HERB – DRUG INTERACTION: THE EFFECT OF *TINOSPORA CRISPA* MIERS. ON DRUG METABOLISM IN RAT LIVER

by

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Parents, my mentor, I appreciate
Husband, my companion, I treasure
Son (DK), my hope, I confide
USM, my fundamental, I pursue

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LIST OF ABBREVIATIONS

5'-GMP	guanosine-5'-monophosphate
AAN	4-aminoantipyrine
AIDS	Acquired Immune Deficiency Syndrome
ANOVA	one-way analysis of variance
ATP	adenosine triphosphate
BfArM	The German Federal Institute for Drugs and Medical Devices
BITC	benzyl isothiocyanate
CaM	calcium / calmodulin
cAMP	cyclic 3',5'-adenosine monophosphate
CE-ED	capillary electrophoresis electrochemical detection
cGMP	cyclic guanosine monophosphate
Cl_{int}	intrinsic clearance
CNS	central nervous system
CYP	cytochrome
CYP450	cytochrome P450
DAG	diacylglycerol
DMSO	Dimethylsulfoxide
DNA	deoxyribonucleic acid
DR	diabetic rats
DSCE	dry stem crude extract
E. coli	*Escherichia coli*
ESR	electron spin resonance
FDA	Food and Drug Administration
GC	guanylate cyclase
GDP	guanosine diphosphate
GSH	glutathione S-transferase hydrogen
GSSG	glutathione conjugation
GTP	guanosine triphosphate
H_2O_2	hydrogen peroxide
HBBS	Hank's Balanced Salt Solution
HCHO	formaldehyde
HIV	human immunodeficiency virus
HTS	high-throughput screening
IBS	irritable bowel syndrome
IC_{50}	50 % inhibitory concentration
IgG	Immunoglobulin G
IP	intraperitoneal
K_i	constant for substrates
K_m	constant for inhibitors
MAO	monoamine oxidase
MAPK	mitogen-activated protein kinase
NADH	reduced nicotinamide adenine dinucleotide
NADPH	nicotinamide adenine dinucleotide phosphate
NCE	new chemical entity
nm	nano meter

NO	nitric oxide
NOAEL	no-observed-adversed-effect-level
NOS	nitric oxide sythase
NR	normal rats
NSAIDs	non-steroidal anti-inflammatory drugs
NYHA	New York Heart Association
O_2	oxygen
OGD	oxygen-glucose deprivation
OTC	over-the-counter
PDE	phosphodiesterase
PKA	protein kinase A
PKC	protein kinase C
PKG	protein kinase G
PLA_2	phospholipase A_2
PLC	phospholipase C
PMN	polymorphonuclear cells
PP1	phophatase 1
PP2A	phosphatase 2A
PPs	protein phosphatases
PTK	protein tyrosine kinase
rpm	revolution per minute
RPTK	receptor protein tyrosine kinase
RYR	cardiac ryanodine receptor
SAR	structure/activity relationship
SD	Sprague Dawley
S.D.	standard deviation
S.E.M.	standard error of the mean
SR	sarcoplasmic reticulum
STZ	streptozotocin
TCM	Traditional Chinese Medicine
*TCREt*s	the hypoglycaemic action of transcervical resection of the endometrium

INTERAKSI HERBA-DRUG: KESAN *TINOSPORA CRISPA* MIERS. TERHADAP METABOLISME DRUG DALAM TIKUS HATI

ABSTRAK

Tinospora crispa Miers. (Famili: Menispermaceae) atau dikenali dengan nama tempatan sebagai Patawali atau Akar Sekuntum, adalah tumbuhan yang digunakan secara tradisional sebagai ubat untuk merawat kencing manis dan gangguan metabolik. Objektif kajian ini ialah untuk mengkaji kesan *in vitro* dan *ex vivo* ekstrak kasar dan ekstrak pelbagai pelarut daripada batang *Tinospora crispa* ke atas aktiviti aminopirin *N*-demetilase di dalam tikus Sprague-Dawley (SD). Ekstrak-ekstrak *n*-heksana, kloroform, *n*-butanol dan akues yang diperolehi daripada residue larutan-metanol *Tinospora crispa* telah digunakan untuk mengkaji kesan mereka ke atas metabolisme fasa I aminopirin dalam hepatosit tikus. Pengasingan hepatosit telah disediakan dengan menggunakan teknik perfusi kolagenase dan pengemparan berperingkat. Pengaruh jantina, umur dan keadaan penyakit (diabetes) juga telah diuji dalam kesan *Tinospora crispa* ke atas aktiviti aminopirin *N*-demetilase. Keputusan menunjukkan ekstrak kloroform *Tinospora crispa*, secara *in vitro*, boleh mempengaruhi *N*-demetilase aminopirin (dan kemungkinan ubat-ubat lain yang melalui tindakbalas fasa I *N*-demetilase yang sama, contohnya diazepam, morfin dan lain-lain). Kajian *ex vivo* ke atas tikus SD normal dan diabetik (aruhan-STZ) telah menunjukkan kesan signifikan ke atas aktiviti aminopirin *N*-demetilase dalam tikus betina muda dan tua (kumpulan normal dan diabetik). Kesan ketoksikan yang mungkin diakibatkan oleh *Tinospora crispa* ke atas hati dan ginjal dalam tikus SD juga telah diuji. Keputusan diperolehi daripada tikus SD normal menunjukkan bahawa langkah berhati-hati perlu diambil

apabila mengambil dos 500 mg/kg dan ke atas ekstrak kloroform *T. crispa* secara jangka panjang kerana ia mungkin mengakibatkan ketoksikan ke atas hati. Walau bagaimanapun, dalam tikus SD diabetik (aruhan-STZ) tiada perubahan signifikan dalam analisis biokimia klinikal dilihat pada 10 dan 100 mg/kg ekstrak kloroform *T. crispa*. Berat badan yang diperolehi dalam kajian ini menunjukkan keputusan berbeza dan kesan antara kumpulan dipengaruhi oleh faktor yang berbeza contohnya jantina, umur dan keadaan penyakit. Sejumlah 6 kematian telah dilaporkan dalam beberapa kumpulan yang diberi 500 mg/kg ekstrak kloroform *T. crispa*. Mekanisme tindakan yang memberikan kesan *in vitro* ekstrak kloroform *T. crispa* ke atas metabolisme aminopirin dalam hepatosit tikus jantan muda diabetik mungkin diperantarakan melalui beberapa lintasan molekul i.e. lintasan cAMP pada kepekatan rendah; bertindak sebagai perangsang fosfatase dan perencat protein kinase C. Keputusan yang diperolehi dalam kehadiran furafylline juga mencadangkan bahawa ekstrak kloroform daripada *T. crispa* telah meningkatkan aktiviti sitokrom $P450_{1A2}$. Kesimpulannya, ekstrak kloroform *T. crispa* telah mempengaruhi fasa I aminopirin *N*-demetilase dalam kumpulan yang tertentu dalam hati tikus. Penggunaan ekstrak kloroform *T. crispa* pada dos 500 mg/kg dan ke atas perlu diberi perhatian sekiranya ia diambil secara jangka panjang kerana ia mempamerkan kesan signifikan ke atas paras ALT (alanine aminotransferase) dalam kumpulan tikus dewasa jantan normal.

