CHAPTER II

LITERATURE REVIEW

2.1 *Cassia siamea*

Cassia siamea Lamk. is in the genus Cassia, Family Leguminosae and found growing throughout the tropical countries. In Thailand, it has various local names such as Khi-lek-luang, Khi-lek-barn, Khi-lek, Khi-lek-yai, Phak-chee-lee or Ya-ha. *Cassia siamea* Lamk is a medium-sized tree having young branches striate and finely pubescent. Leaves are paripinnate, composed of 7-10 pairs of leaflets; petiole 2-3 cm long; rachis 10-25 cm long, stipules minute, subulate and caducous (Figure 2.1). Leaflets are 3-7 by 1-2 cm, oblong, with base rounded, apex rounded or emarginate with a shot mucronate tip. The upper surface is glabrous while the lower surface is more or less finely pubescent. The petiolules are very short. Flowers are in large terminal panicles on robust 5-7 cm long peduncle. The bracts are obovate, with long acute apex, 5 mm long. The pedicels are velutinous, 2-3 cm long. There are 5, orbicular, thick, unequal sepals, 2 outer ones 5 mm long, 3 inner up to 9 mm long, hairy on outer side. The petals are yellow, broadly obovate, 1.5-2 cm long, short clawed, Figure 2.2. There are 10 unequal stamens, 2 long 5 short and 3 reduced. The ovary is finely pubescent; with glabrous style. Fruit is a flat pod, 20-30 by 1-1.5 cm. There are 20-30, flat, oval, light brown seeds, with 10-15 by 5-6 mm in size (Farnsworth & Bunyapraphatsara, 1992).

Flowers and young leaves of *Cassia siamea* have been used as a vegetable and different parts of the plant have been used for various medical purposes. For

example, the root is used as an antipyretic; the bark is used to treat skin disease and haemorrhoids; the leaves are used in the treatment of constipation, diabetes, dysentery, helminthiasis, hypertension and insomnia, and the flowers are used for the treatment of insomnia and asthma, and are used as an anthelmintic and an antidandruff remedy (Bunyapraphatsara and Chokechaicharoenporn, 1996).

Figure 2.1 Leaves of *Cassia siamea*

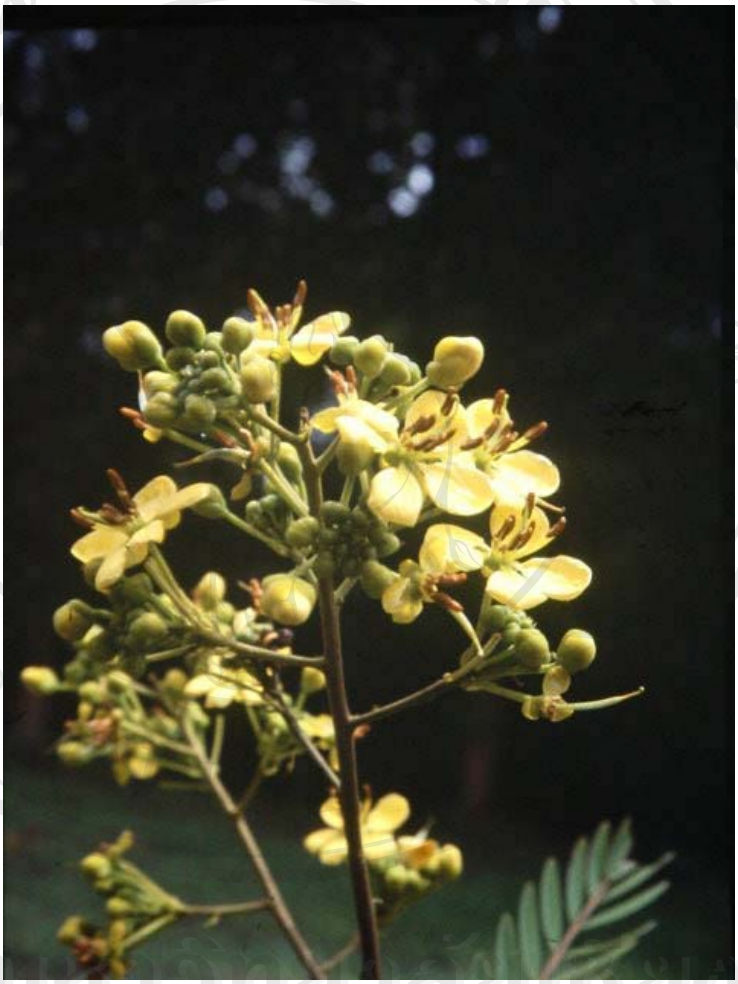


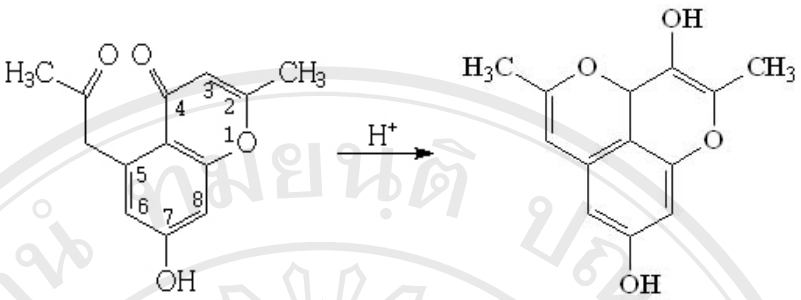
Figure 2.2 Flowers of *Cassia siamea*

2.2 Barakol

2.2.1 Chemical structure and properties of barakol

Chromone is the parent compound of important coloring matters of *Cassia siamea* leaves (Wagner et al., 1978). Barakol, a 3a, 4-dihydro-3a,8-dihydroxy-2,5-dimethyl-1,4-dioxaphenalene, was the product of the acid treatment of chromone (Arora et al., 1971) (Figure 2.3). Barakol was first extracted from the leaves and flowers (Hassanali-Walji et al., 1969). The process of isolation of the barakol was later improved by acid extraction which gave better yields by Chaichantyputh (1979) and Kaokeaw (1992) (0.1 and 0.3%, respectively).

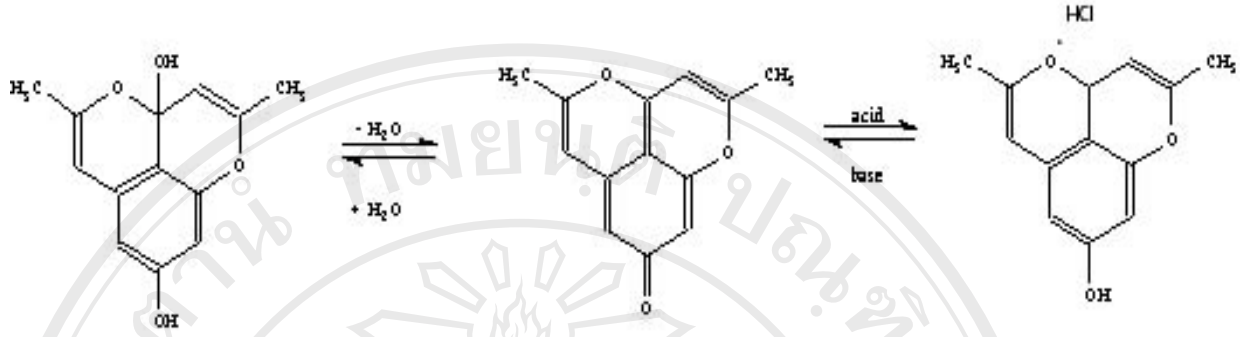
The chemical structure of barakol ($C_{13}H_{12}O_4$) was characterized by Bycroft and colleagues (1970). It contains a tricyclic ring structure and can be crystallized from aqueous methyl alcohol or ethyl alcohol as pale yellow needles. Barakol decomposed at 165 ºC to give a brownish black substance. It was soluble in methyl alcohol, ethyl alcohol, acetone, chloroform, dichloromethane, benzene, carbontetrachloride, ethyl acetate and moderately soluble in water (Hassanali-Walji et al., 1969; Chaichantipyuth, 1979).



