PART I
LITERATURE REVIEW

"Whatever you can do or dream, you can begin it!
Boldness has genuine power, Magic in it! - Begin it Now"

– Goethe –
I: LITERATURE REVIEW

1. Herbal drug interactions

Herbal medicine has been an essential component of oriental medicine (OM), which has existed for over two thousand years, guided by principles of Yin Yang, five elements, organs and meridians (Thai, 2004). The herbal medicines include dietary supplements that contain herbs either singly or in mixtures. Also called botanicals, the same are plants or plants products used for their scents, flavor and/or therapeutic properties (Ernst and Pitter, 2002).

Herbal drugs are readily available in the market from health food stores without prescriptions and are widely used in India, China, USA and all over the world (Parmar, 2005). The aforesaid medications have gone in mainstream use and as the sales continue to rise, so do the concerns about their interactions with prescription and over-the-counter drugs (Hu et al., 2005; Lambrecht et al., 2000). Over the past decade, there has been an increased global interest in traditional systems of medicine and herbal medicinal products. In part, this surge has been due to the rare or nonexistent access to modern medicine in developing countries as well as the acceptance of herbal medicines by large populations of people in affluent nations (Barnes et al., 2004; Tindle et al., 2005; Eisenberg et al., 1998).

In developed countries, complementary and alternative medicine (CAM), are often used concomitantly with conventional medicine (Khan, 2006). A relevant safety concern associated with the use of herbal medicines is the risk of interactions with prescription medications (Izzo, 2005; Izzo, 2004; Brazier and Levine, 2003; Izzo and Ernst, 2001; Fugh-Berman, 2001; Markowitz and DeVane, 2001; Williamson, 2003). This issue is especially important with respect to drugs with narrow therapeutic index, such as warfarin or digoxin or drugs used for chronic therapy such as antidepressants and in sensitive patient populations such as older adults, the chronically ill, and those with compromised immune systems (Izzo, 2004; Kaufman et al., 2002).
Recent examinations have indicated that as many as 16% of prescription drug users consume herbal supplements (Kaufman et al., 2002). Moreover, fewer than 40% of patients disclose their herbal supplement usage to health care providers and many physicians are unaware of the potential for herb–drug interactions (Klepser et al., 2000). This lack of information, combined with the fact that herbal medicines are usually mixtures of more than 100 active ingredients, obviously increases the likelihood of interactions. Herbal drug interactions can results in unexpected concentration of therapeutic drug and lead to the undesired effects. Thus, contrary to the popular belief that “natural are safe” (Kaufman et al., 2002); herbal medicines can cause significant toxic effects, drug interactions and even morbidity or mortality (Parmar, 2005).

**Nature of herbal drug interactions**

Most natural products, unlike conventional drugs, are a complex mixture of chemical constituents and often a complete characterization of the bioactive compounds from an herbal is unknown (Chavez 2006; Barnes et al., 2004). Additionally, the chemical makeup of natural products varies depending on the part of the plant used (bark, stems, leaves, roots, rhizomes), climate, growing conditions, harvesting, and storage conditions. Combination products composed of multiple natural products complicates matters further. Not only does the complex nature of a natural product complicate the determination of herb-drug interactions, but also the manufacturing process; for example, drying process and extraction methods contributes to the overall complexity. As previously mentioned, because herbal products are not regulated by the food and drug administration (FDA), there are no standards for herbal products. Indeed, herbal products have been found to be misidentified and/or substituted or adulterated with other natural products or unwanted substances (Fugh-Berman, 2000; Cupp, 1999; But, 1994). Moreover, herbal products are classified and marketed as dietary supplements (Anonymous, 1994). However, the same are regulated differently in other countries.
The US FDA mandates that only medicine have to be proven to be safe before being released into market. Herbal products do not fall under the category of drugs as long as they are not marketed for the preventions of any diseases. In United Kingdom, any product that is not granted a license as a medical product by Medicine Control Agency (MCA) is treated as food, and no health claim or medical advice can be given on the label. Labeling of herbal products may not actually reflect the contents and adverse events or interactions attributed to specific herb may be related to mis identification of planst, pharmaceutical drugs or heavy metals (Fugh-Berman, 2000). The issue of herb-drug interactions looms large over the practice of herbal medicine. Since the first such reports emerged a decade ago, a concern has been raised, that we know so little about herbs and their potential for interaction with drugs that these incidents could be just the "tip of the iceberg."

**Mechanisms of herbal drug interactions**

Herbal medicines follow modern pharmacological principles. Hence, the herbal drug interactions are based on the same pharmacokinetic and pharmacodynamic mechanisms as drug–drug interactions (Manzi and Shannon, 2005; Izzo, 2004; Izzo et al., 2002). Drug-drug or herb-drug interactions can occur in several different ways. Pharmacodynamic interactions occur when the object drug’s effect is altered by the interfering drug or herb. These interactions are not due to an alteration in the plasma concentration of either drug but rather because of the net effect that can be additive, synergistic (together the two drugs can achieve better results than the sum of their two actions alone) or antagonistic. These adjectives can refer to alteration in the object drug's intended therapeutic effect, or can refer to the change in the toxicity levels and adverse side-effects as well. On the other hand, pharmacokinetic interactions denote changes in the absorption, distribution, metabolism or elimination of the object drug due to the presence of the interfering drug. Unlike pharmacodynamic interactions, these interactions do result in changes in the plasma concentration of the object drug, and as a consequence, the toxic or sub-therapeutic levels occur more frequently.
A good example of pharmacokinetic interactions which are more extensively studied includes the cytochrome P450 system and/or drug transporters such as p-glycoprotein (Zhou et al.; 2003; Zhou and Lim, 2004). The interfering drug may act as an inducer, inhibitor and/or substrate of the same P450 enzyme that is responsible for the metabolism of the object drugs. A variety of herbal medicines were known to have an influence on drug-metabolizing enzymes (Wanwimolruk et al., 2009). For instance, in people taking both St. John's wort and the indinavir, St. John’s wort induced the production of the P450 enzyme CYP3A4 which metabolizes indinavir, and lowered its plasma levels (Xie and Kim, 2005; Piscitelli et al., 2000). On the other hand, the addition of herbal products to a drug regimen has the potential to diminish or amplify the effect of a drug through pharmacodynamic means. Significant pharmacokinetic and pharmacodynamic interactions between various herbal products and drugs being substrates of cytochrome P450 have recently been reported (Panossian et al., 2009; Rodeiro et al., 2009). Some of the basic mechanisms for herb-drug interaction were described as follows (Kuhn, 2002).

1. Pharmacokinetic interactions

Primary mechanisms of herbal drug interaction involve either induction or inhibition of intestinal drug efflux pumps (e. g. efflux proteins such as P-glycoprotein (P-gp) and multiple resistance proteins (MRPs) and intestinal and hepatic metabolism by cytochrome P450s (CYPs) (Ioannides, 2002; Evans, 2000; Wilkinson, 1997).

There are several places in our body where such interactions happen. a. Stomach (Gastro-intestinal tract) - When herbs and drugs are taken orally, they are usually absorbed into the bloodstream through the stomach and intestines. Herbs can affect the way in which drugs are absorbed, leading to changes in the amount of drug that enters the bloodstream. For example, some herbs can change the physical environment of the stomach, such as the pH level, while others might chemically bind to drugs, causing them to remain in the stomach instead of entering the bloodstream.
Some herbs, such as laxatives can speed up the digestive process, reducing the amount of time a drug is present to be absorbed by the stomach.

b. Liver - Once in the bloodstream, many drugs need to be metabolized (chemically altered) by the liver either in order to become therapeutically active or to be removed from the bloodstream. Liver therefore, plays an important role in controlling the level and effectiveness of drugs in our body. Herbal therapies (and drug therapies, too) can change liver metabolism. By inducing or inhibiting liver enzymes, herbs can alter the amount of therapeutically active drug in the blood. A good example of these pharmacokinetic interactions includes the cytochrome P450 system. The interfering drug may act as an inducer, inhibitor and/or substrate of the same CYP P450 enzymes that are responsible for the metabolism of the object drugs. This is the most important mechanism for interactions between herbal therapies and anti-retroviral drugs.

c. Kidney - Some drugs are eliminated from the bloodstream through the kidney. Herbs that affect the functioning of the kidney can change the level of drug in the blood. If the herb reduces kidney function, the level of drug may increase. If the herb increases kidney functioning, the level of drug may decrease.

2. Pharmacodynamic interactions

Pharmacodynamic interactions refer to the mutual actions of herbs and drugs inside the body. When taken at the same time, herbs and drugs may work together (synergistically) or in opposition (antagonistically). For example, separately, they can have the same toxic effects, so that when taken together, they cause increased side effects. Pharmacodynamic interactions may result in additive, synergistic, or antagonistic effects of the supplement combination with a drug. Medication possessing antiplatelet activity, or with the potential for depressing the central nervous system, or with the potential to cause organ toxicity, could be of further risk when used with dietary supplements that share these pharmacologic activities (Boullata, 2005). Many herbal drug interactions fall into this category. Pharmacodynamic interactions are said to be difficult to predict or prevent.
Figure 1.1. A chart on mechanisms of herbal drug interactions.
The chart presented, first divides interaction concerns into broad subgroups, the main ones being related to pharmacodynamic interactions (mostly involved with herbs and drugs yielding similar effects or counteracting one another) and pharmacokinetics (such as changing the rate of absorption or elimination of a drug) (Refer to figure 1.1. A chart on mechanisms of herbal drug interactions).