HERB-DRUG INTERACTION: THE EFFECT OF *TINOSPORA CRISPA* MIERS. ON DRUG METABOLISM IN RAT LIVER

ABSTRACT

Tinospora crispa Miers. (Family: Menispermaceae) or locally known as Patawali or Akar Seruntum, is a plant that has been used locally as a remedy for diabetes mellitus, and metabolic disorders. The objective of this study is to investigate the *in vitro* and *ex vivo* effects of the crude extract and different solvent extracts of the stem of *Tinospora crispa* on aminopyrine *N*-demethylase activity in Sprague-Dawley (SD) rat liver. *n*-Hexane, chloroform, *n*-butanol and aqueous extracts derived from methanolic-soluble residue of *Tinospora crispa* were used to investigate their effects on aminopyrine Phase I metabolism in rat hepatocytes. Isolated hepatocytes from SD rat liver were prepared using the collagenase perfusion technique and differential centrifugation technique. The influence of gender, age and disease (diabetes) were examined on the effects of *Tinospora crispa* on aminoyrine *N*-demethylase activity. Results obtained showed that the chloroform extract of *Tinospora crispa*, *in vitro*, could affect the *N*-demethylation of aminopyrine (and probably other drugs that undergo similar Phase I *N*-demethylation reaction such as diazepam, morphine and etc). The *ex vivo* study of normal and diabetic (STZ-induced) SD rats demonstrated that in young female & old female (for both normal & diabetic groups) there was a significant effect on aminopyrine *N*-demethylase activity. The possible toxic effect of the chloroform extract of *T. crispa* in liver and kidney of SD rats was determined. The results in normal SD rats showed that caution should be exercised for long term intake at

doses 500 mg/kg and more of the chloroform extract of *T. crispa* because it may cause toxicity to the liver. However, in diabetic (STZ-induced) SD rats, no significant change in the clinical biochemistry analysis was observed at 10 and 100 mg/kg of chloroform extract of *T. crispa*. The body weights of the rats were variable between the groups, depending on different factors, such as gender, age & disease condition. A total of 6 deaths were reported in several groups which were administered with 500 mg/kg of the chloroform extract of *T. crispa*. The *in vitro* effect of the chloroform extract of *T. crispa* on aminopyrine metabolism in young male diabetic rat hepatocytes was probably mediated through various molecular pathways i.e. via the cAMP pathway at lower concentrations; as a phosphatase stimulant and an inhibitor of protein kinase C. In the presence of furafylline, the chloroform extract of *T. crispa* enhanced the activity of cytochrome $P450_{1A2}$. In conclusion, the chloroform extract of *T. crispa* affected Phase I aminopyrine *N*-demethylation in certain groups of rat liver. Caution must be exercised when using the chloroform extract of *T. crispa* at doses equal to or greater than 500 mg/kg for the long-term because it significantly elevated the ALT (alanine aminotransferase) level in adult male rats.

CHAPTER 1: INTRODUCTION

1.1 Herb-Drug Interactions

Nowadays, people are aware of the herb-drug interaction, especially for those patients on co-morbidity medication. Herb-drug interaction is a hot topic of debate because many herbs have not been studies or reported. The concerns are multiplied for those patients currently taking multiple medications, often prescribed by multiple physicians who may or may not be in communication with each other regarding their patient's medication history (Subhuti, 2000). Non-error, adverse effects of medications account for the fourth leading cause of death in the United States at 106,000 deaths per year (Starfield, 2000; Castleman, 1998; Subhuti, 2000), while annual deaths from herbs until 2002 were only a handful nationwide (Duke, 1985; 2001 TESS Annual Report, 2002). The increased use of herbal medicines has resulted in increased concern about the efficacy and the safety of these products. Indeed, there has been a number of reports of toxic effects from traditional and herbal medicines – the WHO database currently lists 8985 adverse drug reaction reports in which herbal preparations are suspected of being implicated (Global standardization for herbal medicines, 1998). It is important for herbalists, pharmacists and physicians to understand the nature of herb-drug interactions, and to defend their profession and their products from unjustified attack.

Many whole herbs have remained in the pharmacopoeias of nations that now rely on modern medicine, but the difficulties in procuring and handling the high-quality herbal materials has led to replacements by isolates and synthetics (Subhuti, 1998). Within the context of using intensively regulated, pure chemical

drugs, medical doctors have correctly raised questions about the identity and variability of herbs that have been provided as alternatives without adequate clinical testing and without monitoring of adverse effects once on the market. On the other hand, herbs (as well as other natural healing approaches) were sometimes dismissed under the incorrect premise that modern medicine had already provided a satisfactory replacement for all the earlier health care practices that had been relied upon for centuries (Subhuti, 1998).

Herbs have been in demand for the past few years. Normally herbs served in the market are in the form of beverage teas which can produce a good taste, and typically provided in convenient tea bags. However, as the demand of herbs grew, some medicinal herbs have been sold in the form of capsules and tablets which often containing standardized extracts or specified levels of one active ingredient. More people then turned to herbs in these dosage forms as an alternative to pharmaceutical drugs (e.g. take echinacea instead of an antibiotic; St. John's wort instead of an antidepressant) (Subhuti, 2000). However, as the use of drugs has increased dramatically in recent years, especially among the elderly, herbs have also been increasingly used with other drugs rather than in place of drugs, raising the concern about the interaction of herbs and drugs. From the perspective of the modern medical profession, if an herb or herbal mixture can have a therapeutic activity that is comparable to that of a drug, it can also interact with other drugs (Leake, 1975; Sionneau, 1995; Subhuti, 2000).