5-acetonyl-7-hydroxy-2-methylchromone Barakol

Figure 2.3 Acid treatment of 5-acetonyl-7-hydroxy-2-methylchromone into barakol (Arora, 1971).

Barakol (Figure 2.4a) is usually unstable in normal condition, vacuum or over phosphorous pentoxide by loosing water molecule and become the dark green amorphous compound, anhydrobarakol ($C_{13}H_{10}O_3$) (Figure 2.4b). The resulting anhydrobarakol was extremely unstable. However, this substance can be easily reconverted to barakol by dissolving in aqueous methanol. Barakol is stable in hydroxylic solvents or in moist atmosphere. A relative stable salt of barakol, anhydrobarakol hydrochloride (Figure 2.4c), can be prepared by addition of concentrated hydrochloric acid to a methanolic solution of barakol giving an anhydronium salt (Hassanali-Walji et al., 1969; Bycroft et al., 1970). With strong acids, barakol forms anhydro-salt which does not decompose at room temperature in the solid state. The basic chemical properties of barakol is shown in Table 1.



Barakol (a) Anhydrobarakol (b) Anhydrobarakol hydrochloride (c)

Figure 2.4 Conversion reaction of barakol (a), anhydrobarakol (b) and anhydrobarakol hydrochloride (c) (Bycroft et al., 1970).

Table 2.1 Basic chemical properties of barakol

	Barakol	Anhydrobarakol	Anhydrobarakol hydrochloride
Formular	$C_{13}H_{12}O_4$	$C_{13}H_{10}O_3$	$C_{13}H_{12}ClO_3$
MW	232	214	251.4
Colour	Greenish yellow	Dark green	Lemon yellow
Melting point	165 °C	163 °C	208-210 °C

Adapted from Thongsaard (1998) and Chaichantipyuth (1979).

2.2.2 Physiological and pharmacological properties of barakol

Various studies have been done in order to investigate physiological and pharmacological properties of barakol and are summarized as follows:

2.2.2.1 Antimicrobial effects

Barakol has a low anti-microbial effect to gram-positive (*S. aureus* and *B. subtilis*) and gram-negative (*E. coli, S. typhi, S. dysenteriae* and *P. aeruginosa*) bacteria and yeast-like fungus (*C. albican*) (Gritsanapan et al., 1989).

2.2.2.2 Effects on the cardiovascular system

Barakol, 0.5-15 mg/kg iv, causes a significant dose dependent hypotension in both systolic and diastolic blood pressure in rats and cats. In *in vitro* study, barakol (10^{-5}-10^{-3} M) produced significant reduction in the contraction of isolated rat thoracic aorta induced by phenylephine. Barakol (10 mg/kg i.v.) reduced heart rate and decreased the systemic blood pressure in anaesthetized rats. The hypotensive effects of barakol could be due to peripheral vasodilatation (Suwan et al., 1992).

Pretreatment with barakol (10 mg/kg, i.v.) reduced the incidence of acetonitrile-induced ventricular fibrillation and ventricular tachycardia (Chen et al., 1999). Acetonitrile prolongs the open state of sodium channels, leading to an accumulation of intracellular Na^+ (Sawanobori et al., 1987). Na^+ accumulation eventually results in intracellular Ca^{2+} overload, causing a delay after depolarization which may produce cardiac arrhythmias (Leder and Tsien, 1976). The mechanism of the protective effects of barakol on acetonitrile-induced cardiac toxicity may related to the prevention of intracellular Na^+ accumulation (Chen et al., 1999).

2.2.2.3 Effects on isolated tissue preparations

Barakol has no effect on the contraction of isolated guinea pig ileum (Gritsanapan et al., 1989). It has no effect on the contraction of the porcine tail artery, rat vas deferens and guinea pig ileum produced by field stimulation (Thongsaard et al., 1997a).

2.2.2.4 Effects on central nervous system

In sleeping behavior, delta and theta waves are correlated with sleep stages while alpha and beta waves are correlated with vigilance state. The increase in (delta+theta)/(alpha+beta) energy ratio indicating that the rat was sleeping (John, 1977). Barakol (25 mg/kg, i.p.) modified sleep architecture by significant increasing of (delta+theta)/(alpha+beta) energy ratio, while 100 mg/kg-dose caused no significant change in energy ratio (Bulyalert, 1993).

Thongsaard and colleagues (1996) showed that barakol (10 mg/kg, i.p.) significantly increased all behavioral parameters of the elevated pluz-maze test (unconditional animal model for anxiety) similar to that of diazepam. However, it has been reported contrarily later that there was no evidence of an anxiolytic effect barakol in the same test and it failed to affect any primary behavioral measurements of anxiolytic in the shock-probe (conditional animal model for anxiety) test (Fiorino et al., 1998; Sukma et al., 2002). A clinical trial on *Cassia siamea*, syrup and tablets (contained barakol 10 mg/dose) were found to be helpful in the insomniac patients (Muangman et al., 2000).

For rodent behaviors, barakol (50-100 mg/kg, i.p.) reduced spontaneous locomotor activity, increased the number of sleeping animals and

prolonged the thiopental-induced sleeping time, indicating a sedative effect (Jantarayota, 1988; Sukma et al., 2002).

Barakol (5, 10 and 20 mg/kg, i.p.) failed to antagonize the effects of convulsion induced by bicucullin (GABA receptor antagonist) and strychnine (glycine selective competitive antagonist) suggesting that the sedative effect may not be induced via the GABA or glycine systems (Jantarayota, 1988; Tongroach et al., 1992; Sukma et al., 2002). But a high dose of barakol (100 mg/kg, i.p.) prolonged the latency of the clonic convulsion induced by picrotoxin (blocks chloride ion channel) (Sukma et al., 2002). The sedative effect of barakol may be produced via the chloride ion channel, like barbiturates (Wilcox and Gonzales, 1995), or other pathways.

Barakol at higher dose (100-200 mg/kg, i.p.) showed analgesic effects by increasing nociceptive threshold in hot-plate test (Jantarayota, 1988).

2.2.3 Actions of barakol on neurotransmitters associated with depression

2.2.3.1 Effect of barakol on 5-hydroxytryptamine (5-HT)

Serotonin (5-HT) is the primary neurotransmitter modulating the excitatory catecholamine systems in the CNS; serotonin system control memory, mood, sex drive, appetite, etc. Decreased serotonin levels at the synapses in the brain are associated with decreased mood whereas serotonergic agents (i.e., fluoxetine) tend to improve mood (Griebel, 1995; Charney, 1998). Disruption of serotonergic tone can affect the exploratory behavior of animals (Walters, 2003).