There are also two specialty groups: therapies involving obstetric/gynecological/hormonal matters and Chinese herbs. Very little information about actual interactions is imbedded in the chart presentation. There is mention of "xanthines" (e.g., caffeine) under neuroendocrine, reflecting the concern that caffeine containing herbs (e.g., coffee and tea) could counteract the action of sedatives or produce excessive stimulation with stimulant drugs (Blumenthal, 2000; Lambrecht et al., 2000; Jellin, 1999). Under the cardiovascular heading, there is a mention of "glycoside-containing." The intended meaning is the specific class of cardiac-glycosides, as there are abundant glycosides that do not have significant cardiac effects whereas, the cardiac glycosides are often quite potent, and can not only enforce or counteract the action of cardiac drugs but can themselves cause cardiac problems if the dosage is large. Generally, the field of herbal medicine has been purged of ingredients with cardiac glycosides; there remains a concern that one of the cardiac glycoside-containing herbs may find its way into a product by mistake. Under the heading hematological, there is mention of "coumarin containing," which makes reference to the fact that some herbs contain coumarins which might act along with warfarin, a coumadin (binary coumarin, much more potent than coumarins), or along with other blood thinners (Heck et al., 2000). The table also makes passing mention of tannins (an herb component with several health benefits that can bind up drugs in the intestinal tract and make them less available). Under Chinese herbs, there is mention of three issues: the possible interaction of minor Bupleurum in combination with interferon in treatment of hepatitis to cause an immune response leading to lung damage; the possible interaction of salvia (danshen) and warfarin, leading to excessive blood
thinning and the possible interaction of aristolochic acid and diuretic drugs (or others) to cause renal failure (Izzat, 1998).

**Interactions of herbs with drugs affecting central nervous system**

The interactions between herbal remedies and conventional drugs affecting the central nervous system are summarized in tables (Table 1. Some common herbs affecting CNS, their common uses and possible interactions) and (Table 2. Potential herb-drug interactions based on drug therapeutic class). Patients mixing synthetic and herbal anxiolytic or antidepressant drugs are at the highest risk for having interactions (Izzo, 2004). The herb, St. John’s wort is effective in the treatment of mild to moderate depression (Di Carlo et al., 2001). Both St. John’s wort and synthetic antidepressants have a high probability of concomitant use. St. John’s wort and serotonin re-uptake inhibitors (i.e. sertraline, paroxetine, fluoxetine, nefazodone and venlafaxine) may result in symptoms characteristic of central serotonin excess (e.g. mental status changes, tremor, autonomic instability, gastrointestinal upset, headache, myalgias and motor restlessness) as highlighted by case series and case reports (Bressler, 2005; Prost et al., 2000; Lantz et al., 1999; Gordon 1998). These effects could be the result of an additive effect on 5-hydroxytryptamine (5-HT) because hyperforin in St. John’s wort inhibit the re-uptake of several brain neurotransmitters including 5-HT. A concomitant use of St. John’s wort and sertraline has been reported to cause a manic episode in a 28-year-old man (Barbenel et al., 2000) and the case was classified as possible, in a report reliability score reported in a study (Fugh-Berman and Ernst, 2001). A clinical study showed that co-medication with St. John’s wort decreased plasma and urine concentration of amitriptyline in 12 patients (Johne et al., 2002). In addition to being a P-glycoprotein substrate, the demethylation and subsequent hydroxylation of amitriptyline is catalysed by CYP2C19 and CYP3A4 which were reported to be induced by St. John’s wort (Roby et al., 2000; Obach, 2000; Durr et al., 2000). Other herbs which may interact with conventional antidepressants include ginkgo and ginseng.
An 80-year old woman with Alzheimer’s disease fell into a coma after taking a low
dose of the atypical antidepressant trazodone with ginkgo (Galluzzi et al., 2000). The
case was classified as ‘possible’ (Fugh-Berman and Ernst, 2001). One case report
described a patient who experienced insomnia, headache, tremulousness and mania
after co-administration of ginseng with phenelzine (Shader and Greenblatt, 1985). Here,
causality was reported as likely because inadvertent re-challenge resulted in similar
symptoms (Jones and Runikis, 1987). The mechanisms of such interactions were not
known or explained.

Lithium salts are used prophylactically in treating manic-depressive patients and in the
treatment of manic episodes. Patients taking isphagula or psyllium were found to have
lower blood levels of lithium, possibly because the plant product was alleged to trap
lithium in the gut (Perlman, 1990). In addition, a case was reported wherein a 26-year-
old woman stable on lithium for 5 months, experienced dizziness, grogginess and
diarrhoea after taking a combination of herbal diuretics (juniper, buchu, horsetail, corn
silk, bearberry, parsley, bromelain and paprika) (Pyevich and Bogenschutz, 2001). The
adverse events were associated to increased plasma lithium levels. As there were
several herbal diuretics used along in the preparation, the mechanism of action of each
is elusive, it was impossible to determine which herb caused the lithium toxicity.

Extrapyramidal symptoms occurring in a schizophrenic patient who was maintained on
depot neuroleptic medication following a period of heavy betel nut were described in a
well-documented case report (Deahl, 1989). The underlying mechanism of this
interaction was based on the pharmacological antagonism of the anticholinergic agent
procyclidine (which is given to treat acute neurological adverse effects resulting from
the use of neuroleptic drugs) by arecoline (an acetylcholine receptor agonist), the active
ingredient of the betel nut (Ernst, 2000). Seizures were reported in two patients taking
the phenothiazine fluphenazine with evening primrose oil and in one patient taking
placebo with evening primrose oil in a study of 23 patients with schizophrenia (Holman
and Bell, 1983). Evening primrose oil contains ω-linolenic acid, which lowers the
seizure threshold. However, phenothiazines themselves are known to be epileptogenic. During the course of routine plasma drug level monitoring; an unexpected loss of seizure control and reduction in plasma phenytoin levels were noticed in two patients who were also taking the Ayurvedic multi-herb syrup Shankhapushpi (Dandekar et al., 1992). Subsequent animal experiments confirmed that the herbal remedy decreased the antiepileptic activity of phenytoin without lowering its plasma level (Dandekar et al., 1992). The mechanism of this interaction was also not known. In addition, the benzodiazepines alprazolam and midazolam were used experimentally as probe for CYP3A4 activity because they are entirely metabolized by intestinal and hepatic CYP 3A4. Consistently, clinical studies have shown that St. John’s wort decreased alprazolam and midazolam plasma levels in healthy volunteers (Markowitz et al., 2004; Hall et al., 2003; Dresser et al., 2003; Wang et al., 2001). St John’s wort decreased plasma levels of midazolam; the effect being considerably less after intravenous administration than after oral administration. These findings indicated that enzymatic induction occurs both in the intestine and in the liver (Dresser et al., 2003). Moreover, a clinical study performed on 12 healthy subjects showed that echinacea increased the oral availability of midazolam (CYP3A probe), which was consistent with inhibition of intestinal CYP3A by the herb (Gorski et al., 2004). An inadequately documented case report described a semi comatose state in a patient taking kava and alprazolam (Fugh-Berman, 2000; Almeida and Grimsley, 1996). Kava was reported to inhibit human CYP P450 activities (Mathews et al., 2002) and thus its possibility of increasing alprazolam plasma concentrations. Moreover, it was suggested that the pharmacodynamic mechanism could not be ruled out as both kava and benzodiazepines interfere with GABA receptors (Izzo and Ernst, 2001). Another anxiolytic drug reported to have possibility of interaction with herbal medicines is buspirone, a 5-HT1A receptor agonist. A case of a possible serotonin syndrome was reported after combination of buspirone and St. John’s wort (Dannawi, 2002).
An additive effect on 5-HT receptors (St. John’s wort inhibit 5-HT re-uptake) was put forward to explain such interaction (Markowitz and DeVane, 2001; Di Carlo et al., 2001). Moreover, a case of a female patient experiencing hypomania after adding St. John’s wort and ginkgo to her regimen of buspirone was reported (Spinella and Eaton, 2002). Interactions with anti-parkinson drugs levodopa is a metabolic precursor of dopamine. An increase in the duration and number of ‘off’ periods in a patient with Parkinson’s disease treated with levodopa has also been reported (Schelosky et al., 1995). The reduced efficacy of levodopa was attributed to dopamine receptor antagonistic properties of kava (Dinh et al., 2001; Schelosky et al., 1995). The causality in this case was reported as likely in the study reported (Fugh-Berman, 2001).