Herbs were a dominant medical therapy in the Chinese culture during the 20th century, and pharmaceutical drugs were a relatively recent addition to their medical field (Subhuti, 1998). But, it is in a different situation in the western countries. Herbs had been almost entirely replaced by drugs during the 20th century, and were later reintroduced once drugs had become a dominant feature of modern health care. In the west, the replacement of herbs by drugs took place over a period of many decades during which there was a prevailing attitude that drugs were more reliable than herbs. The re-introduction of herbs brings with it suspicions and concerns about their unreliability and the lack of adequate knowledge about them (Subhuti, 1998). The herbs listed in **table 1.1** are used in the Chinese tradition. Warfarin is the drug most often suggested to have interactions with other herbs (Subhuti, 1998; Subhuti, 2000).

Nowadays, doctors and pharmacists are provided with courses and educational materials which outline the potential problems associated herbs, with the need of herb-drug interaction. There is a considerable amount of speculation about herb-drug interaction, based on knowledge which is also quite limited pertaining to drug-drug interactions and food-drug interactions.

1.1.1 Concepts of Herb-Drug Interactions (Definition)

A basic problem is that the phrase "herb-drug interaction" routinely appears in the media, without definition and an assumption that everyone knows what it meant. There are several distinct categories of interactions defined in pharmacology texts. The basic concept of interaction is that consumption of two or more substances, either herbs, drugs, foods, or liquids at the same time, or within a short time of each other, will result in physiological effects that are different from a simple summation of the effects of either taken separately, which is the basic definition of a non-linear effect. The vast majority of so-called "interactions" is probably not interactions at all and is simple linear effects, but result from individual users' ignorance of the total known clinical effects of a substance, published in standard clinical materia medica. Besides, relatively less common type of interaction is a pharmaceutical interaction in which the two substances interact by direct chemical action with each other, either before ingestion, or while mixed together in the stomach and intestines (Roger, 2004).

The nature of herb-drug interactions is not a chemical interaction between a drug and an herb component to produce something toxic (Subhuti, 2000). Instead, the interaction may involve having an herb component cause either an increase or decrease in the amount of drug in the blood stream. A decrease in the amount of drug could occur by herb components binding up the drug and preventing it from getting into the blood stream from the gastrointestinal tract, or by stimulating the production and activity of enzymes that degrade the drug and prepare it for elimination from the body. An increase in the drug dosage could occur when an herb component aids absorption of the drug, or inhibits the

enzymes that break down the drug and prepare it for elimination. A decrease in drug dosage by virtue of an interaction could make the drug ineffective; an increase in drug dosage could make it reach levels that produce side effects. Alternatively, an herb might produce an effect that is contrary to the effect desired for the drug, thereby reducing the drug effect; or, an herb might produce the same kind of effect as the drug and give an increase in the drug effect (without increasing the amount of the drug) (Subhuti, 2000).

Examples of concerns about herb-drug interactions that have been raised (Subhuti, 2000) are that an herb might:

- increase or decrease the effect of a blood thinner such as warfarin and lead to either a bleeding episode or formation of a dangerous clot;
- decrease the effect of a blood pressure medication, leading to high blood pressure and a stroke;
- decrease the effect of an anti-infective agent, letting the infection get out of control; or
- increase the effect of an anti-diabetics drug and plunge blood sugar to dangerously low levels.

Such responses can occur with drug-drug interactions and with food-drug interactions, so the finding of some instances of herb-drug interaction would not be surprising (Refer to **Table 1.2**).

Table 1.1: Some Herbs Recently Mentioned as Having Potential for Drug Interactions (taken from Subhuti, 2000)

Herb	Source	Interactions Reported or Suspected
St. John's wort *tianjihuang*	*Hypericum perforatum* (tops)	warfarin (to cause bleeding); serotonin-uptake inhibitors (to cause mild serotonin syndrome); indinavir (increased bioavailability); digitoxin, theophylline, cyclosporin, phenprocoumon, and oral contraceptives (all with reduced bioavailability)
Ginseng *renshen*	*Panax ginseng* (root)	antidepressants such as phenelzine sulfate (to cause manic episodes, headaches); warfarin (to cause bleeding or to decrease effectiveness); corticosteroids (potentiation); estrogens (potentiation)
Ginkgo *yinxingye*	*Ginkgo biloba* (leaf)	warfarin (to cause bleeding)
Ginger *jiang*	*Zingiber officinale* (rhizome)	sulfaguanidine (enhance absorption)
Garlic *dasuan*	*Allium sativum* (bulb)	warfarin (to cause bleeding)
Tang-kuei *danggui*	*Angelica sinensis* (root)	warfarin (to cause bleeding)
Salvia *danshen*	*Salvia miltiorrhiza* (root)	warfarin (to cause bleeding)
Rhubarb *dahuang*	*Rheum officinale* (root)	cardiac glycosides and antiarrhythmic agents (potentiating by reducing potassium via laxative effect)
Aloe *luhui*	*Aloe ferox* (leaf sap)	cardiac glycosides and antiarrhythmic agents (potentiating by reducing potassium via laxative effect)
Ma-huang *mahuang*	*Ephedra sinica* (leaf)	MAO (monoamine oxidase) inhibitors (to cause hypertension); cardiac glycosides or halothane (to produce cardiac arrhythmia); caffeine (to intensify cardiovascular side effects)
Astragalus *huangqi*	*Astragalus membranaceus* (root)	cyclosporine, azathioprine, methotrexate (to impair intended immuno-suppressive effects).
Bupleurum *chaihu*	*Bupleurum falcatum* (root)	sedatives (potentiation)
Licorice *gancao*	*Glycyrrhiza uralensis* (root)	corticosteroids and thiazide diuretics (potentiation); digitalis or other cardiac glycosides (increased sensitivity)

Table 1.2: Chinese Herbal Concept of Interaction of Drugs
The common term used for interaction is *xiang*, literally, mutual or reciprocal action (that is, the drugs act upon one another) (taken from Subhuti, 2000)

Type of Interaction	Examples
Xiangfan (incompatibility): yields toxic reaction or side effects.	Raw aconite is incompatible with raw pinellia; licorice is incompatible with sargassum; veratrum is incompatible with scrophularia.
Xiangwu (antagonism): reducing the desired effect of one drug by combining with another drug.	Raphanus inhibits the action of ginseng; ginseng inhibits the action of pteropus; clove inhibits the action of curcuma.
Xiangsha (detoxification) or *Xiangwei* (inhibition): one drug reduces the toxicity or side effects of another.	Fresh ginger and alum each reduce the toxicity of pinellia and arisaema.
Xiangshi (enhancement): one drug enhances the effect of the other.	An herb that tonifies the spleen, when combined with an herb that is diuretic will enhance the diuretic action.
Xiangxu (synergism): drugs of similar nature reinforce each other's effects.	Rhubarb and mirabilitum are each purgatives that produce a more reliable and stronger purgative action when used together.