Barakol (25 mg/kg, i.p.) enhanced serotonergic activity by increasing head shake behavior in rats produced by injection of 5-hydroxytryptophan, the 5-HT

precursor. But at higher dose (50-100 mg/kg, i.p.), barakol suppressed serotonergic activity by decreasing head shake behavior (Jantarayota, 1988). This suggested that effects of barakol on 5-HT system might depend on dose.

From the study of brain tissue extraction (hippocampus and nucleus accumbens) after administration of barakol, the results indicated that barakol has no effect on the levels of 5-HT and its metabolites (Thongsaard, 1997). The ratio of a 5-HT metabolite, 5-hydroxyindolacetic acid, over 5-HT (5-HIAA/5-HT) which is used as an index of the turnover of the neurotransmitter (Curzon and Marsden, 1975; Lavielle et al., 1978) also were not affected by barakol suggesting the compound may have no effect on the activities of enzymes in 5-HT synthesis and metabolism in the brain. The results with barakol on *in vitro* endogeneous 5-HT release from rat hippocampal slices demonstrated that barakol (1 μM) significantly increased 20 mM[K+]-stimulated 5-HT release (Thongsaard, 1997). The increase in release may result from an antagonist acting of barakol at the terminal 5-HT autoreceptor in the rat preventing feed-back inhibition (Thongsaard et al., 1997a).

2.2.3.2 Effect of barakol on dopaminergic system

Dopamine is a neurotransmitter that modulates reward. It is one of a monoamine that may involved in depression. Low levels of dopamine may be presented in patients with depression. It thus appears that the mesolimbic dopamine system plays a permissive role in the antidepressant activity (Fielding and Lal, 1978; Cervo et al., 1990; Charney, 1998).

Dopamine receptor antagonist (i.e., fluphenazine), administered either systemically or into the nucleus accumbens septi, blocks the effect of various

antidepressants in the forced swimming test (Borsini et al., 1984; Cervo and Samanin, 1987; Cervo et al., 1990).

In rats treated with unilateral 6-hydroxydopamine lesions of the nigrostriatal pathway, barakol enhanced dopaminergic activities by increase dopamine receptor stimulation effect of apomorphine (Jantarayota, 1988), a direct acting dopamine receptor agonist (Anden et al., 1967). And at low dose (10 mg/kg, i.p.) barakol increased spontaneous locomotor behavior (Thongsaard, 1998).

Barakol (25-100 mg/kg, i.p.) could suppress methamphetamine (1 mg/kg, i.p.)-induced hyperlocomotor activity in a dose-dependent manner, indicating an effect on the dopaminergic system. In a microdialysis study, the dose of barakol (100 mg/kg, i.p.) that inhibited spontaneous locomotor activity in mice did not affect the basal levels of extracellular dopamine or its metabolites in the striatum. Pretreatment with barakol (100 mg/kg, i.p.) decreased the maximal dopamine release and dopamine turnover induced by methamphetamine (1 mg/kg, i.p.) (Sukma et al., 2002). This finding indicates that the inhibitory effect of barakol on dopamine release may account for the blocking effect of barakol on the striatum-related behavior induced by methamphetamine (Sukma et al., 2002). At higher dose (100 mg/kg, i.p.), this effect might be mediated by D2-like presynaptic receptors because barakol decreased striatal dopamine efflux.

From the suggested that the tissue levels of dopamine (DA) and metabolites, as well as, the ratios of DA metabolite, dihydroxyphenylacetic acid (DOPAC) over DA (DOPAC/DA) which is an index of the turnover of the neurotransmitters (Lavielle et al., 1978). This ratio was not change after barakol administration (Thongsaard, 1997). The results suggested that barakol may have no

effect on the activities of enzymes in dopamine synthesis and metabolism. The behavioral effect of barakol are not the result of gross changes in dopamine availability but may due to a direct effect on dopamine release (Thongsaard, 1998).

The investigation of the effects of barakol on the *in vitro* release of endogenous and radiolabelled dopamine from rat striatal slices in comparison with the dopamine receptor agonist, quinlorane and pergolide, and the dopamine receptor antagonist, eticlopride. The [3H]DA release is sensitive to temperature, K(+)-stimulation, and to both a dopamine agonist (pergolide) and an antagonist (eticlopride). Endogenous dopamine release was also stimulated by high K+ (20 mM) and sensitive to a dopamine agonist. Pergolide reduced both [3H]DA and endogenous dopamine release, while eticlopride increased [3H]DA, but not endogenous dopamine release (Thongsaard et al., 1997b). Barakol (1, 10, 100 µM) reduced K(+)-stimulated endogenous dopamine release as did the dopamine D2 receptor agonist but had no effect on [3H]dopamine release. The inhibition of barakol (10 µM) on K(+)-stimulated endogenous dopamine release was antagonized by a dopamine D2 receptor antagonist, eticlopride. Barakol (0.1 nM-10 µM) had no effect on [3H]DA uptake except at the highest concentration (100 µM) when inhibition was observed.

Barakol inhibited in vitro dopamine release in a similar manner to quinelorane and pergolide (Thongsaard et al., 1997a). Quinelorane is a dopamine D3 receptor-preferring agonist (Duarte et al., 2003) and pergolide also exhibits high affinity for the dopamine D3 receptors (Strange, 1994). The results indicate that barakol might act as a dopamine agonist to inhibit endogenous dopamine release without a change in dopamine uptake (Thongsaard et al., 1997a). Barakol may have

a dopamine D2-like receptor agonist properties at both pre- and post-synaptic nerve terminal. The interesting point is that barakol, however, significantly reduced extracellular dopamine but produced different types of the behavior indicating that the change in dopamine release may not be the only factor involved in the behavioral effects of barakol.

Propose mechanisms of action of barakol in dopamine function

From the above observations of barakol in dopamine function, it is suggested that barakol may act as both pre- and post-synaptic dopamine D2 and/or D3 receptors. At pre-synaptic nerve terminal, barakol may act at both dopamine D2 and D3 receptor to inhibit dopamine release. At post-synaptic nerve terminal, barakol at low dose may act at the D2 receptor to exhibit exploratory and hyperlocomotor behaviors, while higher dose of barakol may act at the D3 receptor to produce hypolocomotion and sedation (Thongsaard, 1998).

2.2.4 Toxicity of barakol

Toxicity is defined as any harmful effect of a chemical or drug on a target organism. The Organization for Economic Cooperation and Development (OECD) panel of experts (OECD Test Guidelines, 1981) defines acute toxicity as “the adverse effects occurring within a short time of administration of a single dose of a substance or multiple doses given within 24 hours” and subchronic toxicity as “the adverse effects occurring as a result of the repeated daily dosing of a chemical to experimental animals for part (not exceeding 10%) of the life span”. Although opinions differ on the length of exposure, exposure in subchronic study generally ranges from 1 to 3 months. The National Academy of Science (NAS) (1977) defines

subchronic exposure from a few days to 6 months. When these times are translated in terms of human exposure, acute toxicity represents life threatening crises of accidental catastrophes, overdoses, or suicidal attempts. Chronic toxicity, on the other hand, represents daily ingestion of additives or agricultural chemical residues in food (Chan et al., 1982).

