formation of 6β-hydroxycortisol (Ged *et al.*,1985), which is excreted in urine and has been used as a clinical marker for P-450 activity (Ohnhaus & Park, 1979). The regulation of metabolic processes ultimately depends upon the control of enzyme activity (Martin, 1987). It has been shown in a very wide range of different cell types that a rise cyclic AMP in response to a hormone produces a rise in the activity of the cyclic AMP-dependent protein kinase. However, there is, apparently, a discrepancy between the basal protein kinase activity and the resting level of cyclic AMP in the cell (Martin, 1987).

1.5.2 Cytochrome P-450

Cytochrome P-450 is a hemeprotein containing one molecule of iron-protoporphyrin IX as its prosthetic group (Omura & Sato, 1964). It is readily identified by a pronounced absorbance band at 450 nm in the Soret region of the visible spectrum when the carbon monoxide adduct of the reduced hemeprotein is formed hence the name P-450. This property of cytochrome P-450 is attributed to the presence of a thiolate group as a ligand of the hemeprotein (Peisach & Blumberg, 1970). Cytochrome P-450 is classified into two general categories, class A represents the type of P-450 that require an FAD - containing flavoprotein and an iron - sulfur protein for electron transport from reduced pyridine nucleotide (NADPH) to the hemeprotein (Estabrook *et al.*, 1973). This class of P-450 is most frequently found associated with the mitochondria of steroid - metabolizing tissues and also is typical of the P-450s associated with bacteria (Peterson & Griffin, 1972). The P-450 of class A are usually involved in the metabolism of endogenous compound such as cholesterol, deoxycorticosterone, or cholecalciferol and generally possess a high degree of substrate specificity. The class B types of cytochromes P-450 require an unusual type of flavoprotein, which contains both FAD and FMN as prosthetic groups (Vermilion & Coon, 1974., Yasukochi & Masters, 1976), for the transfer of reducing equivalents from reduced pyridine nucleotide to the hemeprotein. In general, this class of P-450 is found associated with the microsomal fraction of tissue homogenates in close association with another hemeprotein, termed cytochrome b_5, in some tissue similar type of P-450 is associated with the nucleus (Bresnick *et al.*, 1977).

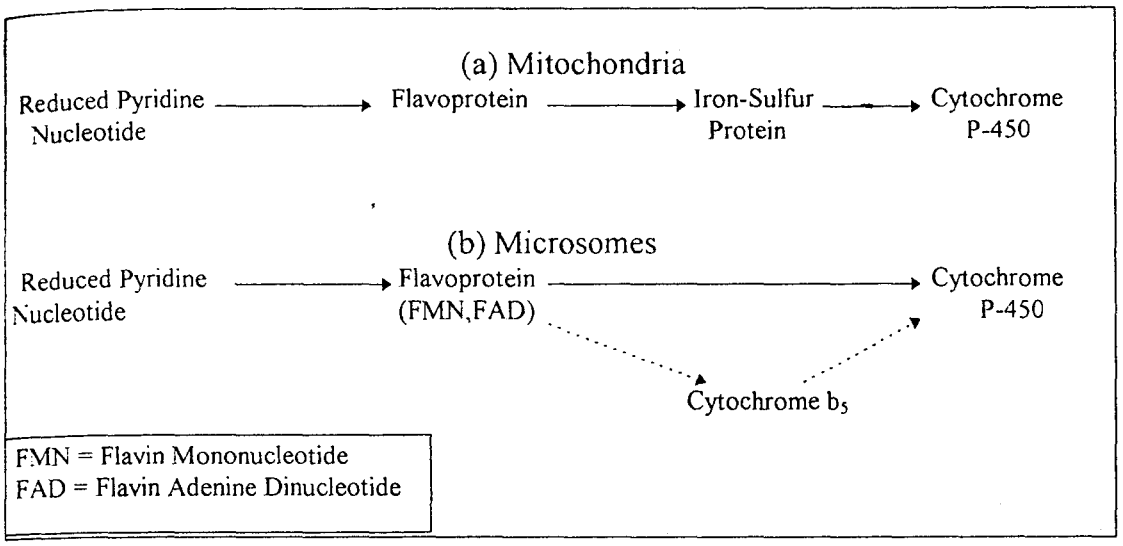

Figure 1.1 Two general categories of cytochrome P-450. The specific reduced pyridine nucleotide interacting flavoprotein required for the transfer of electron are designated, and the role of the iron- sulfur protein is indicated [(a) Estabrook *et al*, 1973; (b) Yasukochi & Masters, 1976].