1.1.2 Current Issues - Synergies and Antagonisms

It has been observed by some herbalists and phytochemists that, in many cases, the presumed "active" ingredients of an herb, when extracted and isolated, do not have quite the same pharmacological effects as the whole plant preparation (Roger, 2000). That this is a common experience is often plausibly explained as the result of complex chemical synergies within the whole herb preparation. Complexity in the number and type of chemical constituents may merely mean that the net effect of all together may be difficult to predict, and part of this difficulty may or may not be due to true synergies, or non-linear interactions between constituents (Subhuti, 2000). Perhaps the most convincing evidence of phytochemical synergies within a given plant species is discussed by Duke (1998). He points out various examples of pairs of chemical constituents having a measured pharmacological effect different from that expected from each constituent administered individually and discusses the theory that plants have evolved to make the most efficient use of their biochemical machinery. If clusters of closely related or complementary chemical constituents create adaptive synergistic effects, then such clusters will tend to be favored by evolution. If this theory is true, it weighs heavily against the interests of pharmaceutical companies in isolating a few active ingredients and then selling them to the public, if the whole raw herbs provide the advantages of inherent synergies of intra-species phytochemical groups (Duke, 1998).

Even assuming that synergistic effects among clusters of phytochemicals within a species might be common, if the net effect of such clusters is to maximize the adaptive pharmacologic effects for the survival of the plant itself, it does not

necessarily follow that combining herbs together will result in even more synergies. There would not necessarily be any additional adaptive benefit for two plant species to evolve merely so that humans could gain synergistic effects when consuming them together. The only exceptions to this might be in plants that coexist in nature and have a symbiotic or commensal relationship. If such effects do exist, they are much more likely to be accidental (Subhuti, 2000).

Herbs can have significant positive effect when used as part of a program of drug therapy, such as reducing the side effects as mentioned in Subhuti's review (1998), but the concerns being raised by doctors and patients are for the potential adverse responses. The interactions depicted here (**Table 1.3**) may involve foods, herbs, or nutritional and non-nutritional supplements; the information stated in terms of herbs should be applied to any substance with a potential for drug interactions.

Table 1.3: Information for Practitioners to Relay to Patients about Interactions with Drugs (taken from Subhuti, 1998)

Type of Interaction	Examples	Patient Information
Drug absorption inhibited by binding, resulting in low drug levels.	Tetracycline with minerals; alkaloids with tannins; pectins, resins, and fibers may bind several drugs.	Take herbs at least one hour apart, preferably 1.5 hours apart from taking drugs.
Drug absorption inhibited by rapid transit time, resulting in low drug levels.	Diarrhea or frequent bowel movements due to colitis or laxative intake speeds transit of all materials through the intestinal tract.	Treat diarrhea and avoid excessive use of laxatives. Induction of diarrhea is an intended treatment strategy in Chinese medicine for nephritis.
Drug absorption and/or elimination modified.	Saponins may improve absorption and elimination of drugs, altering the blood levels and rate of change of drug levels; strongly acid or alkaline herbs may alter absorption of drugs.	Take herbs at least 1.5 hours apart from drugs; avoid herbal preparations that have high saponin content.
Drugs metabolized too slowly resulting in elevated drug levels.	Grapefruit juice and herbs that inhibit CYP (cytochrome) enzyme system can result in much higher levels of drugs in the bloodstream, and longer persistence of the drugs.	Take herbs at least 1.5 hours apart from drugs, preferably taking the drugs first (so that drug metabolism is already under way by the time the herbs can inhibit enzyme systems).
Potassium decreased when using cardiac drugs, resulting in adverse cardiac conditions.	Laxative and diuretic herbs may reduce potassium; these types of herbs are often given together for weight loss.	Avoid any strong laxative or diuretic action while using cardiac drugs. To compensate for mild diuretic or laxative treatments, consume high-potassium foods.
Drug action is intensified by similar effect of herbs.	Blood vitalizing herbs and blood thinning drugs may prevent adequate clotting; hypoglycemic herbs and hypoglycemic drugs may lower blood sugar too far; caffeine or ephedrine containing herbs and central nervous system (CNS) stimulants disturb nerve functions.	When the drug therapy is already addressing a particular therapeutic goal, avoid adding an herbal therapy with the same goal. Intensify monitoring of blood conditions affected by the drugs.

1.2 Monograph of *Tinospora crispa* Miers.

Tinospora crispa Miers. (Family: Menispermaceae) is a native of South-East Asia (Perry, 1980). The plant family Menispermaceae consists of 65 genera and 350 species which are mainly tropical twining herbs, shrubs and trees. *Tinospora crispa* is commonly known as Glunchanb or Tinospora in English and Guduchi in Sanskrit. The virtues of this plant, which were long known to native physicians, attracted the attention of Europeans in 1827 (James & Thomas, 1993). In Malaysia, it is known as Patawali, Putarwali or "akar seruntum" in Malay (Muhamad & Mustafa, 1994); Baw-ra-pet, borapet or heart-leaved moonseed in Thailand (Naomichi *et al.*, 1983; Scott, 1998); ratnawali in Brunei; Makabuhai in Philipines; and K'uan Chu Hsing in Chinese (Pankaj, 2003). It is widely distributed throughout tropical Indian subcontinent and China, ascending to an altitude of 300 miles. In Hindi, the plant is commonly known as Giloe, Ambervel or Giloya, which is a Hindu mythological term that refers to the heavenly elixir that have saved celestial beings from old age and kept them eternally young. Through the centuries, it has been extensively used in various Ayurvedic preparations for the treatment of various ailments (Kirtikar & Basu, 1933; Thatte *et al.*, 1987). According to the ayurvedic lexicons, *T. crispa* is referred to as "Amrita", which means its ability to impart youthfulness, vitality and longevity to its patron. Thus, *Tinospora* species is categorized in Ayurveda as "Rasayana".