2.2.4.1 Toxicity of crude *Cassia siamea*

Wells (1919) reported that there was a poisonous alkaloid, 3β-acetoxy-4α-hydroxy-2β(p-methylbenzyl)pyrrolidine ($C_{14}H_{19}NO_3$), in the branch, leaf, and pod of *Cassia siamea*. Intraperitoneal injection of 1 ml of 5% solution of this alkaloid into animals caused toxicity and fatality. Chivapat and colleagues (2001) found that oral administration of crude extract of Cassia siamea leaves for 6 months in rats caused a decrease in red blood cell count, hematocrit and neutrophil. In addition, hepatic cells were degenerated and necrosis more prominent in high doses (2,000 mg/kg). Some cases were reported for acute hepatitis occurred in patients after taking grounded-dried leaves of *Cassia siamea* 2-4 tablets (equivalent to barakol 20-40 mg) for 4-60 days (Hongsirinirachorn et al., 2003).

2.2.4.2. Toxicity of barakol

Barakol when given intra-peritoneally (i.p.) produced acute toxicity and death in mice. The median convulsion dose (CD50) and median lethal dose (LD50) of barakol intraperitoneal administered were 296.17 (265.25-331.56) mg/kg, and 324.09 (302.36-347.39) mg/kg, respectively. At higher doses in mice, barakol produced continuous locomotion and hyperreflexia (150-200 mg/kg, i.p.), hyperventilation and spastic contraction of body muscle (225-250 mg/kg, i.p.), spastic contraction of body muscle, piloerection, hyperreflexia, straub tail, tonic

arching of the tail, tremor, hopping movements, jerks and/or violent twirls, extensor spasm, flexor spasm (275-325 mg/kg, i.p.). At a dose of 375 mg/kg, i.p., all mice died after injection of barakol (Jantarayota, 1988).

The information on the toxicity of oral administration of barakol in animals have not been found to be reported.

2.3 Depression

2.3.1 Description of depression

Depression is a potentially life-threatening mood disorder that affects approximately ten percent of men and nearly twenty-five percent of women at least once in their lifetime. Depression is often difficult to diagnose because it can manifest in so many different ways. For example, some depressed individuals seem to withdraw into apathy, while others may become irritable or even agitated. Eating and sleeping patterns can be exaggerated to either extreme, excessive or almost eliminated. Observable or behavioral symptoms may be minimal despite profound inner turmoil. Depression is a holistic disorder, generally affecting body, feelings, thoughts, and behaviors with varying degrees. Symptoms of depression include the following: (American Psychiatric Association, 1994)

- Persistently sad, anxious, or empty moods;
- Loss of pleasure in usual activities (anhedonia);
- Feelings of helplessness, guilt, or worthlessness;
- Crying, hopelessness, or persistent pessimism;
- Fatigue or decreased energy; loss of memory, concentration, or decision-making capability;

- Restlessness, irritability; sleep disturbances;
- Change in appetite or weight;
- Physical symptoms that defy diagnosis and not respond to treatment (especially pain and gastrointestinal complaints);
- Thoughts of suicide, death, or suicide attempts; poor self-image or esteem (as illustrated, for example, by verbal self-reproach).

To establish the diagnosis of major depression, a patient must express one of the first 2 and at least 5 of the other symptoms listed above. Such disturbances must be presented nearly daily for at least 2 weeks. Symptoms can last for months or years. Symptoms can cause significant personality changes and changes in work habits, making difficulty for others to empathize with the depressed individual. Some symptoms appear to be precipitated by life crises. People with depression may be unable to eat or even to get out of bed.

Symptom episodes may occur only once in a lifetime or may be recurrent, chronic, or longstanding; in some cases they seem to last forever. Occasionally, symptoms appear to be precipitated by life crises or other illnesses; at other times, they occur at random. Clinical depression commonly occurs concurrently with other medical illnesses and worsens the prognosis for these illness. Even diagnosis of concurrent illness is made much more difficulty by the presence of depression. Physical signs of depression may include the following:

- Psychomotor retardation or agitation, such as slowed speech, sighs, and long pauses;
- Slow body movements, even to the extent of motionless or catatonia;

- Pacing, hand wringing, and pulling on hair; appearance of preoccupation;
- Lack of eye contact; tearfulness or sad countenance;
- Self-deprecatory manner; memory loss, poor concentration, and poor abstract reasoning.

2.3.2 The neurobiological factors associated with depression

There are several biological factors that contribute to the increased susceptibility to depression. While the environment does play an important role in the development of depressive symptoms, genetic influences are also important. In addition, the role of certain neurotransmitters, called monoamines, in depression is immensed. Norepinephrine and serotonin play an important role in depression. The levels of norepinephrine and serotonin in the brains of the depressed patients were lower than the levels of the nondepressed patients. Dopamine is a third monoamine that may be involved in depression. Low levels of dopamine may be presented in patients with depression. Drugs that increase dopamine levels reduce the severity of depression that a patient may feel. However, monoamines are not the only substances that may mediate the disorder (Kaplan and Sadock, 1998; Akiskal, 2000)

2.3.3 Description of amphetamine withdrawal

Amphetamine is a CNS stimulant; it is structurally related to the naturally occurring stimulant ephedrine and the hormone epinephrine. It was synthesized in 1887 and used for the bronchial dilator (Tyler, 1995). Amphetamines produce feeling of euphoria and relief from fatigue, and may improve performance on some

simple tasks, increase activity levels, and produce anorexia (King and Ellinwood, 1997). Amphetamines have been abused almost since their introduction. Taken intravenously, the abuse potential of amphetamines is comparable to that of heroin or cocaine (Kramer et al., 1967; Gawin and Ellinwood, 1988). The abuse of amphetamines undoubtedly results from its euphoric and psychomotor-stimulating properties. Due to reflected counteradaptive changes in reward system, the acute drug withdrawal symptoms is associated with a negative state including anhedonia and depression. The individual usually has prolonged hypersomnia, followed by a period of atypical depression.

Amphetamine withdrawal is a common problem with a prevalent rate of 87% among amphetamine users (Cantwell and McBride, 1998; Schuckit et al., 1999). This prevalence is as high as those of opiate withdrawal (91%) and cocaine withdrawal (86%). Although the duration of amphetamine withdrawal tends to be much longer than cocaine withdrawal, the Diagnostic and Statistical Manual of Mental Disorders (DSM-IV) diagnostic criteria for amphetamine withdrawal are the same as those for cocaine withdrawal (Table 2) (American Psychiatric Association, 1994). Although the symptoms occurring during amphetamine withdrawal may be over in four of five days, some of the symptoms may continue for weeks or months. As amphetamine users are usually required to stop their amphetamine use before receiving any treatment, amphetamine withdrawal should be considered as a common problem for health professionals providing treatment for amphetamine abusers (Watson et al., 1972; Hofmann, 1983).

Table 2.2 Diagnostic criteria for amphetamine withdrawal

A. Cessation of (or reduction in) amphetamine (or a related substance) use that has been heavy and prolonged.
B. Dyspholic mood and two (or more) of the following physiological changes, developing within a few hours to several days after Criterion A:
(1) fatigue
(2) vivid, unpleasant dreams
(3) insomnia or hypersomnia
(4) increased appetite
(5) psychomotor retardation or agitation
C. The symptoms in Criterion B cause clinically significant distress or impairment in social, occupational, or other important areas of functioning.
D. The symptoms are not due to a general medical condition and are not better accounted for by another mental disorder.