References to what the herbs "may" do when combined with certain drug groups, (e.g., valerian may increase the effects of certain anti-seizure medications or prolong the effects of anaesthetic agents) often refer to pharmacology studies rather than actual clinical experience. For example, when one wishes to demonstrate that valerian, used traditionally for seizures and for analgesic effects, is likely to accomplish what has been claimed, laboratory animal studies are conducted. A standard procedure is to test the herb extract alone and to also test it with drugs that cause the same effect. If the drug effect is increased or prolonged by the herb, it is implied that the herb has a similar effect, even though it may have a different mechanism. Thus, a study intended to demonstrate that a traditional claim for an herb is true and turns out to be a source of worry regarding an issue of herb-drug interactions. However, the amount of herb used in the pharmacology experiments of these types are often far higher than the amount normally used in clinical practice; the likelihood of herb-drug interactions occurring with normal use of the herb may be minimal. Still, if one wishes to consider possible herb-drug interactions under a variety of scenarios, including excessive use of the herb and use of the herb by individuals who are more sensitive to the possible interaction, then such data must be included. When published reports alluding to adverse herb reactions (but not interactions) and to pharmacology studies only are eliminated, one is
left with few instances of reported herbal drug interactions. This is likely due to the low
dose of any individual herb component usually consumed and the simple absence of
significant interaction at any reasonable dose.

**Evaluation of herbal drug interactions**

Herbal constituents are substrates for drug metabolizing enzymes as they contain
several chemicals that are metabolized by phase 1 and phase pathways as well as
substrates for certain hepatic and extra hepatic transporters (Venkataramanan et al.,
2006).

The potential for involvement of drug metabolizing enzymes and transporters in the
handling of herbal components leads to a predisposition of herbal drug interactions.
Induction and inhibition of drug metabolizing enzymes and transporters by herbal
component has been documented in several *in vitro* studies (Pal and Mitra, 2006). Such
herb drug interactions may involve inhibition of drug metabolizing enzymes and/or
drug transporters resulting in increased levels of one or both drugs leading to adverse
drug reactions. Conversely, the induction of these same enzyme systems leads to a
decrease in the overall body exposure to the drug creating a situation where the patient
will be under dosed. While these studies offer a system to determine the potential for
the herbal component to alter the pharmacokinetics of a drug, they can not always be
used to predict the magnitude of any potential effect *in vivo* studies which are the
ultimate way to determine the clinical importance of such interactions
(Venkataramanan et al., 2006).
# Table 1. Some common herbs affecting CNS, their common uses and possible interactions.

<table>
<thead>
<tr>
<th>Name of Herb</th>
<th>Source and Common Uses</th>
<th>Possible Drug Interactions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ephedra (Ma-Huang, Leaf)</td>
<td>It is used in many over-the-counter diet aids as an appetite suppressant. For asthma or bronchitis (Cupp, 1999).</td>
<td>Ephedra may interact with certain antidepressant medications or certain high blood pressure medications to cause dangerous elevation in blood pressure or heart rate or may cause death in certain individuals (Cupp, 1999).</td>
</tr>
<tr>
<td>Ginger (Zingiber officinale, Rhizome)</td>
<td>Anti-nausea, anti vomiting and vertigo (Feltrow and Avila, 1999; Jellin, 1999).</td>
<td>Ginger may increase bleeding, especially in patients already taking certain anti-clotting medications (Lambrecht et al., 2000).</td>
</tr>
<tr>
<td>Ginkgo (Ginkgo biloba, Leaf)</td>
<td>Increasing blood circulation and oxygenation and for improving memory and mental alertness. (Foster, 1996).</td>
<td>Ginkgo may increase bleeding, especially in patients already taking certain anti-clotting medications (Rosenblatt and Mindel, 1997).</td>
</tr>
<tr>
<td>Ginseng (Panax ginseng, Roots)</td>
<td>It increases physical stamina and mental concentration. (Olin, 2005).</td>
<td>Ginseng may cause decreased effectiveness of certain anti-clotting medications. Persons using ginseng see increased heart rate or high blood pressure or bleeding in women after menopause. Also, it may increase psychoactive stimulation if used with drugs like phenelzine (Janetzky and Morreale, 1997; Jones and Runikis, 1987).</td>
</tr>
<tr>
<td>Kava-kava (Piper methysticum, Leaf)</td>
<td>Anti anxiety, As a sedative or restlessness; A muscle relaxant. (Schelosky et al., 1995)</td>
<td>Kava-kava may increase the effects of certain anti-seizure medications and/or prolong the effects of certain anaesthetics and CNS depressants and alcohol. It may increase the risk of suicide for people with certain types of depression (Almeida and Grimsley, 1996).</td>
</tr>
<tr>
<td>St. John's Wort (Hypericum perforatum, Tops)</td>
<td>A mild to moderate depression or anxiety and sleep disorders. (Di Carlo et al., 2001; Butterweck, 2003).</td>
<td>St. John's Wort may prolong the effect of certain anaesthetic agents, sedative, antidepressants and antipsychotic medications (Bressler, 2005; Mannel, 2004).</td>
</tr>
<tr>
<td>Valerian (Valeriana officinalis, Roots and rhizomes)</td>
<td>Valerian is used as a mild sedative or sleep-aid, Antispasmodic (Jellin, 1999).</td>
<td>Valerian may increase the effects of certain anti-seizure medications or prolong the effects of sedative and certain anaesthetic agents (Lambrecht et al., 2000).</td>
</tr>
<tr>
<td>Conventional drugs</td>
<td>Herbs</td>
<td>Potential interaction</td>
</tr>
<tr>
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<tr>
<td>Analgesics</td>
<td>-Herbs with diuretic activity (eg. corn silk, dandelion, juniper). -Herbs with corticosteroid activity (eg, licorice, bayberry) -Herbs with sedative effects (eg, calamus, nettle, ground ivy, sage, borage).</td>
<td>-Increased risk of toxicity with anti-inflammatory analgesics. -May induce reduction of plasma salicylate concentration. -Possible enhancement of sedative effects.</td>
</tr>
<tr>
<td>Anticonvulsants</td>
<td>-Herbs with potential sedative effects (eg, calamus, nettle, ground ivy, sage, borage). -Herbs containing salicylates (eg, poplar, willow). -Ayurvedic Shankapuspi.</td>
<td>-Possible increase in sedative side effects. Increase risk of seizure. -Potentiation of phenytoin action. -Decrease phenytoin half life.</td>
</tr>
<tr>
<td>Antidepressants</td>
<td>-Herbs with sympathomimetic amines (eg, agnus castus, calamus, cola, broom, licorice). -Ginkgo biloba.</td>
<td>-Increase risk of hypertension with MAO inhibitors. -May potentiate sedative side effects. -Use with tricyclic antidepressants or other medications which increase the seizure threshold were not advised.</td>
</tr>
<tr>
<td>Antiemetic and antivertigo</td>
<td>-Herbs with potential sedative effects (eg, calamus, nettle, ground ivy, sage, borage). -Herbs with anticholinergic effects.</td>
<td>-May increase sedative effect. -Antagonism.</td>
</tr>
<tr>
<td>Antiparkinsonism</td>
<td>-Herbs with anticholinergic effects. -Herbs with cholinergic effects.</td>
<td>-Potentiation of effects. -Antagonism.</td>
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<tr>
<td>Antipsychotics</td>
<td>-Herbs with diuretic activity (e.g., corn silk, dandelion, juniper, uva ursi) -Herbs with anticholinergic effects -Ginseng, yohimbine, and ephedra</td>
<td>-Potentiation of lithium action; increased risk of intoxication -Reduction of phenothiazine concentrations; increased risk of seizures -Concomitant use with phenelzine and MAO inhibitors may result in increased side effects.</td>
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<tr>
<td>Antimaniac Lithium</td>
<td>-Green tea, Gaurana.</td>
<td>-Increase lithium levels.</td>
</tr>
<tr>
<td>Anxiolytics, Sedatives, Hypnotics, Phenobarbital</td>
<td>-Herbs with sedative effects (eg, Calamus, nettle, ground ivy, sage, borage). -Valerian, Ginger, Goldenseal. -Thujone-containing herbs (e.g., wormwood, sage) or gamolenic acid-containing herbs (e.g., evening primrose oil, borage).</td>
<td>-Potentiation. -Increased sedation. -May lower seizure threshold.</td>
</tr>
<tr>
<td>CNS stimulants</td>
<td>-Herbs having CNS stimulatory effects like Ephedra, Green tea, Gaurana.</td>
<td>-Potentiation. -Increased stimulatory effect.</td>
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</table>
In vitro and in vivo approaches for evaluation of herbal drug interactions

Several approaches (in vitro and in vivo are used to evaluate herbal drug interactions. Recently, there has been an increase in the use of in silico approaches to drug metabolism, drug transport and drug interaction studies. Most research on such interactions has focused on the in vitro evaluation of herbal constituents in microsomal systems, supersomes, cytosols, expressed enzymes or cell culture systems such as transfected cell lines, primary cultures of human hepatocytes and tumor derived cells (MacGregor et al., 2001). These studies are valuable for evaluating multiple products and multiple components, provide mechanistic information about any potential interaction and are simple to conduct. It has certain limitations as to the simple component used in the test; a typical of higher concentrations than clinical relevance and it does not account for the poor bioavailability of the active component or the binding of the same in vivo to plasma proteins (Venkataramanan et al., 2006). In addition, studies have also been carried out in vivo in animals (normal, transgenic, humanized) and in humans (primarily healthy individuals). Most of the studies reported have used the commercially available products or a crude extracts of the herbal product or isolated purified individual components. These studies so far have paid particular attention to the effect of herbal components on CYP enzymes. Only a small number of studies have examined the effects of herbal products on phase II metabolism or drug transport.