1.2.1 Taxonomy

Division: Spermatophyla

Sub Division: Angiospermae

Class: Dicotyledonae

Order: Ranunculales

Family: Menispermaceae

Genus: Tinospora

Species: *Tinospora crispa* Miers. (Muhamad & Mustafa, 1994)

Synonyms: *Tinospora tuberculata* Beumée; *Tinospora rumphii* Boerl. *Tinospora cordifolia* Miers.; *Tinospora baenzigeri*; *Tinospora fragosa* (Fukuda *et al.*, 1993; Teresita *et al.*, 1995; Cavin *et al.*, 1998)

1.2.2 Morphology

Tinospora crispa Miers. is a climbing herb with spotted stems and about 1.0 to 1.5 centimeters in diameter. It is usually grown in the backyards with climbing on other vegetation or on the fences as a medicinal plant (see **Plates 1.1 & 1.2**). The young stem of the plants appears green and gradually turns to brown and woody when old. The characteristic feature of the plant is the surface of the stem which is warty. The stems shows a radiating pattern, which twine around the other plants for its physical support. The bark is corky and tubercled and the leaf is broad cordate and acuminate with 5 to 14 centimeters long, and 3 to 10 centimeters wide, and the leaf veins are very obviously seen. It has petiole in 3 to 10 centimeters long and oppositely arranged. The flower is dioeciously, pedicellate and in pale yellow, and the main flower stalk is a long raceme. The female flower is isolated and has oblong-spatulate petals, small stamen, and carpel in three or usually less. The male flower is in small fasciles with six sepals, and all is in two rows; the outer row consists of short and rounded sepals while the sepals in the inner row is twice as long, obovate, broad, convex and smooth as petals and stamens (Zhari *et al.*, 1999).

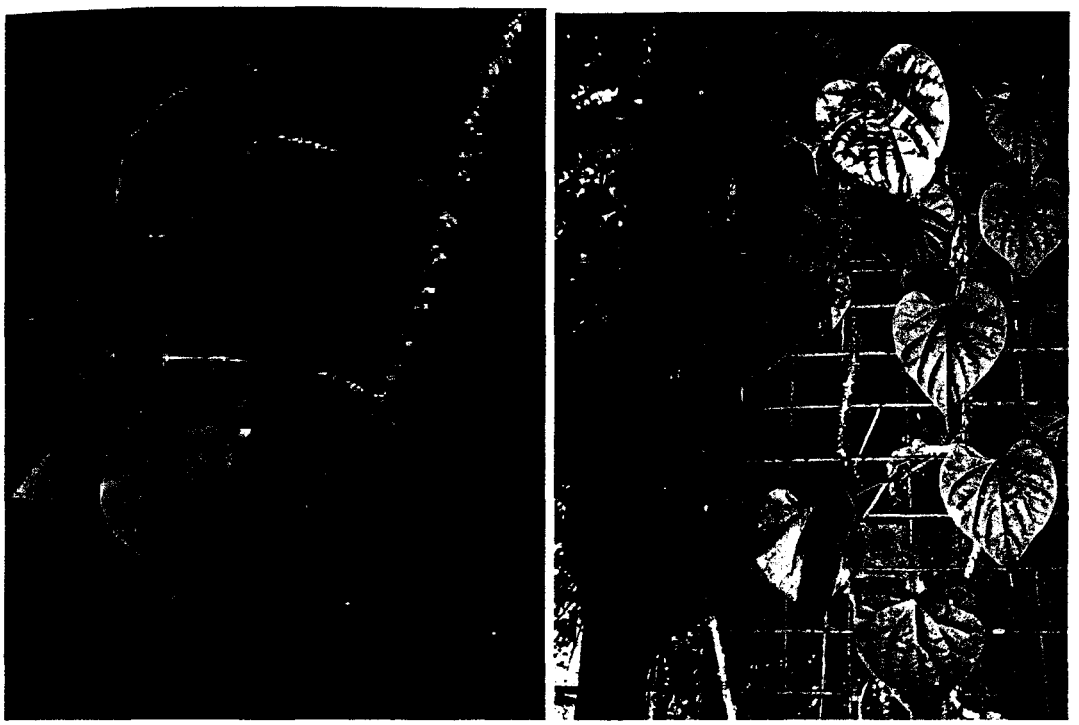


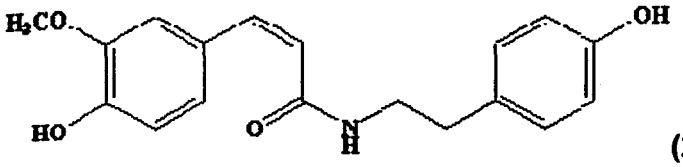
Plate 1.1 & Plate 1.2: The stems of *Tinospora crispa* plant obtained from Balik Pulau, Penang, Malaysia. The plant has been identified and voucher specimen was deposited in the herbarium of the School of Pharmaceutical Sciences, Universiti Sains Malaysia, Penang, West Malaysia. (Picture Source: Personal)

1.2.3 Constituents of *Tinospora crispa*

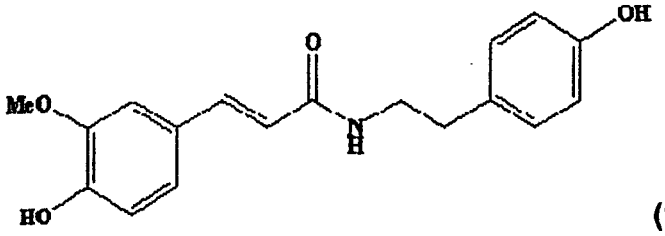
A variety of constituents have been isolated from *Tinospora crispa* and other *Tinospora species*, and their structures were elucidated. They belong to different classes such as alkaloids, diterpenoid lactones, glycosides, steroids, sesquiterpenoids, phenolics, aliphatic compounds and polysaccharides. The leaves of this plant are rich in protein (11.2 %) and are fairly rich in calcium and phosphorus (Khosa & Prasad, 1971). The bitter principles have been identified by some researchers such as columbin, a diterpenoid furanolactone (Swaminathan *et al.*, 1989a), chasmanthin and palmarin (Bhide *et al.*, 1941).

1.2.3.1 Alkaloids

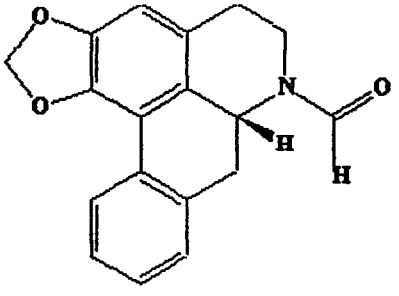
The plant family Menispermaceae has long served as a rich source of alkaloids. Several compounds has been isolated from *Tinospora* species such as N-trans-feruloyl-tyramine **(1)**, N-cis-feruloyl-tyramine **(2)** (Fukuda *et al.*, 1983), N-formylannonaine **(3)**, N-formylnornuciferine **(4)**, N-acetylnornuciferine **(5)** (see Figure **1.1**) (Pachaly & Adnan, 1992), palmatine, berberine, jatrorrhizine, tembetarine, choline (Bisset & Nwaiwu, 1983), magnoflorine (Qudrat-I-Khuda *et al.*, 1964), tinosporin, N-formyl- and N-acetyl-aporphine (Pachaly & Adnan, 1992) isocolumbin, and tetrahydropalmatine (Sarma *et al.*, 1998).