(American Psychiatric Association, 1994)

Amphetamine is an indirect sympathomimetic agent. It blocks the uptake of and directly releases dopamine and norepinephrine from newly synthesized pools. In addition, high doses of amphetamine releases serotonin and may affect serotonergic receptors (Chiueh and Moore, 1974). Behavioral evidence indicates that 5-HT receptors can modulate dopamine-mediated behaviors (Costall et al., 1987), and 5-

HT receptor play a role in withdrawal for drugs of abuse (Costall, 1993). Withdrawal from chronic amphetamine administration results in depleted these neurotransmitter levels (Fuller et al., 1973).

Methamphetamine is one of the many amphetamine derivatives. It was first synthesized in Japan in 1919 and then used medically in German in the 1930s (Ogata, 1991). After its introduction into the medical community, methamphetamine has been used as an appetite suppressant, and an energy booster, as well as in the treatment of narcolepsy (Lynch and House, 1992). During World War II, methamphetamine was used by soldiers and factory workers in the warring countries because of its stimulant properties. After the war ended, a surplus of methamphetamine was released into the world markets for medical, recreational, and antifatigue purposes (Ellinwood, 1973).

An epidemics of amphetamine abused emerged, peaking in Japan in 1954 and peaking in the United States in 1965 (Greberman and Wada, 1994). Methamphetamine is a long-lasting stimulant which affects the central nervous system. Methamphetamine has become increasingly more popular because of its low production costs and its longer lasting euphoric effects when compared with cocaine (Darlet and Heischober, 1990; Winger et al., 1992; Beebe and Walley, 1995). Symptoms for methamphetamine withdrawal are the same as those for amphetamine withdrawal (Cadet, 2000).

Methamphetamines withdrawal and major depression share many behavioral commonalties in humans. Therefore, the examination of the behavioral effects of amphetamine withdrawal in rodents may provide insights into the neurobiological

mechanisms underlying both disorders and aid in the development of animal models of depression that are sensitive to antidepressant agents (Cryan et al., 2003).

2.3.4 Testing methods for potential antidepressant drugs

The attempt to simulate depression using a single physiology or (more usually) behavioral manipulation may be counterproductive, since few of the identified etiological factors appear sufficiently potent to precipitate depression in an otherwise risk-free individual. Basically, an ideal model would be one which there are no false positives and no false negatives; that is, when a drug works in animals it is predictive of its clinical effects in humans and vice versa. Unfortunately, there is never 100 percent correspondence between the effects of a drug in an animal models with established high empirical validity. One method is not enough for predicting antidepressant activity of the drug. There are many types of models for screening antidepressive activity. They can be divided into 3 categories (Akiskal, 1985; Willner, 1985; Willner, 1990; Vogel and Vogel, 1997).

2.3.4.1 *In- vitro* methods

This method uses tissue preparation to evaluate whether agent affected on neurotransmitters or receptors associated with depression. Some of *in vitro* methods are described as below:

2.3.4.1.1 Inhibition of [^{3}H]-norepinephrine uptake in rat striatal synaptosomes

The neuronal reuptake mechanism for norepinephrine is the most important physiological process for removing and inactivating norepinephrine in the synaptic cleft. This uptake is inhibited by cocaine, certain phenylethylamines

and antidepressants. This mechanism is considered as one of the most important models of action of antidepressants leading to receptor down-regulation. In the brain, the hypothalamus shows the highest level and greatest uptake of norepinephrine. Therefore, this region is used for testing potential antidepressant drugs (Hertting and Axelrod, 1961).

2.3.4.1.2 Inhibition of [^{3}H]-dopamine uptake in rat striatal synaptosomes

High affinity, saturable, temperature and sodium-dependent transport of ^{3}H-dopamine has been observed in various tissue preparations from different brain regions. The area striatal has a high content of dopamine and is suitable for uptake experiments. The ^{3}H-dopamine uptake is inhibited by cocaine, certain phenylethylamines and antidepressants like nomifensine and bupropion, but not by tricyclic antidepressants. The test can be used to characterize the mode of action of antidepressant drugs (Heikkila et al., 1975).

2.3.4.1.3 Inhibition of [^{3}H]-serotonin uptake in synaptosomes

Patients with serotoninergic hypofunction constitute a subgroup of depression and claim that altered serotoninergic function determines the mood changes associated with affective disorders. A number of clinically effective antidepressants block the reuptake of serotonin. ^{3}H-serotonin transport in brain has been found to be saturable, sodium- and temperature-dependent. This transport is inhibited by several agents, such as ouabain, tryptamine analogs, and tricyclic antidepressants. Apparently, the serotonin uptake can be differentiated from catecholamine uptake. Therefore, the test can be used to detect compounds that

inhibit serotonin uptake into rat brain synaptosomes and may be potential antidepressants (Biegon and Marthis, 1993).

2.3.4.2. Behavioral tests

The term “animal model” often denoted such an attempt to reproduce a psychiatric disorder in a laboratory animal. Animal models of depression have played a significant role in the development of current treatments for this disorder. Animal models can be divided into two categories as illustrated below.

Models in this category use behavioral and/or physiological responses of animals to assess processes. The animals show responses similar to those seen in clinical depression. They may manifest only particular response that appears similar to that seen in clinical depression. For example, rats show turning (rotational) behavior when receptors for dopamine in the brain are stimulated. The activity of the dopaminergic neurons can be assessed in animals by measuring turning behavior : thus, this response of the animal serves as an assay for a physiological process of importance in behavioral pathology. It can be noted that the responses observed and/or measured in an animal assay model, being essentially a "readout" of some process of interest, may bear no resemble to what is seen in the disorder that the model is relevant to.

A. Forced swimming or despair swim test

Behavioral despair was proposed as a model to test for antidepressant activity. The forced swimming test (FST) developed by Porsolt and colleges (1977a, 1978) has gained considerable acceptance. It is a behavioral test which predicts the efficacy of antidepressant treatments (Porsolt et al., 1977b; 1978) The test consists of placing a rodent in a cylinder tank of water for a 15-min

"pretest", and then returning the animal to the water 24 hours later for a 5-min "test". Rats response vigorously during the early part of the test, but then display little motor activity during later portions of the test period. The characteristic behavior of the test, termed immobility, develops when a rodent has been placed in a tank of water for an extended period of time and makes only those movements necessary to keep its head above water (Porsolt et al., 1977b). If antidepressant drugs are administered between the pretest and test periods, the rats are more active, and less immobile in the test (Porsolt et al., 1977a; 1978; Porsolt, 1981). The FST is sensitive to the effects of all of the major classes of antidepressant drugs (Detke et al., 1995a). Immobility time is reduced by clinically relevant doses of tricyclic and atypical antidepressants, 5-HT uptake inhibitors and monoamine oxidase inhibitors in mice and rats (Porsolt et al., 1977b; Lucki et al., 1994).

Though, a single exposure to forced swimming can be used to discriminate between antidepressant and saline-treated rats. Daily exposure to variable stressors has been shown to produce escape deficits, increased immobility in the Porsolt test and a reduction in activity in response to a noise stress (Katz et al., 1981; Garcia-Marquez & Armario, 1987; Murua et al., 1991). Daily exposure to forced swimming for 3 days caused a decline in struggling behavior and swimming, while increasing immobility and the defecation rate (Armario et al., 1988). The repetition of the procedure of forced swimming tends to increase immobility to a certain level (Abel and Hannigan, 1992; Abel, 1993) and to produce anhedonia, similarly to procedures which produce chronic stress (Katz, 1982; Willner et al., 1987).