In vitro studies using microsomes

Many studies on herbal drug interactions using microsomes were reported (Obach, 2000; Beckmann-Knopp et al., 2000; Zuber et al., 2002). Studies in microsomes while providing information on the potential of a chemical to alter enzyme activity were limited in that they were useful only to evaluate acute inhibition of metabolism and not induction of metabolism as they are not intact cell systems. Furthermore, it was not possible to evaluate the effect of herbal components on transporters using microsomes.
Since excess co-substrate was added in the system it was not possible to evaluate co-substrate depletion as a potential mechanism of any interactions. Microsomal studies also do not provide complete mechanistic information of any interactions (effects on mRNA or protein and the potential role of any metabolite formed).

**In vitro studies using primary cultures**

Primary cultures are valuable *in vitro* tools in evaluating herbal drug interactions (Venkataramanan et al., 2006). The use of more physiologically relevant *in vitro* models, such as primary cultures of human hepatocytes (PCHH) are necessary if better predictions of such interactions are to be made in humans. These systems also facilitate determination of whether there is a need to conduct more demanding clinical studies. Primary cultures of human hepatocytes are viable for up to 2 weeks (or one month if placed in a three-dimensional culture) and retain all co-factors and co-substrates necessary for phase I and phase II metabolic pathways and transporter function, making them a versatile *in vitro* system to study induction and inhibition of drug metabolizing enzymes and certain transporters (Hellum et al., 2009; Gebhardt et al., 2003). As the use of PCHH has advanced, modified culturing techniques have enabled the examination of other processes involved in drug metabolism, namely the uptake and efflux of drugs and their metabolites by hepatic drug transporters.

**In vivo studies**

*In vivo* studies in humans have been carried out with various experimental designs (Nagai et al., 2009; Tomlinson et al., 2008; Venkataramanan et al., 2006; Gurley et al., 2005; DiCenzo et al., 2003). Typically subjects receive a single dose of a test drug or a cocktail of drugs that are markers for various enzymes on day 1. This is followed by multiple daily dose treatment with the herbal product (typically one week) and on the last day of treatment; administration of the test drug or the cocktail of drugs is accomplished. A comparison of the various pharmacokinetic parameters or phenotypic measures is used as a method to evaluate the effect of herbal products on the pharmacokinetics of test drug or activity of various drug metabolizing enzymes.
(Pekthong et al., 2009). One such study was reported recently suggested that the long-term use of *Ginkgo biloba* extract significantly influenced talinolol disposition in humans, likely by affecting the activity of P-glycoprotein and/or other drug transporters (Fan et al., 2009).

Some ideal *in vivo* study designs include:

1. Evaluating the composition of the herbal product used.
2. Evaluating the disintegration and dissolution property of product used.
3. Using chronic dosing of the herbal products (at least one week).
4. Co-administering herb and drug product on study day to maximize potential for interaction.
5. Using positive controls in the study design (for example, rifampin to document induction and ketoconazole to document inhibition and give a comparative effect of the herbal product being tested).
6. Measuring some herbal component in the blood or plasma to verify systemic levels of some components from the herbal product.

While *in vitro* studies in microsomal system provide limited information, human hepatocyte system offers a unique opportunity to evaluate herbal drug interactions and will help in focusing and minimizing *in vivo* human studies. A lack of effect in human hepatocyte would suggest lack of *in vivo* effect. A positive response in human hepatocyte culture would indicate a need for further assessment in vivo in humans. While other systems provide useful information, *in vivo* studies in humans are suggested as the only definitive way to assess the magnitude and implications of herbal–drug interactions (Venkataramanan et al., 2006). With an increased understanding of the mechanism of such interactions, it should be feasible to minimize or avoid therapeutic failures or increased toxicity of conventional drug therapy.
2. Plant profiles

2.1. *Bacopa monniera*

**Introduction**

In the folklore of Indian medicine, several herbal plants have been used traditionally as brain or nerve tonics. One of the most popular of these herbs is *Bacopa monniera* (BM), a well-known memory booster. BM, also referred to as *Herpestis monniera* or water hyssop, is locally known as brahmi or Jalanimba. The plant has been used for centuries in the Ayurveda, a holistic system of medicine originating from India, where it has been classified as under ‘Medhya rasayana’, i.e., medicinal plants rejuvenating intellect and memory (Rai et al., 2003). The name brahmi is derived from the word ‘Brahma’, the mythical ‘creator’ in the Hindu pantheon. Because the brain is the centre for creative activity, any compound that improves the brain health is called brahmi, which also means ‘bringing knowledge of the supreme reality’. The herb has long been used medicinally and as an aid to meditation (Kumar, 2006). In India, BM is largely treasured as a revitalizing herb used by Ayurvedic medical practitioners for almost 3000 years. The plant has been mentioned in several ancient Ayurvedic treatises including the “Charaka Samhita” since 6th century AD, in which it is recommended in formulations for the management of a range of mental conditions including anxiety, poor cognition and lack of concentration, as a diuretic and a tonic for the nervous system and heart (Mukherjee and Dey, 1966). Specific uses include the treatment of asthma, insanity and epilepsy (Chopra, 1958). It has been utilized extensively as a nootropic, digestive aid, and to improve learning, memory and respiratory function (Nadkarni, 1988; Kirtikar and Basu, 1918).

**Description of the plant**

*Bacopa monniera* (BM), from a family *Scrophulariaceae* is a small creeping herb with numerous branches, small oblong leaves, and light purple or small and white flowers, with four or five petals. (Refer, figure 1.2. A herb plant- *Bacopa monniera*).
The leaves are succulent, relatively thick, and oblanceolate and are arranged oppositely on the stem.

Figure 1.2. A herb plant - *Bacopa monniera*.

The plant is found in wetlands throughout the Indian subcontinent in wet, damp and marshy or sandy areas near streams in tropical regions. The genus *bacopa* includes over 100 species of aquatic herbs distributed throughout the warmer regions of the world, apart from India, Nepal, Sri Lanka, China, Taiwan, and Vietnam, and is also found in Florida and other southern states of the USA where it is recognized as weeds in rice fields (Barrett and Strother, 1978; Russo and Borrelli, 2005). The herb can be found at elevations from sea level to altitudes of 4,400 feet, and is easily cultivated if adequate water is available. Propagation is often achieved through cuttings. Flowers and fruit appear in summer. The entire plant is used medicinally (Satyavati et al., 1976).

**Active constituents**

Compounds responsible for the pharmacological effects of BM include alkaloids, saponins, and sterols. Detailed investigations first reported the isolation of the alkaloid “brahmine” from BM (Bose and Bose, 1931). Later, other alkaloids like nicotine and herpestine have also been reported (Chopra, 1956). Subsequently, the isolation of D-mannitol, and a saponin, hersaponin and potassium salts were reported (Shastri et al., 1959). The major chemical entity shown to be responsible for neuropharmacological effects and the nootropic action or antiamnestic effect of BM, is bacoside A, assigned as 3-(α-L-arabinopyranosyl)-O-b-D-glucopyranoside-10,20-dihydroxy-16-ketammar-24-ene (Chatterji et al., 1965).
Bacoside A usually co-occurs with bacoside B; the latter differing only in optical rotation and probably an artefact produced during the process of isolating bacoside A (Rastogi, 1990). On acid hydrolysis, bacosides yield a mixture of aglycones, bacogenin A1, A2, A3 (Kulshreshtha and Rastogi, 1974; Kulshreshtha and Rastogi, 1973; Chandel et al., 1977), which are artefacts, and two genuine sapogenins, jujubogenin and pseudojujubogenin and bacogenin A4, identified as ebelin lactone pseudojujubogenin were isolated (Rastogi et al., 1994). Successively, a minor saponin bacoside A1 and a new triterpenoid saponin, bacoside A3 were isolated (Rastogi et al., 1994). Later, three new dammarane-type triterpenoid saponins of biological interest, bacopasaponins A, B and C, pseudojujubogenin were isolated and a new dammarane-type pseudojujubogenin glycoside, bacopasaponin D, were identified by spectroscopic and chemical transformation methods (Garay et al., 1996). In view of the increasing interest on this herbal plant, yet two new pseudojujubogenin glycosides designated as bacopaside I and II were isolated from glycosidic fraction of the methanol (Chakravarty et al., 2001). Subsequently, three new saponins from BM, designated as bacopasides III, IV and V were isolated (Chakravarty et al., 2003). In addition, the isolation of three new phenylethnoid glycosides, viz. monnierasides I–III along with the known analogue plantainoside B was reported from the glycosidic fraction of BM (Chakravarty et al., 2002). Moreover, an isolation of a new saponin, a jujubogenin, named bacopasaponin G, and a new glycoside, phenylethyl alcohol was also reported (Hou et al., 2002). The chemical structures (Deepak, 2003) of some saponins isolated from BM are shown. (Refer, figure 1.3. Chemical structures of some well known saponins of *Bacopa monniera*).
Bacoside A (Levorotatory)