The series of cytochrome P-450 directed reaction is concluded when the product of substrate oxidation dissociates from cytochrome P-450, thereby reforming the ferric hemeprotein. Essentially nothing is known about this reaction although it is recognized that it may be of importance during the steady state of drug metabolism. In this way, a cycle of substrate binding, reduction, oxygenation, further reduction, rearrangement (assisted perhaps by protonation), and product dissociation accomplishes the catalytic oxidative transformation of the substrate together with the regeneration of cytochrome P-450 in a form capable of interacting with yet another molecule of substrate. Associated with the function of microsomal-bound cytochrome P-450 is the operation of other oxidation - reduction reaction such as those catalyzed by the microsomal hemeprotein, cytochrome b_5, or a variety of microsomal flavoproteins.

The cytochrome P-450 of mammalian tissues are associated with membrane structures, either the endoplasmic reticulum or the mitochondria. Integration of P-450 and its requisite companion electron transfer proteins into a membrane structure may impose limitations on their mode of function and interaction. In addition, since such membranes are rich in phospholipids, and most of the substrates metabolized by P-450 are highly lipophilic, considerable interest has developed on the role of membrane structure relative to the metabolism of xenobiotics (Mitchell & Horning, 1984).

1.5.2.1 Phase I metabolic reactions

Drugs are generally metabolise in two phases, namely the so-called Phase I or 'biotransformation' in which new function groups are introduced to the lipophilic chemical, and Phase II or 'conjugation' in which syntheses occur by addition of small endogenous molecules to the functional groups of the drug or its Phase I metabolites, making the molecules less lipophilic, more polar, and more readily excreted from the cell. The major group of Phase I metabolic reactions or biotransformation are oxygenation or hydroxylation reactions which affect the addition of oxygen to carbon, nitrogen or sulphur, N-and O-dealkylation, and deamination. These hydroxylation reactions are catalysed by the mixed function oxidases of the endoplasmic reticulum of the liver and of certain other tissues, such as the lungs and skin (Park, 1978).

Dealkylation occurs very readily with drugs containing a secondary or tertiary amine, an alkoxy group on an alkyl substituted thiol. The alkyl group is lost as the corresponding aldehyde. In the examples of N-demethylation, diazepam, lignocaine,

ephedrine, **aminopyrine**, morphine, procainamide, thioridazine, chlorpromazine, phenothiazine, tamoxifen,...etc. are converted to N-demethylated of the substrate with the loss of formaldehyde. The reaction is considered to occur in two steps. The first being hydroxylation of the methyl group on the nitrogen, and the second a decomposition of this intermediate.

1.5.2.2 Assay of aminopyrine N-demethylase activity

N-demethylation of drugs is a common metabolic pathway and usually proceeds by initial hydroxylation at the α-carbon atom and subsequent breakdown of carbinolamine intermediate liberating formaldehyde (see fig. 4.1 a). The produced formaldehyde could be measured, this would yield an appropriate assay for the N-demethylase activity. It should be noted that both aminopyrine and monomethyl-4-aminoantipyrine are metabolised by other pathways (including additional demethylation reaction) and therefore this particular assay does not reflect the overall metabolism of the substrate (Gibson & Skett, 1994). However, the aminopyrine assay test is a useful marker of liver enzyme, correlates with the activity of the hepatic mixed-function oxidase system. The main attraction of using the aminopyrine assay test lies in its simplicity, and aminopyrine had been proposed as a useful test compound for the assessment of hepatic function (Park & Kitteringham, 1988).

1.5.3 Signal transduction in drug metabolism

1.5.3.1 General aspects and roles of cyclic AMP

One of the major reasons for the growth of interest in protein phosphorylation was the discovery that cyclic AMP can often stimulate the reaction. Cyclic AMP is, of course, the 'secondary messenger' responsible for the control of many metabolic processes, and many hormones have been shown to act by increasing the concentration of cyclic AMP in the target tissue. The properties and functions of cyclic nucleotides will be discussed later but it may be pointed out here that in a number of cases it has been found that the action of cyclic AMP is mediated through protein phosphorylation. This has caused considerable interest in the mechanism by which cyclic nucleotides can stimulate protein kinase activities. Most of enzymes which are more sensitive to cyclic AMP than to other cyclic nucleotides by definition are 'type (a_1) protein kinases'. Some of the enzymes are more sensitive to cyclic GMP. These are referred to as 'type (a_2) protein kinases'. Type a_1 protein kinases respond to other cyclic nucleotides besides cyclic AMP but are much more sensitive to cyclic AMP, although at high concentration all cyclic nucleotides often give the same stimulation of activity. In general the order of sensitivity appears to be:

Cyclic AMP>Cyclic IMP >Cyclic GMP >Cyclic UMP >Cyclic CMP

There are, however, two regions of the cyclic AMP molecule which are vital for activity. Any substitution of the 2'- OH group inactivates cyclic AMP. In addition, substitution of the exo-NH_2 group may cause some loss of activity.

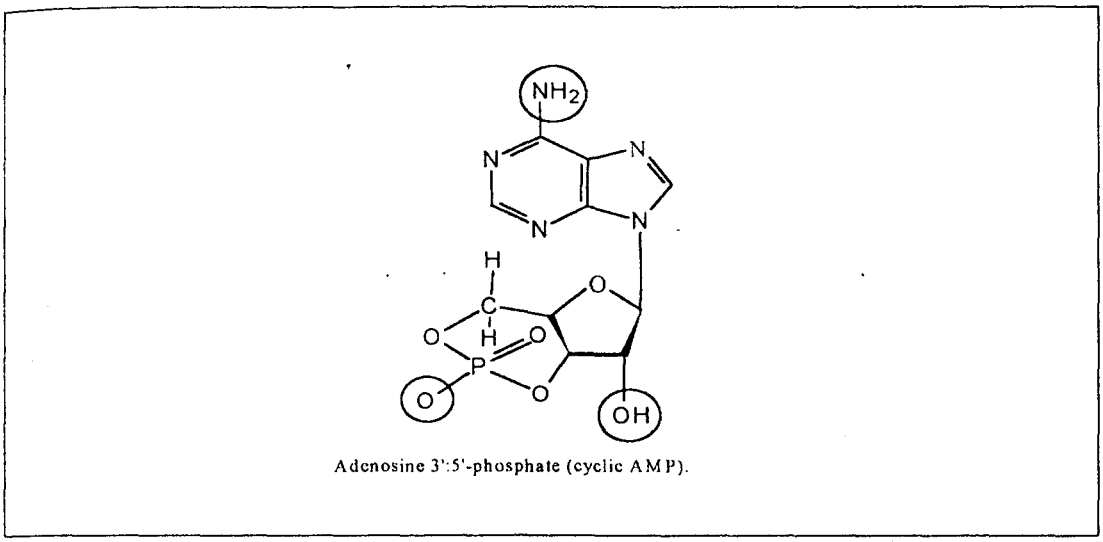

Figure 1.2 The region of the molecule which are important for activating type a_I protein kinase are circled.

It is perhaps too early to state that the dissociation of regulatory and catalytic units is the only mechanism by which protein kinases are stimulated by cyclic AMP but no other system has yet been demonstrated. The degree of stimulation caused by cyclic AMP depends on the type of protein used as substrate and also on the source and purity of the enzyme. If the enzyme has become partly dissociated during preparation, and contains a high proportion of free catalytic units, it will show relatively high activity in the absence of cyclic AMP and be only slightly stimulated (Weller, 1979). Very high concentrations of cyclic AMP (in the order of 1mM) generally inhibit type a_I protein kinases despite the fact that lower concentration cause activation. Since such high

concentration are, of course, completely unphysiological, this observation is of limited interest. It is possible that high concentrations of cyclic AMP compete with ATP. Of more interest is the observation that, in the presence of Ca^{2+} (instead of Mg^{2+}), cyclic AMP inhibits rather than stimulates the activity of type a_1 protein kinases. It should be emphasised that, because a hormone or other substance causes an increase in the concentration of cyclic AMP in a tissue, this does not mean that all the effects of the substance are mediated by cyclic AMP. It is necessary to demonstrate that the effect in question is mimicked by cyclic AMP at near-physiological concentration (Weller, 1979).

1.5.3.2 Metabolism and function of cyclic AMP

Cyclic AMP is formed from ATP in a reaction catalyzed by the enzyme adenylate cyclase.