In addition, prolongation of pharmacological treatments to more than a day increases the anti-immobility effects of antidepressant drugs (Kitada et al., 1981). Daily exposure to variable stressors has been shown to produce escape deficits, increase immobility in the Porsolt test (Katz et al., 1981; Murua et al., 1991). Given that prolonged antidepressant administration has been found to specifically reverse these behavioral aberrations, chronic exposure to either the same or variable stressors frequently has been employed as an animal model of depression (Willner, 1984). The anti-immobility effects of selective serotonin reuptake inhibitors (SSRIs) were initially observed by some authors in very specific conditions. SSRIs were seen to decrease immobility if given for prolonged periods (Okada et al., 1997). It was shown that chronic but not acute treatment of rats with the antidepressant fluoxetine displayed significantly longer times of mobility (Uz and Manev, 2001). Therefore, these modifications in an effort enhance the sensitivity of the traditional forced swimming test (Cryan et al., 2003).

The results from the FST could be related to the mood state of the animal (Willner, 1984) and brain levels of noradrenaline, dopamine, serotonin and cholinergic neurotransmitters (Borsini et al., 1984; Cervo and Samanin, 1988; Cervo et al., 1990; Detke et al., 1995b). Active behaviors of climbing and swimming changed specifically in two antidepressant groups (Detke et al., 1995a). SSRIs decreased immobility but increased swimming behavior (Detke et al., 1995a; Detke and Lucki, 1996). Enhancement of serotonin neurotransmission may mediate swimming behavior in the forced swimming test, whereas enhancement of norepinephrine neurotransmission may mediate climbing (Detke et al., 1995a). Differing from desipramine, imipramine produces a mixed effect, equally increasing

both mobility-type behaviors, climbing and swimming. As a predominantly serotonin reuptake inhibitor one would expect imipramine to increase swimming, but this mixed effect is probably explained by its metabolism in mammals, changing to desipramine, which blocks norepinephrine reuptake and increase climbing (Ferigolo et al., 1998; Clenet et al., 2001).

Dopamine receptor-mediated played a necessary enabling role for the expression of swimming behaviors (Fielding and Lal, 1978; Borsini et al., 1984; Cervo and Samanin, 1987; Cervo et al., 1990). Enhancement of dopamine neurotransmission reduce floating and increase struggling behavior (Cervo et al., 1992a, 1992b).

B. Tail suspension test in mice

The "tail suspension test" has been described by Steru and colleges (1985) as a facile means of evaluating potential antidepressants. The immobility displayed by rodents when subjected to an unavoidable and inescapable stress has been hypothesized to reflect behavioral despair which in turn may reflect depressive disorders in humans. Clinically effective antidepressants reduced the immobility that mice displayed after active and unsuccessful attempts to escape when suspended by the tail.

C. Learned helplessness in rats

Animals exposed to inescapable and unavoidable electric shocks in one situation later fail to escape shock in a different situation when escape is possible (Overmier and Seligman, 1967). This phenomenon was evaluated as a potential animal model of depression (Sherman et al., 1979).

2.3.4.3 Pharmacological test or antidepressant tests based on the mechanism of action

These models endeavor to re-create the human disorder in animals. In these models, drugs are administered to animals in order to reproduce some of the phenomenology of human depressive symptoms. A related approach is to use certain drugs to produce a set of changes in animals, changes that do not necessarily resemble human depression but have high empirical validity in terms of predicting clinical drug responses (McKinney et al., 1971). Some pharmacological models are showed as below:

A. Reserpine-induced hypothermia in mice

Depletion of biogenic amines (noradrenaline, serotonin, dopamine) in the brain induces not only catalepsy and ptosis but also hypothermia in rodents. The decrease of body temperature induced by reserpine is antagonized by antidepressants, MAO-inhibitors and central stimulants. The subcutaneous administration of 2 mg/kg reserpine leads to a decrease of core temperature in mice to 20-23 °C after 18 hours. The fall in temperature can antagonized by antidepressants but also by amphetamine-like drugs. However, the time course is different: tricyclic antidepressants have a slow onset of action and a long lasting effect, whereas amphetamine-like drugs have a quick onset of action and a short-lasting effect (Cox and Lee, 1981; Alpermann et al., 1992).

B. Reserpine-induced reduction of motor activity

Reserpine and reserpine-like compounds inactivate the ability of synaptic vesicles to retain monoamines; as a consequence of this action, release of monoamines follows administration of these drugs, and then a long-term reduction in

monoamine stores occurs, resulting in depletion of dopamine, norepinephrine, epinephrine, and serotonin in the brain and periphery. The depletion of amines produces a variety of physiological and behavioral effects, and antidepressants have been shown to counteract some of these physiological and behavioral effects (Domenjoz and Theobald, 1959; Costa et al., 1960).

C. Chronic amphetamine withdrawal model

Amphetamines are associated with the neurobiology of reward, motivation and emotion processes. Alterations in the neuronal mechanisms that underline these processes lead to some of the signs and symptoms associated with several psychiatric disorders, e.g., depression, schizophrenia, and drug dependence. Chronic amphetamine administration can produce profound and long-lasting changes in brain neurochemical and neuroendocrine systems. Withdrawal from chronic amphetamine is characterized by deficits in reward that resemble some symptoms of depression. Antidepressants, both tricyclics and monoamine oxidase inhibitors, have been found to potentiate these responses (Carlton, 1961; Halliwell et al., 1964).

Withdrawal from chronic amphetamine administration is characterized by deficit in reward. Depression of reward system may be associated with states of psychological depression (Kokkinidis et al., 1980; Fibiger, 1984), dysphoria (Frank et al, 1992) or anhedonia (Wise et al., 1978; Wise, 1982) and that such depression might be correlated to psychomotor stimulant withdrawal distress and craving in chronic stimulant users (Dackis and Gold, 1985b; Koob et al., 1989). Reward deficits can represent an animal model of the symptom of "diminish interest

or pleasure (anhedonia)" with construct, convergent and predictive validities (Kokkinidis, 1986; Geyer and Markou, 1995).

Various chronic amphetamine treatment regimens had been used to induce some symptoms of depression, i.e. three injections 1-5 mg/kg per day for 1, 2, 4 or 6 days (Lin et al., 1999); amphetamine escalating dose, 1-8 mg/kg before multiple daily injections of high doses of the drug (8 mg/kg/2 hr x 4 injections) (Segal and Kuczenski, 1997); the twice-daily, for 6 weeks at dosages escalating from 1 to 10 mg/kg per i.p. of d-amphetamine (Wise and Munn, 1995); 4-day escalating dose of d-amphetamine administration 1-12 mg/kg, i.p. (Barr et al., 1999). On the basis of these results, the magnitude and duration of amphetamine withdrawal were proportional to the duration and dose of amphetamine treatment prior to withdrawal (Lin et al., 1999).

2.3.5 Effects of barakol on antidepressant animal models

The information on the effects of barakol on antidepressant animal models remains unseen.

2.4 Principle of pharmacokinetics (Shargel,1980; Renwick, 2000; Ballantyne et al., 2000).

Biopharmaceutic and pharmacokinetic studies of drugs and drug products are useful in understanding the relationship between the physicochemical properties of the drug product and the pharmacological or clinical effect. Pharmacokinetics involve the kinetics of drug absorption, distribution and elimination (i.e. excretion and metabolism).