Bacoside B (Dextrorotatory)

(Triterpenoid saponin)  (Bacogenin A4)

Figure 3.3. Chemical structures of some well known saponins of Bacopa monniera.
Mechanism of actions based on preclinical studies

The BM extracts and isolated bacosides have been extensively investigated for their neuropharmacological effects. The triterpenoid saponins and their bacosides are responsible for BM’s ability to enhance nerve impulse transmission. It was suggested that bacosides induce membrane dephosphorylation, with a concomitant increase in protein and RNA turnover in specific brain areas (Singh et al., 1988). The other proposal that was put forward was that BM enhances protein kinase activity in the hippocampus which may also contribute to its nootropic action and thus it would aids in repair of damaged neurons by enhancing kinase activity, neuronal synthesis, and restoration of synaptic activity and ultimately nerve impulse transmission (Singh and Dhawan, 1997).

Sedative and tranquillizing properties

Earlier studies reported a sedative effect of glycosides named hersaponins (Malhotra and Das, 1959). Subsequent study has found that the alcoholic extract, and to a lesser extent the aqueous extract of the whole plant exhibited tranquilizing effects on albino rats and dogs (Aithal and Sirsi, 1961). On the other hand, it has been found that the alcoholic extract of the plant and chlorpromazine improved the performance of rats in motor learning (Prakash and Sirsi, 1962). A previous study has reported that a single dose of the glycoside hersaponin is better than pentobarbitone in facilitating acquisition and retention of brightness discrimination reaction (Sinha, 1971).

Cognition

A team of other researcher reported that a standardized bacosides-rich extract of BM, reversed the cognitive deficits induced by intracerebroventricularly administered colchicines and injection of ibotenic acid into the nucleus basalis magnocellularis (Bhattacharya et al., 1999a). In the same study, BM was also shown to reverse the depletion of acetylcholine, the reduction in choline acetylase activity and decrease in muscarinic cholinergic receptor binding in the frontal cortex and hippocampus. The cognition facilitating activity of the BME was attributed to the saponins, bacoside A
and bacoside B, which were effective in much lower doses in various model studies, including tests for conditioned taste aversion and conditioned shock avoidance response (Singh et al., 1988; Singh and Dhawan, 1982). BM’s antioxidant properties and its ability to balance super oxide dismutase (SOD) and Catalase levels were postulated to be responsible for these effects (Sairam et al., 2001).

**Antidepressant and Antianxiety effects**

Research using a rat model of clinical anxiety demonstrated that a BME containing 25-percent bacoside A exerted anxiolytic activity comparable to lorazepam, a common benzodiazepine anxiolytic drug and it was noted with attention that the BME did not induce amnesia, the side effects associated with lorazepam, but instead had a memory-enhancing effect (Shankar and Singh, 2000; Bhattacharya and Ghosal, 1998). The antidepressant potential of BM has been evaluated in an earlier study wherein it showed a significant antidepressant activity in the most commonly used behaviour paradigms in animal models of depression, namely, forced swim test and learned helplessness test (Sairam et al., 2002). In the study, the BME in the doses of 20 to 40 mg per kg was given once daily for five days and were found to be comparable to that of standard antidepressant drug imipramine in rodents for antidepressant activity. The same study has postulated the role of serotonin and GABA (gamma amino butyric acid) as the mechanism of action attributed for its antidepressant action along with its anxiolytic potential, based on the compelling evidence that the symptoms of anxiety and depression overlap each other (Shader and Greenblatt, 1995).

**Anti-epileptic effects**

Although BM has been indicated as a remedy for epilepsy in Ayurvedic medicine (Shanmugasundaram et al., 1991), research in animals showed anticonvulsant activity only at high doses over extended periods of time. Early research in India demonstrated that hersaponin (an active constituent) exhibited protection against seizures in mice and mentioned the possibility of its use as an adjuvant in treatment of epilepsy (Martis and Rao, 1992).
One Indian study examined the anticonvulsant properties of BM extracts in mice and rats. Researchers determined that intraperitoneal injections of high doses of BME (close to 50 percent of LD$_{50}$) given for 15 days demonstrated anticonvulsant activity. When administered acutely at lower doses (approaching 25 percent of LD$_{50}$), anticonvulsant activity was not observed (Ganguly and Malhotra, 1967). It was postulated in the study that the anti-convulsive effects could be mediated through GABA which is involved in neural impulse transmission, because substances which stimulate GABA are known to possess anticonvulsant, pain relieving and sedative activity.

**Antioxidant and adaptogenic properties**

BME or bacosides have shown an antioxidant activity (Kapoor et al., 2008; Singh et al.; 2006, Bafna and Balaraman, 2005; Rohini et al., 2004; Sumathy et al., 2001; Pawar et al., 2001; Bhattacharya et al., 2000; Tripathi, et al., 1996) and antistress (Bhakuni et al., 1969). A previous study suggests an involvement of the GABA-ergic system in the mediation of these central nervous system effects of BM (Singh et al., 1996). Based on animal study results, bacosides were shown to have antioxidant activity in the hippocampus, frontal cortex, and striatum (Bhattacharya et al., 2000). Animal research has shown that the BME modulate the expression of certain enzymes involved in generation and scavenging of reactive oxygen species in the brain (Govindarajan et al., 2005).

It was suggested that the adaptogenic properties of the herb would be beneficial in the management of stress related conditions as BM showed the potential to be effective in stress in one study on rats (Chowdhuri et al., 2002). In the study, BME was not only found to induce the constitutent expression of heat-shock protein (HSP 70) but also induce the CYP 450 enzymes in all regions of brain. The level of HSP 70 was found to be increased in brain as a response to stress. On the other hand, the group that was pre-treated for one week with 20 to 40 mg/kg/daily, before giving stress, the HSP 70 was found to be in lower concentration.
An increase in the activity of CYP 450 dependent enzymes \textit{7-pentoxyresorufin-odealkylase} (PROD) and \textit{7-ethoxyresorufin-o-deethylase} (EROD) was observed in all the brain regions after exposure to stress alone and with both doses of BME although the magnitude of induction observed was less with a higher dose of the same. Therefore, it was suggested that the BM primed the brain for stress by stockpiling these useful enzymes even before stressful conditions and that our susceptibility to stress could be lowered by using this medicinal herb. It was speculated that this induction may be an adaptive response to the stress which needs further investigation. The level of SOD was also increased in brain in the groups pre-treated with BME. The data indicated that BME has a potential to modulate the activities of HSP 70, \textit{CYP 450} and SOD and thereby possibly allowing the brain to be prepared to act under adverse condition like stress.

Researchers concluded that BM helps in coping with combined hypoxic, hypothermic and immobilization stress that could lead to onslaught of “free radicals" (Rohini et al., 2004). The results of the above mentioned study have indicated that this extract exhibits interesting antioxidant properties, expressed by its capacity to scavenge superoxide anion and hydroxyl radical, and to reduce H$_2$O$_2$-induced cytotoxicity and DNA damage in human fibroblast cells (Rai et al., 2003; Tripathi et al., 1996; Seiss et al., 1993). BME has shown neuroprotective effect against aluminium induced oxidative stress in the hippocampus of rat brain (Jyoti and Sharma, 2006). An \textit{aqueous} extract of BM was shown to reduce nicotine-induced lipid peroxidation (LPO) and conferred geno protection in Swiss mice in one study (Vijayan and Helen, 2007). Yet another study suggested that BME reduces amyloid levels in PSAPP mice and can be used in the therapy of Alzheimer's disease (Holcomb et al., 2006). One of the recent studies has shown the protective role of bacoside A against chronic cigarette smoking induced oxidative damage in rat brain (Anbarasi et al., 2006). This antioxidant activity of BM is able to explain, at least in part, the reported antistress, cognition-facilitating and antiaging effects produced by it in experimental animals and in clinical situations (Aloe
et al., 2002; Singh et al., 1996) and may justify further investigation of its other beneficial biological properties.

**Miscellaneous studies**

*In vitro* research has shown a protective effect of BM against DNA damage in astrocytes (Russo et al., 2003a) and human fibroblasts (Russo et al., 2003b). *In vitro* research has suggested that an anticancer effect of BM extracts is possibly due to inhibition of DNA replication in cancer cell lines (Elangovan et al., 1995).