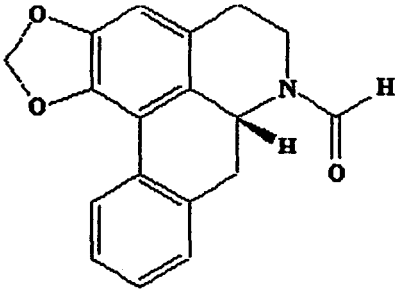
(2) N-cis-feruloyl-tyramine



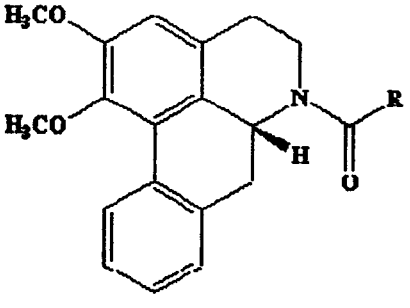
(1)N-trans-feruloyl-tyramine



(3E) N-formylannonaine

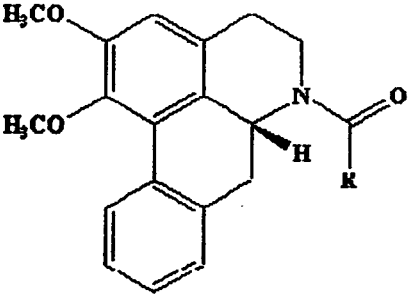


(3Z) N-formylannonaine



(4Z) N-formylnornuciferin; R=H

(5Z) N-acetylnornuciferin; R=CH_3



(4E) N-formylnornuciferin; R=H

(5E) N-acetylnornuciferin; R=CH_3

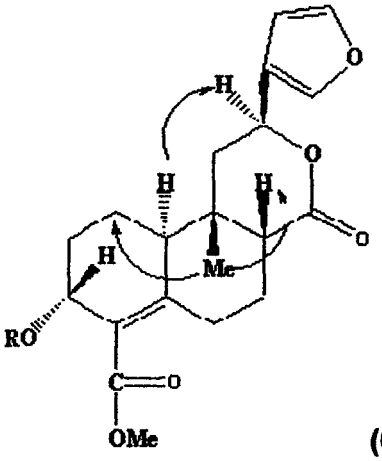
Figure 1.1: Alkaloids isolated from *Tinospora cordifolia*

1.2.3.2 Glycosides

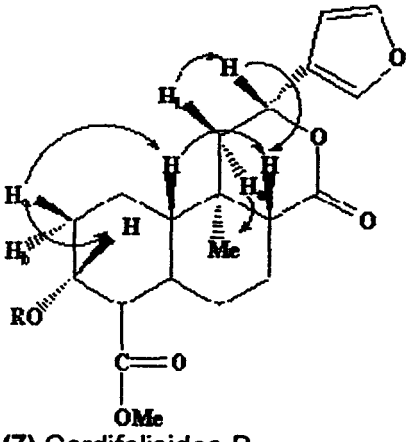
The more polar *n*-butanol fraction from *Tinospora* species has produced some glycosides compounds. There were cordifolisides A **(6)**, B **(7)**, C **(8)** (see Figure **1.2**); norditerpene furan glycosides (Gangan *et al.*, 1994), cordifoliside D & E (Gangan *et al.*, 1995), syringin, syringin-apiosylglycoside, 18-norclerodane glucoside (Khan *et al.*, 1989), tinocordiside (Ghosal & Vishwakarma, 1997), tinocordifolioside (Maurya & Handa, 1998), cordioside (Wazir *et al.*, 1995), and cordifolioside B (Gangan *et al.*, 1994; Maurya *et al.*, 1996).

1.2.3.3 Diterpenoid lactones, Clerodane-derived terpenes & Sesquiterpenes

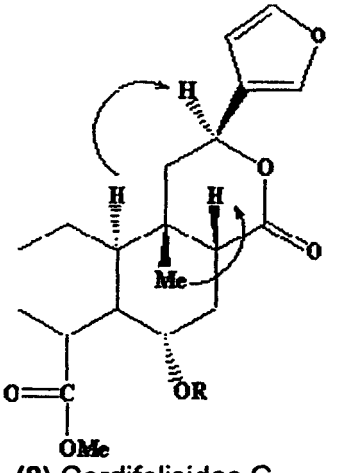
Tinospora cordifolia has been claimed to be the species which is rich in clerodane derived diterpenes (Pachaly & Adnan, 1992; Fukuda *et al.*, 1993; Gangan *et al.*, 1995; Maurya, 1996). Several new clerodane furano diterpene glucosides have been designated as amritosides A **(9)**, B **(10)**, C **(11)**, (see Figure **1.3**) and D (Maurya *et al.*, 2004). Other compounds isolated from this species such as tinosporaside **(12)**, tinosporide **(13)** (Swaminathan *et al.*, 1989; Hanuman *et al.*, 1986), furanolactone (Hanuman *et al.*, 1988), tinocordifolin (Maurya & Handa, 1998), tinosponone, tinocordioside (Maurya *et al.*, 1995), (5R,10R)-4R,8R-dihydroxy-2S,3R:15,16-diepoxycleroda-13 (Pachaly & Schneider, 1981),14-dieno-17,12S:18,1S-dilactone (Swaminathan *et al.*, 1988), columbin **(14)** (see Figure **1.4**) (Swaminathan *et al.*, 1989a), tinosporon (Qudrat-I-Khuda *et al.*, 1966), and jateorine (Bhatt & Sabata, 1990).