The basic pharmacokinetic parameters will relate to the underlying physiological and biochemical processes. Each of the basic process involved in pharmacokinetics, i.e. absorption, distribution and elimination, may be described by parameters which define the usually calculated from the concentrations of the chemical and/or its metabolites in biological fluids, such as whole blood, plasma or urine, measured at known times after the administration of known doses. Most pharmacokinetic parameters are based on the measurement of the chemical in plasma samples collected at various times after dosing. A key requirement for the use of pharmacokinetic principles is some measure of the drug concentration at the site of action and the site(s) of elimination of the drug. Fortunately, the concentration of drug in the plasma (C) is a reasonable approximation of these concentrations (in most situations) and is easy to measure. The concentration in plasma is a function of the rate of input of the drug into the plasma (by absorption), the rate of distribution to the peripheral tissues (including the target organ), and the rate of elimination from the body.

2.4.1 Absorption

Absorption is the process of transfer of drug from the site of administration into the general circulation. The process of absorption occurs whenever the compound is given by a route other than direct intravascular injection. The rate of absorption depends on both the nature of the chemical and the site of administration. The kinetic parameters which describe the absorption rate are the absorption rate constant (k_a) and its associated absorption half-life.

The parameter which describes the extent of absorption is the bioavailability (F), which is defined as the fraction of the dose which is transferred from the site of administration into the general circulation as the parent compound. By definition, it is 1.0 (or 100%) in the case of intravenous administration. After administration by non-IV routes, bioavailability is generally reduced by incomplete absorption and first-pass metabolism, which occur before the drug enters the systemic circulation. Incomplete absorption (F<1) may be due to an inability of the chemical to cross the lipid barrier of the epithelial membrane so that the parent compound is unabsorbed and eliminated in the faeces, or it may be due to metabolism prior to reaching the general circulation. The latter is referred to as first-pass metabolism since it usually occurs on the first passage through the liver during the absorption process.

The bioavailability can be calculated from the area under the plasma concentration-time curve (AUC) between administration and infinity following both routes of administration as

$$F = \frac{AUC_{oral} \times dose_{iv}}{AUC_{iv} \times dose_{oral}}$$

The use of AUC data assumes that there is no saturation of elimination, and that the intravenous dose with its possibly higher plasma concentrations does not produce cardiovascular, renal or metabolic effects which could alter the plasma clearance of the compound. Bioavailability does not have any units; it is expressed as a fraction or a percentage.

2.4.2 Distribution

Distribution is the process of reversible transfer of drug from the general circulation into the body tissues. The process may be characterized as both rate and extent; the corresponding parameters are the distribution rate constants and the apparent volume of distribution. The distribution is usually rapid. The distribution rate constant is measured following the administration of a single rapid (bolus) intravenous dose. The rate of distribution into tissue may be slow for two possible reasons. First, distribution will be slow if the drug has a high affinity for and accumulates in a tissue or organ which is only slowly perfused, e.g. fat or muscle. For such a compound the rate at which the tissues and blood can reach equilibrium will be limited by the blood flow to the tissues. Second, the drug may be polar so that its rate of entry into the intracellular fluid of all tissues will be limited by its solubility in the lipid of the membrane.

A drug's ability to distribute is reflected by the value of its apparent volume of distribution or V_d. The units for volume of distribution are volume or volume per weight (eg, L/kg). V_d is a parameter which relates the concentration in plasma (C_0) to the amount of drug in the body (Dose) with which it is in equilibrium, i.e.

$$V_d = \frac{\text{Dose}}{C_0}$$

2.4.3 Elimination and clearance

A. Elimination

There are two principle mechanisms of elimination of foreign compounds from the body. Metabolism eliminates the drug from the body by

converting it into a metabolite, which is a different chemical species. The resulting metabolite may itself undergo further metabolism or it may be removed from the body by an excretory process.

The general goal of drug metabolism is to transform such compounds into more polar water soluble products. The liver plays a central role in metabolizing most drugs, which usually require biotransformation for pharmacologic activity or excretion. Metabolism generally occurs in two stages:

Phase I reactions convert the parent drug to a metabolite by oxidation, reduction, or hydrolysis.

Phase II reactions create a polar excretory product by coupling the drug or metabolite with an endogenous substrate (eg, glucuronide, sulfate).

The elimination of a drug can be divided into the rate and extent of elimination. The rate of elimination is limited by two biological processes: i) the ability of the organs of elimination to extract the drug from the circulation and to remove it from the body by metabolism or excretion; ii) the extent to which the drug remains in the circulation and is available for elimination rather than entering tissues. If the drug has entered the body tissues to a major extent, so that at any time only a very small fraction of the total body load remains in the blood and is available for elimination, then the rate at which it is transferred back from the tissues into the circulation may become the main variable determining the elimination rate.

B. Clearance

Extent of elimination is of less importance than the rate. Following a single, the extent of elimination will eventually be 100% of the dose. This is even true for drug which bind covalently to tissue macromolecules, because the parent

drug is eliminated by the formation of the adduct. During chronic intake, the body load increases until the extent of elimination per day equals the daily intake of the drug. Kidneys are capable of eliminating drugs which are low in molecular weight, or which are polar and fully ionized at physiologic pH. Most drugs do not fit these criteria, but rather are fairly large, unionized or partially ionized, lipophilic molecules.

The clearance (*Cl*) of a chemical is perhaps the single most important pharmacokinetic parameter. It is defined as

$$Cl = \frac{\text{Rate of elimination of the chemical}}{\text{Plasma concentration}}$$

or

$$Cl = \frac{\text{Dose}}{\text{AUC}}$$

or

$$Cl = \frac{\text{Dose (iv)}}{\text{AUC(iv)}} = \frac{\text{Dose(oral) x F}}{\text{AUC(oral)}}$$

Pharmacokinetic Compartments

One compartment model

All drugs initially distribute into a central compartment (*V1*) before distributing into the peripheral compartment (*V2*) (Figure 2.5). If a drug rapidly equilibrates with the tissue compartment, then, for practical purposes, we can use the much simpler one-compartment model which uses only one volume term, the

apparent volume of distribution, V_d Serum level plot for a 1-compartment model yields a monophasic straight line when using a log scale on the y-axis.

Two compartment model

Drugs which exhibit a slow equilibration with peripheral tissues, are best described with a two compartment model. Serum level plot for a 2-compartment model yields a biphasic straight line when using a log scale on the y-axis.