A study in mice demonstrated high doses (200 mg/kg) of BME increased the thyroid hormone, T4, by 41 percent when given orally. T3 was not stimulated, suggesting the extract may have directly stimulated the synthesis and/or release of T4 at the glandular level, while not affecting conversion of T4 to T3. While this study indicated that BME did have a stimulatory effect on thyroid function, the doses were very high and it was assumed that the typical 200-400 mg daily dose in humans may not have the same effect (Kar et al., 2002).

BM was also reported to stabilize mast cells *in vitro* (Samiulla et al., 2001) and possesses anti-inflammatory activity via inhibition of prostaglandin synthesis and lysosomal membrane stabilization (Channa et al., 2006; Jain et al., 1994). Studies were also reported for its antiulcerogenic activity (Dharmani and Palit, 2006; Dorababu et al., 2004; Rao et al., 2000; Jain et al., 1994; Goel and Sairam, 2002) and its potential usefulness in intestinal spasm such as irritable bowel syndrome (IBS) (Dar and Channa, 1999; Dar and Channa, 1997), bronchoconstrictive and allergic conditions which warrant human studies (Channa et al., 2004; Samiulla et al., 2001).

**Clinical studies**

**Cognition**

Numerous clinical studies have been carried out to date, to establish the efficacy of BM in memory and attention disorders and to study its acute and chronic effects clinically on cognitive function. A study was conducted to measure the effect of BME on human memory (Roodenrys et al., 2002).
In seventy six adult volunteers, aged between 40 and 65 years in double-blind randomized placebo control study, the results showed a significant effect of BM on the test for the retention of new information. In the follow-up tests it was found that the rate of learning was unaffected, suggesting that BM decreases the rate of forgetting of newly acquired information. In adults, only chronic administration was shown to enhance cognitive effects. In a double-blind, placebo-controlled trial of 38 healthy volunteers (ages 18-60), subjects were given a single dose of 300 mg BME (standardized to 55-percent combined bacosides A and B) or placebo (Nathan et al., 2001). As demonstrated in a double-blind, placebo-controlled, 12-week trial which utilized the same patient selection criteria and same dose of BME (300 mg daily) containing 55-percent combined bacosides (Stough et al., 2001). Forty-six healthy volunteers (ages 18-60) were randomly and evenly divided into treatment and placebo groups. The same series of tests administered in the acute dosage trial were administered at baseline, five, and 12 weeks after treatment began. At the end of the 12-week study, results indicated a significant improvement in verbal learning, memory consolidation, and speed of early information processing in the treatment group compared to placebo. These effects were not observed at baseline or at five weeks. These results were attributed to BM’s antioxidant properties and/or its effect on the cholinergic system (Stough et al, 2001). BM’s ability to modulate or enhance cognitive function has also been studied in children (Negi et al, 2000; Sharma et al., 1987). In one double-blind, placebo-controlled randomized study, the efficacy of standardized BME (SBME) in subjects with age-associated memory impairment (AAMI) without any evidence of dementia or psychiatric disorder was evaluated. SBME was found to be efficacious in subjects with age-associated memory impairment (Raghav et al., 2006).

**Anxiety and depression**

The traditional use of BM as an anti-anxiety remedy in Ayurvedic medicine is supported by both animal and clinical research. A one-month, limited clinical trial of 35 patients with diagnosed anxiety neurosis demonstrated that administration of brahmi
Syrup (30 mL daily in two divided doses, equivalent to 12 g dry crude extract of BM resulted in a significant decrease in anxiety symptoms, level of anxiety, level of disability, and mental fatigue, and an increase in immediate memory span (Singh and Singh, 1980). Other changes noted were increased body weight, decreased respiration rate, and decreased systolic blood pressure. In one latest study, effects of a SBME (300 mg/day) on cognitive performance, anxiety, and depression in the elderly were evaluated in a randomized, double-blind, placebo-controlled clinical trial with a placebo run-in of 6 weeks and a treatment period of 12 weeks (Calabrese et al., 2008). In fifty-four participants aged 65 or older (mean 73.5 years), without clinical signs of dementia, were recruited and randomized to BM or placebo. Forty-eight (48) completed the study with 24 in each group. BM participants were found to have enhanced Auditory Verbal Learning Test (AVLT), delayed word recall memory scores relative to placebo, decreased Center for Epidemiologic Studies Depression scale (CESD-10) depression scores, combined state plus trait anxiety scores, and heart rate over time compared to that of placebo group. This study provided further evidence that BM has a good potential for safely enhancing cognitive performance in the aging.

Side effects and toxicity

BM has a record of several hundred years of relatively safe therapeutic use in Ayurvedic medicine. A double-blind, placebo controlled clinical trial of healthy male volunteers investigated the safety of pharmacological doses of isolated bacosides over a four-week period. Concentrated bacosides given in single (20-30 mg) and multiple (100-200 mg) daily doses were well tolerated and without adverse effects (Singh and Dhawan, 1997). The LD₅₀ of aqueous and alcoholic extracts of BM in rats were found to be 1000 mg and 15g/kg by the intraperitoneal route, respectively (Martis and Rao, 1992) and the aqueous extract given orally at a dose of 5 g/kg did not show any toxicity. The LD₅₀ of the alcoholic extract was reported as 17g/kg given orally. Both extracts have not produced any gross behavioural changes at these dose levels (Monograph, 2004).
Dosage

The daily doses of BM, generally recommended in traditional practice are 5-10 g of non-standardized powder, 8-16 mL of infusion, and 30 mL daily of syrup (brahmi) (Monograph, 2004). Dosages of a 1:2 fluid extract are 5-12 mL per day for adults and 2.5-6 mL per day for children ages 6-12. For BME standardized to 20-percent bacosides A and B the dosage is 200-400 mg daily in divided doses for adults, and for children, 100-200 mg daily in divided doses. The recommended daily dose of Bacopin® (standardized to contain a minimum of 20% Bacosides A and B) for an adult is 100 mg - 3 times daily and children 50 mg - 3 times daily (Monograph, 2004). In India, the Ayurvedic doctors use it without any ill effects in children, pregnant ladies and breast feeding mothers but no parallel studies of its use in children, pregnant and breast feeding women regarding toxicity or herb-drug interactions, unlike modern pharmacological drugs are available, so its use may warrant precautions.

Herbal drug interactions

In vitro and animal studies have demonstrated that the BM extracts might potentiate the effect when taken with some synthetic drugs or it might have a protective effect against certain drugs and their negative side effects. BM has been noted in animal models to decrease the toxicity of morphine and phenytoin (Sumathy et al., 2001). In this study the effects of an alcohol extract of BM on morphine-induced hepatotoxicity in rats was examined, as measured by lipid peroxide accumulation and antioxidant enzyme levels. Administration of BME with morphine was found to significantly decrease lipid peroxidation, in addition to increased levels of antioxidant enzymes and glutathione in rat hepatic tissue, when compared to morphine alone. These results suggested a protective effect for BM on the hepatic antioxidant status in morphine-treated rats. In mice, BM administration with phenytoin significantly reversed phenytoin-induced cognitive impairment, as noted by improved acquisition and retention of memory (Smith, 1991). In study, the passive avoidance response task (PA), maximal electroshock seizures and locomotor activity were evaluated in mice.
The mice received phenytoin (PHT, 25 mg/kg orally for 14 days). BM (40 mg/kg for 7 days) given along with phenytoin in the second week of the two-week regimen significantly reversed PHT-induced impairment of cognitive function as determined from the PA results. Both acquisition and retention of memory were improved without affecting the anti-convulsant activity of PHT in the study. These effects were found to be independent of motor stimulation. The results suggested a potential corrective effect of BME in phenytoin-induced cognitive deficit (Vohora et al., 2000). It has also been shown, albeit inconsistently; to have a slight sedative effect (Brinker, 2008; Martis and Rao, 1992). Both cold aqueous infusion and standardized 95% alcoholic BME potentiated the sleep, prolonging the hypnotic effect of sodium phenobarbitone in rats in the study. This sedative action observed in rats was attributed to the saponin fraction bacosides. So a caution was raised when the same is administered in combination with other known sedatives.

One in vitro study using guinea pig ileum isolates examined the effect of BME on drug-induced morphine withdrawal. Addition of 1,000 µg/mL BME to the tissue isolates, prior to injection of morphine significantly reduced the naloxone-induced withdrawal effects in the study (Sumathi et al., 2002a), an effect that was attributed to the anticholinergic and calcium antagonistic properties. The same researchers reported a similar effect for brain mitochondrial enzyme activity of morphine treated rats (Sumathy et al., 2002b). Also, since BME appeared to stimulate T4 thyroid hormone activity in animals at high doses (Kar et al., 2002), it was theorized that it may potentiate the activity of thyroid-stimulating drugs or inhibit the effect of thyroid-suppressant drugs. An animal study has found that the effects of chlorpromazine, a drug similar to (perphenazine, prochlorperazine, thioridazine), were enhanced when a BME was given along with it (Ganguly and Malhotra, 1967). So it was cautioned that people taking medications from these family of drugs mentioned above should be careful while taking BM, until more information is available. Moreover, the benefits of BM have been so good in anxiety and depression that it could be used alone for mild to moderate
problems. However if is taken along with other synthetic drugs, a caution should be taken to monitor the response and the dosage.