(6) Cordifolisides A

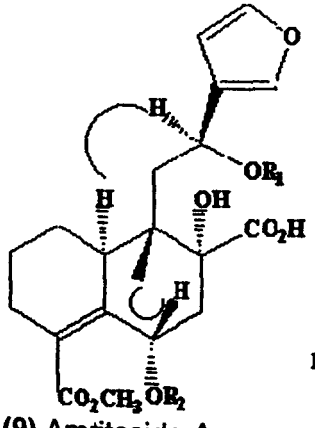


(7) Cordifolisides B

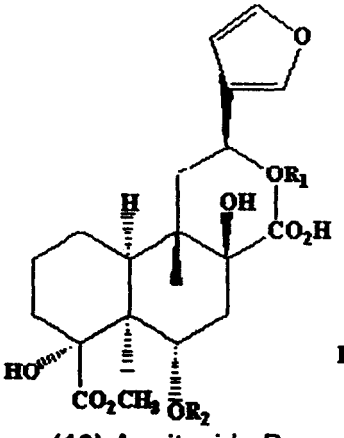


(8) Cordifolisides C

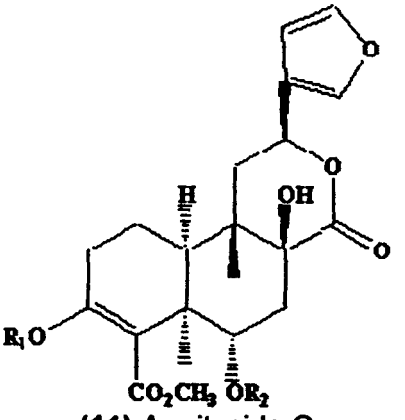
Figure 1.2: Glycosides isolated from genus *Tinospora*



(9) Amritoside A;



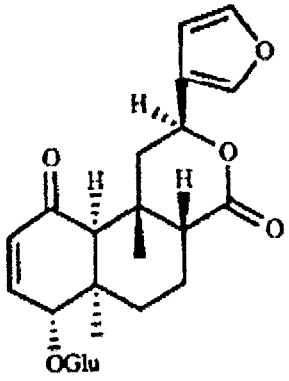
(10) Amritoside B;



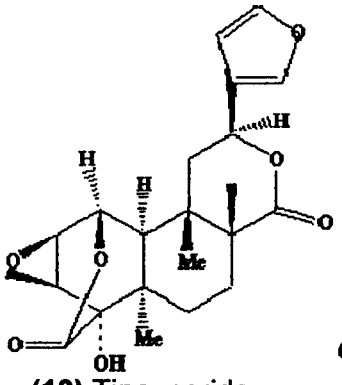
(11) Amritoside C;

R_1=H, R_2=β-D-Glucopyranosyl; Amritoside A/B/C
R_1=Ac, R_2=Tetra-*O*-acetyl-β-D- Glucopyranosyl; Amritoside A/B/C pentaacetate

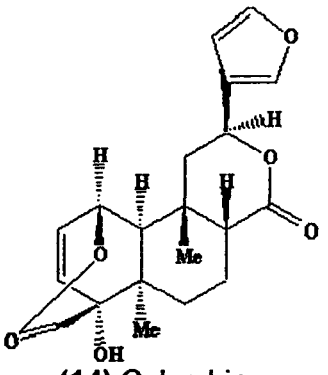
Figure 1.3: Clerodane furano diterpene glucosides from genus *Tinospora*



(12)Tinoporaside



(13) Tinosporide



(14) Columbin

Figure 1.4: Diterpenoid lactones, Clerodane-derived terpenes & Sesquiterpenes isolated from genus *Tinospora*

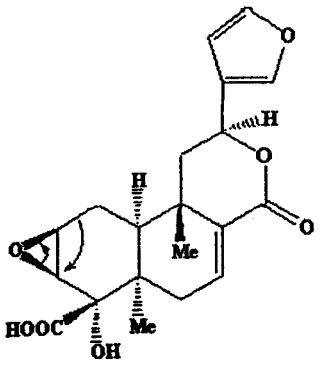
Other species of the genus *Tinospora* have also been isolated and identified such as baenzigeride **(15)** and baenzigeride A **(16)** (see Figure **1.5**) from *T. baenzigeri* (Pittaya *et al.*, 1999), menispermacide, cycloeuphordenol, malabarolide B_1, and 10-α-hydroxycolumbin from *T. malabarica* (Atta-Ur-Rahman *et al.*, 1988; Atta-Ur-Rahman *et al.*, 1992), rumphioside A - F, rumphioside I, and tinoporicide from *T. rumphii* (Teresita *et al.*, 1995; Atta-Ur-Rahman *et al.*, 1991), tinocrisposide, a bitter furanoditerpene glucoside (Pachaly & Adnan, 1992), cycloeucalenol **(17)** and cycloeucalenone **(18)** (see Figure **1.6**) from *T. crispa* (Ngampong *et al.*, 2002).

1.2.3.4 Steroids

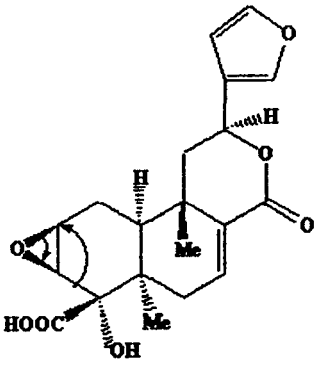
β – Sitosterol, δ-sitosterol, 20β-hydroxy ecdysone, ecdysterone, makisterone A, and giloinsterol were six steroids isolated by Pathak *et al.* (1995), Khaleque *et al.* (1970), Kidwai *et al.* (1949), Pradhan *et al.* (1997), and Gangan *et al.* (1997). However, no further work was carried out to determine their bioactivities.

1.2.3.5 Aliphatic compounds

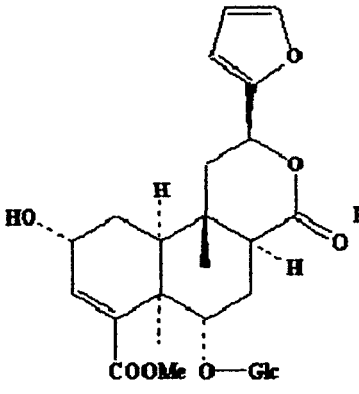
Three aliphatic compounds have been isolated from *Tinospora* species, i.e. octacosanol, heptacosanol and nonacosanol-15-one (Dixit & Khosa, 1971; Hanuman *et al.*, 1986; Khaleque *et al.*, 1970). However, no further work was carried out to determine their bioactivities.