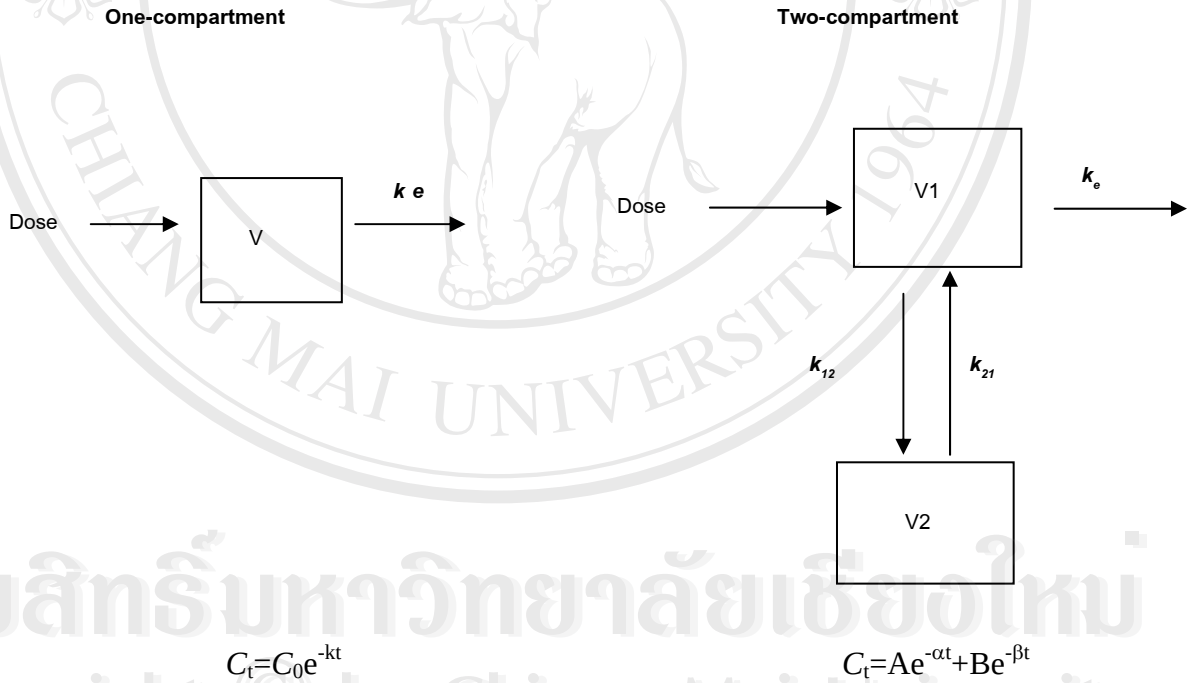


Figure 2.5 Simple one- and two-compartment models of distribution and elimination. k_{12} and k_{21} are first-order rate constants. The constant α and β are rate constants for the elimination and distribution phase, respectively.

Pharmacokinetics parameters

The concentration in plasma is a function of the rate of input of the drug into the plasma (by absorption), the rate of distribution to the peripheral tissues (including the target organ), and the rate of elimination from the body.

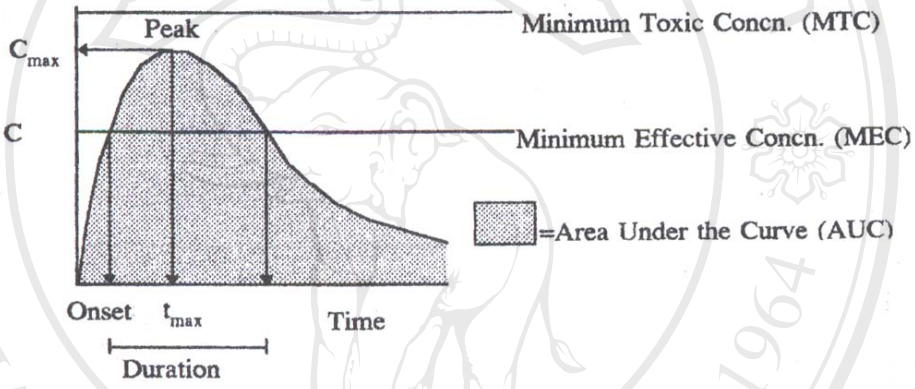


Figure 2.6 The change of plasma concentration with time after administration of a single oral dose.

Maximum concentration (C_{max})

Maximum concentration is the peak of drug plasma concentration after drug administration. From figure 2.6 the interrupted horizontal lines show the minimum effective concentration (MEC) and minimum toxic concentration (MTC). A therapeutic effect can be expected only when plasma level is above the MEC and below MTC. The C_{max} should be between MEC and MTC to obtain maximum therapeutic effect.

Time to peak concentration (t_{max})

Time to peak concentration is the time when C_{max} occurred. This parameter describes the rate of absorption of drug.

Area under the curve (AUC)

The area under the concentration-time curve, AUC, is the parameter which describes the extent of drug transferred from the site of administration into the general circulation as the parent compound.

AUC can be used to calculate overall clearance and half-life values for a drug. In addition, AUC is frequently used to compare drug exposures achieved with different drug doses, or to compare pharmacokinetics in the presence or absence of a drug with the potential to produce a pharmacokinetic drug interaction. Several methods are available for the determination of AUC, but the most common is the trapezoidal rule, which is the sum of the individual areas between observations, from time =0 to the last measured.

Absorption rate constant (k_a)

Absorption rate constant (k_a) is the fraction absorbed per unit time. This parameter describe, in mathematical terms, the absorption of a drug. K_a typically follows first order kinetics. That is, the rate of reaction is proportional to the amount of substrate present. The equation for such reactions is :

$$\frac{dC}{dt} = k_a C$$

where d*C*/d*t* is the rate of change in concentration and *C* is the concentration. The term k_a is the absorption rate constant; the greater the value the greater the rate of the absorption process. K_a can be determined by residual analysis (Figure 2.7). The unit of k_a is time^{-1}.

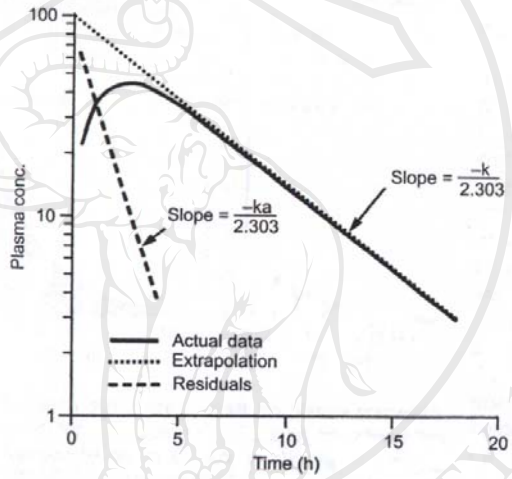


Figure 2.7 Plasma concentration-time curve following oral administration of a compound showing simple first-order absorption. The difference (or residual) between the actual data obtained and the extrapolation line are plotted in order to derive the absorption rate constant.

Elimination rate constant (k_e)

Elimination rate constant (k_e) is the fractional rate of drug elimination from the body. If k = 0.25 h^{-1}, then 25% of the amount of drug in the body will be eliminated per hour at any given moment

The overall rate of elimination (k_e) is independent of the serum concentration under first-order conditions. It depends in two independent variables, the volume of distribution (V_d) and the clearance (Cl). K_e has unit of inverse time.

$$k_e = \frac{Cl}{V_d}$$

Plotting the natural logarithm of the concentration (C_0) against time (t) will give a straight line with a slope of $-k_e$ (Figure 2.7). If $\log_{10}$ is used, then the intercept is $\log C_0$ and the slope of $-k_e/2.303$.

2.4.4 Pharmacokinetic study of barakol

Since the therapeutic and toxic effects of drugs are largely determined by the concentration of drug at its site of action. The pharmacokinetic properties of a drug (its absorption, distribution and elimination) determine a drug's concentration at its site of action, the design of appropriate dosing regimens requires consideration of pharmacokinetics as well as pharmacodynamics. Data obtained from pharmacokinetic studies in healthy volunteers is used to obtain the types of pharmacokinetic parameters as described above. The pharmacokinetic study of barakol remains unseen. Therefore, one of the goal of this study is to define some of these key pharmacokinetic parameters.