**Current findings and future prospects**

In a recent study, the neuroprotective role of BME was investigated in hippocampus of temporal lobe epileptic rats (Khan et al., 2008). The study concluded that BME treatment potentiate the therapeutic effect by reversing the alterations in glutamate receptor binding and NMDA R1 gene expression that occur during epilepsy, resulting in reduced glutamate-mediated excitotoxicity in the over stimulated hippocampal neurons. Apropos to a crucial role that glutamate and its receptors play in consolidation of memory, a current study hypothesized role of glutamatergic synapses as a potential target for bacoside action (Hota et al., 2009). Hence, the effect of chronic bacoside supplementation on the glutamatergic transmission was studied by investigating the expression of NR1 subunit of NMDA receptor and activity of glutamate dehydrogenase that catalyzes glutamate synthesis. In the study, the standardized BME administration was seen to enhance learning ability in rats along with augmentation in memory retrieval and prevention of dendritic atrophy following hypoxic exposure. In addition, it was shown to decrease oxidative stress, plasma corticosterone levels and neuronal degeneration. Bacoside administration also increased cytochrome c oxidase activity along with a concomitant increase in ATP levels and it was suggested that the administration of bacosides could be a useful therapeutic strategy in ameliorating hypobaric hypoxia induced cognitive dysfunctions and other related neurological disorders.

In light of many reports showing important activities of BME or bacosides, the wide variety of neuropharmacological actions of BM opens up interesting avenues for further research and offers new perspectives in the treatment of many diseases. While the activity of BM both as an anxiolytic and antidepressant needs further evaluation, its potential as an anti-epileptic treatment and as a treatment to correct side effects of anti-epileptic drugs is another area to be studied in the future.
Also, the antioxidant capacity of BM which explain, at least in part, the reported antistress, immunomodulatory, cognition-facilitating, anti-inflammatory and antiaging effects produced by it in experimental animals as well as in clinical situations, it may justify further investigation of its other beneficial properties. Moreover, these experimental evidences suggest that because of its antioxidant activity, the plant may be useful in the treatment of human pathologies in which free radical production plays a key role.

The antifertility potential of BM was recently disclosed in male mice wherein it was shown to cause reversible suppression of spermatogenesis and fertility, without producing apparent toxic effects (Singh and Singh, 2008). BM was also shown to have thrombolytic activity in one recent in vitro study (Prasad et al., 2007).

In addition to all pharmacological studies mentioned above, herb-drug and herb-herb interactions of BM need to be studied. In recent years, various case reports and clinical studies in herbal drug interactions have been published which provided a consistent evidence that the interactions between herbal medicines and synthetic drugs exist and can have serious consequences (Fugh–Berman and Ernst, 2001; Izzo and Ernst, 2001, Gohil and Patel, 2007; Patel and Gohil, 2008). Therefore, it is necessary to consider the possibility of BM-drug interactions and the need for exercising requisite precautions while co-medicating the herb extract with synthetic medications.
2.2. *Centella asiatica*

**Introduction**

*Centella asiatica* (CA) is an outstandingly important medicinal herb that is widely used in the orient (Bown, 1995) and is becoming increasingly popular in the west (Chevallier, 1996). Commonly known as mandukparni, Indian pennywort or jalbrahmi, it has been used as a medicine in the Ayurvedic tradition of India for thousands of years and listed in the historic Sushruta Samhita, an ancient Indian medical text (Diwan et al., 1991; Chopra et al., 1986).

In China, known as gotu kola, it is one of the reported "miracle elixirs of life" known over 2000 years ago. In the nineteenth century, CA and its extracts were incorporated into the Indian pharmacopoeia, wherein addition to being recommended for wound healing, it was recommended in the treatment of skin conditions such as leprosy, lupus, varicose ulcers, eczema, and psoriasis, even diarrhoea, fever, amenorrhea, and diseases of the female genitourinary tract (Brinkhaus et al., 2000). CA or gotu kola is not to be confused with kola nut as it does not contain any caffeine and has not been shown to have stimulant properties.

**Description of the plant**

*Centella asiatica* (CA), a clonal, perennial herbaceous creeper belongs to the family *Umbellifere* (*Apiceae*) and is found throughout India in moist places up to an altitude of 1800 m in tropical and subtropical countries in swampy areas, including parts of India, Pakistan, Sri Lanka, Madagascar, South Africa, South pacific and Eastern Europe. About 20 species related to CA grow in most parts of the tropical or wet pantropical areas such as rice paddies, and also in rocky, higher elevations (Bown, 1995). It is a tasteless, odourless plant that thrives in and around water, having small fan-shaped green leaves with white or light purple-to-pink or white flowers and bears small oval fruit. (Refer, figure 1.4. A herb plant- *Centella asiatica*). The whole plant is used for medicinal purposes (Singh and Singh, 2002). It is widely used as a blood purifier as well as for treating a variety of other illnesses.
CA is used to lower high blood pressure, improve memory and promote longevity (Chopra et al., 1986; Brinkhaus et al., 2000). In Ayurveda, it is one of the chief herbs for revitalizing the nerves and brain cells. Eastern healers relied on CA to treat emotional disorders, such as depression, that were thought to be rooted in physical problems (Monograph, 2007). In western medicine during the middle of the twentieth century, CA and its alcoholic extract were shown to have positive results in the treatment of leprosy (Baily, 1945).

Figure: 1.4. A herb plant - *Centella asiatica*

**Active constituents**

The primary active constituents of CA are saponins (also called triterpenoids), include asiaticosides in which a trisaccharide moiety is linked to the aglycone asiatic acid, madecassoside and madasiatic acid (Singh and Rastogi, 1969). (Refer, figure 1.5. Three wound healing components in *Centella asiatica*). These triterpene saponins and their sapogenins are thought to be mainly responsible for the wound healing and vascular effects by inhibiting the production of collagen at the wound site (Singh and Rastogi, 1969). Other components isolated from CA, such as brahmoside and brahminoside, which are postulated to be responsible for CNS and uterorelaxant actions, but are yet to be confirmed by clinical studies. Crude extract that contains glycosides isothankuniside and thankuniside showed antifertility action in mice (Heidari et al., 2007; Duke 1985).
In addition, the total extract contains plant sterols, flavonoids, and other components with no known pharmacological activity, namely, abundant tannins (20-25%), essential acid (0.1% with beta-chariophylen, trans-beta-pharnesen and germachrene D), phytosterols (campesterol, sitosterol, stigmasterol), mucilages, resins, free amino acids (alanine, serine, aminobutyrate, aspartate, glutamate, lysine and treonine), flavonoids (derivates of chercetin and kempferol), an alkaloid (hydrochotine), a bitter component (vallerine), fat acids (linoleic acids, linolnelic, oleic, palmitic and stearic) (Srivastava et al., 1997).

Mechanism of actions based on preclinical studies

Sedative and anxiolytic properties

CA has been described to possess CNS effects in Indian literature such as stimulatory-nerve tonic, rejuvenator, sedative, tranquilizer and intelligence promoting property (Kumar, 2006; Gupta et al., 2003). It has been traditionally used as a sedative agent in many Eastern cultures, with the effects was postulated mainly due to the brahmoside and brahminoside constituents. On the other hand, the anxiolytic activity, in part was attributed due to it’s binding to cholecystokinin receptors (Ramaswamy et al., 1970).

Antidepressant activity

The antidepressant effect of total triterpenes from CA on the immobility time in forced swimming in mice and concentration of amino acid in mice brain tissue were observed.
(Chen et al., 2003). In the study, imipramine and total triterpenes from CA reduced the immobility time and ameliorated the imbalance of amino acid levels confirming the antidepressant activity of CA. Later on, the same authors investigated the possible antidepressant effect of total triterpenes of CA by measuring the corticosterone levels in mice brain (Chen et al., 2005). In this study, the contents of monoamine neurotransmitters and their metabolites in rats cortex, hippocampus and thalamus were evaluated wherein significant reduction of the corticosterone level and increase of the contents of serotonin (5-HT), noradrenaline (NA), dopamine (DA) and their metabolites high 5-hydroxyindoleacetic acid (5-HIAA), 3-methoxy-4-hydroxyphenylglycol (MHPG) in rat brain were observed which further strengthened the postulated involvement of total triterpenes of CA in ameliorating the function of HPA axis and increasing the contents of monoamine neurotransmitters for its antidepressant effects.