ATP ----------> 3': 5'- cyclic AMP + ppi

The most important feature of adenylate cyclase is that it can be stimulated by certain hormones and other substances, causing an increase in the cyclic AMP concentration in the affected tissue. Cyclic AMP is broken down by hydrolysis to 5'-AMP in a reaction catalyzed by cyclic nucleotide phosphodiesterase. The activity of phosphodiesterases may also be controlled by certain regulatory substances in the cell. It should be emphasised that substances can alter cyclic AMP concentration by an action on phosphodiesterase activity, as well as by an action on adenylate cyclase, and this possibility should never be overlooked in any investigation of the effect of

substances on cyclic AMP concentration. The major, if not only, function of cyclic AMP is to act as a messenger from a hormone receptor to various cellular sites with which it interacts to cause the observed effects of the hormone. Not only is cyclic AMP a messenger, allowing the hormone to cause an effect in interior parts of the cell which doesn't normally reach, but it also amplifies the message. The interaction of a molecule of hormone with the receptor on the adenylate cyclase unit allows the synthesis of a number of molecules of cyclic AMP, which spread throughout the cell. In addition, there is a second system of amplification which involves the interaction of cyclic AMP with its target receptor. In a number of cases, cyclic AMP acts by stimulating a protein kinase which phosphorylates the target protein, causing a change in its functional activity. This is obviously a further amplification of the message. One molecule of cyclic AMP interacts with, and activates, one molecule of the first protein kinase [type a_1 protein kinase], which phosphorylates, and activates, a number of molecules of the second protein kinase (phosphorylase kinase), each activate molecule of which interacts with, and activates, a number of molecules of the target protein (phosphorylase b). This cascade system obviously produces considerable amplification, and even more could be obtained if additional protein kinases were intermediate between the kinase containing the original cyclic AMP receptor and the final target protein (Weller, 1979).

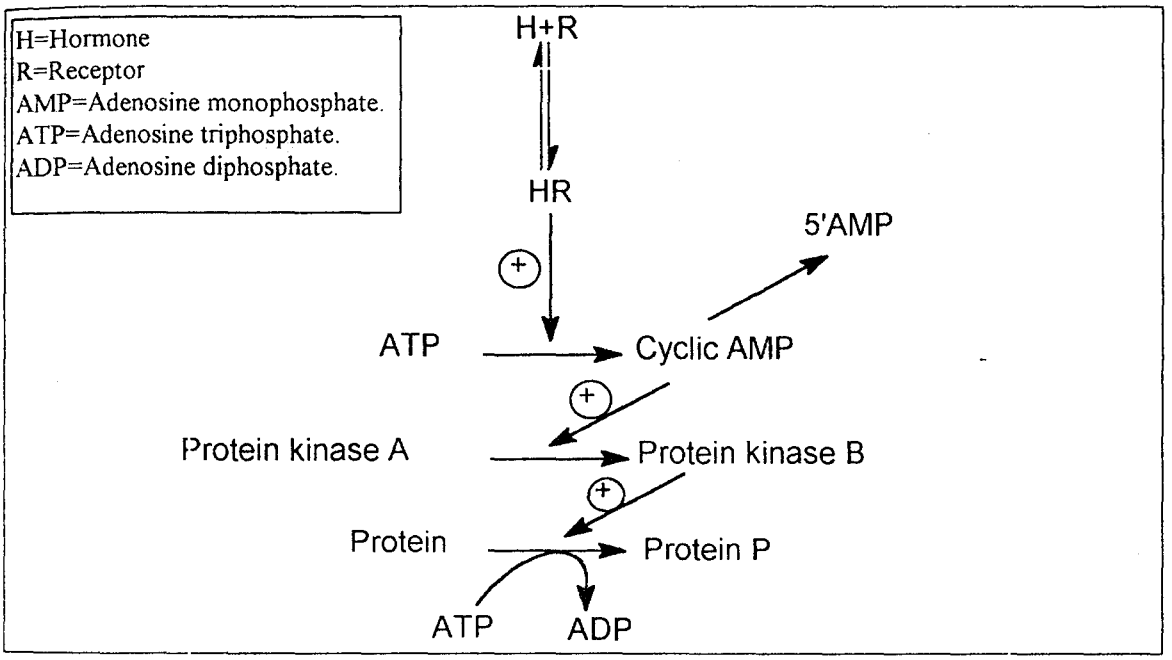

Figure 1.3 The cyclic AMP mediated the signals as a secondary messenger (Martin, 1987)