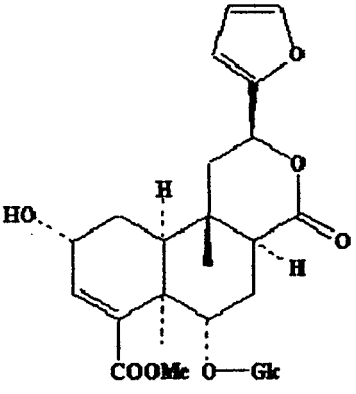
(15) Baenzigeride



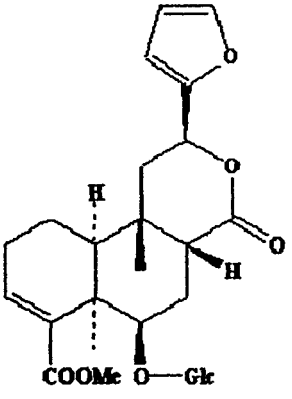
(16) Baenzigeride A



(19) Borapetoside A

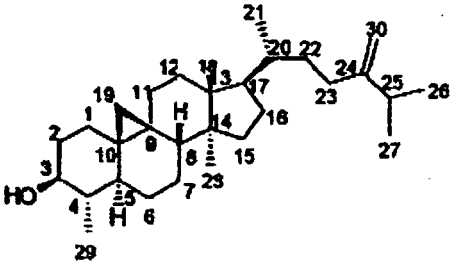


(20) Borapetoside B

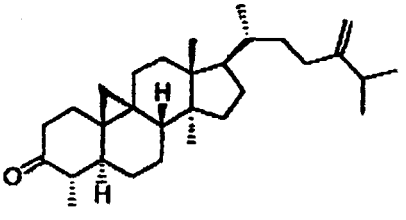


(21) Borapetoside C

Figure 1.5: Diterpenoid lactones, Clerodane-derived terpenes & Sesquiterpenes isolated from genus *Tinospora*



Cycloeucalenol



Cycloeucalenone

Figure 1.6: Isolation of two triterpenes from the stems of *Tinospora crispa*, namely, cycloeucalenol **(17)** and cycloeucalenone **(18)**

1.2.4 Bioactive Properties of *Tinospora crispa*

It has been indicated in Ayurvedic treatment as a tonic, vitalizer and as a remedy for diabetes and metabolic disorders (Nadkarni, 1992; Chopra *et al.*, 1956). All parts of the plant are used as medicine but the stem and root are used most frequently. According to Ayurveda, the stem is bitter, stomachic, tonic, appetizer, antipyretic and expectorant. It is used in the treatment of vomiting, fever, blood disorders, jaundice (Pelia), enlarged spleen, vaginal and urethral discharges, giddiness, piles, anaemia, diabetes, skin troubles and cough. It caught the notice of European physicians in India for its tonic and diuretic properties (Pendse *et al.*, 1977) and the drug became an official preparation in Indian Pharmacopoeia in 1932. Gulancho (*T. crispa*) was also included in Bengal Pharmacopoeia in 1844 and in Indian Pharmacopoeia in 1868. Scientific reports describing anti-diabetic (Gupta *et al.*, 1967), immunomodulatory (Atal *et al.*, 1986), anti-hepatotoxic (Peer & Sarma, 1989), antipyretic (Ikram *et al.*, 1987; Vedavathy & Rao, 1991) and anti-stress activity (Sarma *et al.*, 1995) are available. The leaves of *T. crispa* exhibits anti-diabetic action in alloxan diabetic rabbits (Noreen *et al.*, 1992). Some authors have shown that *T. crispa* roots possess antiulcer effect (Sarma *et al.*, 1995). Recently, Thatte and Dahanukar (1989) have shown that the aqueous extracts of the plant stimulated the phagocytic and bactericidal activity of neutrophils and macrophages.

Tinospora species have been used traditionally for antitoxin action and as a febrifuge (Nadkarni, 1992), and also used for urinary diseases, syphilis, skin diseases, and bronchitis (Kapoor, 1990). Atal *et al.* (1986) showed that an ethanolic extract of *Tinospora species* appeared to improve the phagocytic activity of the reticuloendothelial system in mice. Another recent study analyzed six pure compounds isolated from *Tinospora species* for their immunomodulatory activity (Kapil & Sharma, 1997). The compounds syringin and cordiol inhibited the *in vitro* immunohaemolysis of antibody-coated erythrocytes due to inhibition of the C3-convertase of the classical complement pathway. All six compounds exhibited significant enhancement of IgG (Immunoglobulin G) antibodies, with cordiol having the highest immunostimulating activity. The compounds cordioside, cordiofolioside, and cordiol induced a significant increase in phagocytic activity by activation of the peritoneal macrophages. In an animal model of cholestasis, significant depression of the activities of polymorphonuclear cells (PMN) and peritoneal macrophages was observed (Rege *et al.*, 1989).

It is reported that the daily administration of either alcoholic or aqueous extract of *T. cordifolia* decreases the blood glucose level and increases glucose tolerance in rodents (Gupta *et al.*, 1967; Grover *et al.*, 2001). Aqueous extract also caused a reduction in blood sugar in alloxan induced hyperglycemia in rats and rabbits at a dose of 400 mg/kg. However, histological examination of pancreas has not revealed any evidence of regeneration of β-cells of islets of Langerhans and the possible mode of action of the plant is through glucose metabolism (Raghunathan & Sharma, 1969). The aqueous extract has also

exhibited some inhibitory effect on adrenaline-induced hyperglycemia. Ethyl acetate extract of its roots has afforded a pyrrolidine derivative with hypoglycemic activity in rabbits (Mahajan & Jolly, 1985; Grover *et al.*, 2000; Stanely *et al.*, 2000). Another study has also revealed significant hypoglycemic effect of the extract of leaves in normal and alloxan diabetic rabbits. However, the extract had no significant effect on total lipid levels in normal or treated rabbits (Wadood *et al.*, 1992; Basnet *et al.*, 1995).

1.3 Drug Metabolism & Liver

Drug metabolism or biotransformation is an integral component of the processes that govern the pharmacokinetic profile of therapeutically used drugs. Biotransformation generally converts non-polar, lipophilic pharmacologically active drug molecules into polar, inactive, or nontoxic metabolites that are readily eliminated by the kidneys or other organs. Traditionally, biotransformation pathways are classified into two groups: Phase I reactions that involve functionalization of drug substrates to yield more polar derivatives such as alcohols, phenols, or carboxylic acids; and Phase II reactions involving bimolecular conjugation with endogenous hydrophilic moieties to yield products such as glucuronides and sulfates that are readily excreted by kidneys (Venkatakrishnan *et al.*, 2001).

The liver is the major site of drug metabolism, with intestinal drug extraction playing a secondary yet potentially important role in the pre-systemic elimination of orally administrated agents. The liver has long been recognized as the major organ for all phases of intermediary metabolism (Brauer, 1956; Brauer, 1963).