**Anti-epileptic effects**

Asian CA increases the cerebral levels of $\gamma$-amino butyric acid (GABA), which explains its traditional use as anxiolytic and anti-convulsant. It is known that GABA and its agonists inhibit the central cholinergic action by affecting the turnover rate of acetylcholine in the rat brain. The isolated steroids from the plant have been used to treat leprosy (Hausen, 1993). In one study, the effect of *aqueous* extract of CA (100 and 300 mg/kg) was evaluated on the course of kindling development, kindling-induced learning deficit and oxidative stress markers in pentylenetetrazole (PTZ) kindled rats (Gupta et al., 2003). The administration of CA (300 mg/kg orally) decreased the PTZ-kindled seizures and showed improvement in the learning deficit induced by PTZ kindling as evidenced by decreased seizure score and increased latencies in passive avoidance behaviour. The findings suggested the potential use of *aqueous* extract of CA as adjuvants to antiepileptic drugs with an added advantage of preventing cognitive impairment. The hydroalcoholic extract of CA leaves was also subjected to pharmacological screening using various experimental models and was found to have a
protective action against increase in current electroshock (ICES) and chemoconvulsions, which includes pentylenetetrazol-induced convulsions, pentylenetetrazol-kindled seizures, and strychnine-induced opisthotonus tonic convulsions on oral administration (Ganachari et al., 2004). It also showed a reduction in formation of lipid peroxidation products and in spontaneous motor activity, potentiation in diazepam withdrawal-induced hyperactivity, hypothermia, and potentiation of pentobarbitone sleeping time. The extract (200 mg/kg body weight) completely inhibited pentylenetetrazol-induced convulsions. In pentylenetetrazol-kindled seizures and strychnine-induced convulsions, the extract showed protection at a dose of 100 mg/kg body weight. The doses of the extract selected for remaining studies were based on pilot studies, animal model used, and so forth. These findings suggested its potential anticonvulsant as well as antioxidant as well as CNS depressant actions (Ganachari et al., 2004).

**Cognition and antioxidant properties**

CA is known to re-vitalize the brain and nervous system, increase attention span and concentration and to combat aging (Brinkhaus et al., 2000). A study demonstrated cognitive-enhancing and anti-oxidant properties of CA in normal rats (Gupta et al., 2003). The effect of an aqueous extract of CA (100, 200 and 300 mg/kg for 21 days) was evaluated in intracerebroventricular (i.c.v.) streptozotocin (STZ) -induced cognitive impairment and oxidative stress in rats (Veerendra Kumar and Gupta, 2003). The rats treated with CA showed a dose-dependent increase in cognitive behaviour in passive avoidance and elevated plus-maze paradigms. A significant decrease in malondialdehyde (MDA) and an increase in glutathione and Catalase levels were observed in rats treated with 200 and 300 mg/kg CA. As the oxidative stress or an impaired endogenous anti-oxidant mechanism is an important factor that has been implicated in Alzheimer's disease (AD) and cognitive deficits seen in the elderly and the i.c.v. (STZ) in rats has been linked to sporadic AD in humans and the cognitive
impairment was associated with free radical generation in the model. The findings reported in above study suggested the potential efficacy of CA in preventing the cognitive deficits, as well as the oxidative stress (Gupta et al., 2003). To throw more light on mechanism of the neuroprotection by CA, a recent study reported that the phosphorylation of cyclic AMP response element binding protein (CREB) is enhanced in both a neuroblastoma cell line expressing amyloid beta 1-42 (A beta) and in rat embryonic cortical primary cell culture (Xu et al., 2008). In addition, the contribution of two major single components to the enhanced CREB phosphorylation was examined. Furthermore, inhibitors were applied in this study revealed that extracellular signal-regulated kinase- Ribosomal S6 kinase (ERK/RSK) signaling pathway might mediate this effect of CAE. In another study, oral treatment with 50 mg X kg(-1) day (-1) of crude methanol extract of CA for 14 days significantly increased the anti-oxidant enzymes, like superoxide dismutase (SOD), catalase and glutathione peroxidase (GSHPx), and anti-oxidants like glutathione (GSH) and ascorbic acid decreased in lymphoma-bearing mice (Jayashree et al., 2003). In one study, derivatives of asiatic acid derivatives were shown to exert significant neuroprotective effects on cultured cortical cells by their potentiation of the cellular oxidative defence mechanism. Therefore, these agents were proved to be efficacious in protecting neurons from the oxidative damage caused by exposure to excess glutamate (Lee et al., 2000).

Another study demonstrated the protective effects of asiaticoside derivatives against beta-amyloid neurotoxicity when tested on B103 cell cultures and hippocampal slices. Out of 28 of the asiaticoside derivatives three components, including asiatic acid, showed a strong inhibition of beta-amyloid- and free radical-induced cell death and thus protects neurons from beta-amyloid toxicity. These derivatives were postulated as the potential candidates for a treatment of AD (Mook-Jung et al., 1999).

**Precautions and Safety**

CA has no known toxicity in recommended doses. Side effects reported were rare namely, skin allergy and burning sensations (with external use), headache, stomach
upset, nausea, dizziness, and extreme drowsiness which tend to occur with high doses of the herb (Eun and Lee, 1985). The fresh plant is postulated to have a low potential for skin irritation. A contact dermatitis has been reported on a few occasions using topical preparations and it was suggested that subcutaneous injections can trigger allergic reactions, cause pain at the injection site, or cause discoloration. Side effects occur less often when using intramuscular injections (Eun and Lee, 1985).

Oral consumption of an excessive amount of CA (i.e., overdose) can cause headaches and transient unconsciousness. Also, it was postulated that chronic treatment may cause spontaneous abortion in women (Dutta and Basu, 1968). As there is little or no information regarding the safety of this herb during breastfeeding, nursing mothers are advised to refrain from taking this herb. During prolonged treatment, especially with higher doses, the metabolism of asiaticoside to asiatic acid slows down proportionally to the plasma asiatic acid content and so it was suggested that this pharmacokinetic phenomena should be considered for effective and safe treatment (Grimaldi et al., 1990). The use of CA for more than 6 weeks is not recommended in the literature. People taking the herb for an extended period of time (up to 6 weeks) are advised to take a 2-week break before taking the herb again. The standardized extracts of CA and asiaticoside were well tolerated in experimental animals especially by oral route. Asiaticoside did not show any sign of toxicity up to the dose of 1 mg/kg after oral administration, whereas the toxic dose by intramuscular application to mice and rabbits was reported as 40-50 mg/kg (Kartnig, 1988).

**Herbal drug interactions**

Though, there have been no reports documenting negative interactions between CA and medications to date, the cautions were raised based mainly on the knowledge of mechanism of actions derived from the laboratory studies conducted on animals. Since high doses of CA were found to cause sedation, it was warned that individuals should refrain from taking this herb with medications that promote sleep or reduce anxiety (Monograph, 2007).
Theoretically, CA was postulated to interfere with blood glucose levels and thus it was proposed that the hypoglycemic therapy may possibly interfere with cholesterol lowering herbs like CA (Kartnig, 1986).

**Dosage**

A typical daily dose of CA reported is approximately 600 mg of dried leaves or infusion, single-dose capsules (300 mg to 680 mg, thrice daily) and a 10-mg concentrated extract, also available in capsules. Other preparations include Madecassol tablets 10 mg 3 times daily, Tincture 1 mL, and Emdecassol ointment twice daily (Monograph, 2007). Dried gotu kola leaf as a tea, by adding 1–2 teaspoons (5–10 grams) to about 2/3 cup (150 ml) of boiling water and allowing it to steep for ten to fifteen minutes and three cups (750 ml) are usually suggested per day. Fluid extract (1/2–1 teaspoon (3–5 ml) per day) or a tincture (2–4 teaspoons (10–20 ml) per day) are sometimes recommended and the standardized extracts containing up to 100% total saponins (triterpenoids), 60 mg once or twice per day, are frequently used in modern herbal medicine (Brinkhaus et al., 2000).

**Current findings and future prospects**

*Centella asiatica* is a useful plant having multiple useful clinical effects in CNS disorders. Further long-term studies will help determine the exact mechanism by which CA influences age-related changes in mood and cognitive function. Also the purported anxiolytic activity of CA is intriguing in view of the proposed involvement of cholecystokinin (CCK) in the pathophysiology of fear and anxiety. However, the mechanism of action and possible toxicity needs to be further investigated in a large sample which may bring to the light, the precise mechanisms for ameliorating many other CNS related conditions like depression and sleep disorders apart from anxiety. Moreover, more double blind randomized clinical trials are needed for investigating its sedative, analgesic, antidepressive, antiviral and immunomodulatory effects that have been demonstrated experimentally in animals.
Currently, a pilot study is ongoing on by National Centre for Complementary and Alternative Medicine (NCCAM) in U.S.A, Oregon to investigate the safety, tolerability and effectiveness of *Centella asiatica* Selected Triterpenes (CAST) (Anonymous, 2008b). In this phase II, randomized, double blind study, CA was evaluated as a treatment for diabetic neuropathy using primary outcome measure as total neuropathic symptom score and secondary outcomes as neurological disability score, nerve conduction measurements and quantitative sensory testing. The trial is aimed to be completed by the current year 2009. The therapeutic potential of this plant in terms of its efficacy and versatility is such that further detailed research appears momentous. The growing number of herbal preparations on the market, including CA raised the possibility of complications related to improper use of these products or the lack of medical supervision along with the likelihood of interactions with the drugs and herbs on simultaneous use. Several of the recent cases reported to The Special Nutritional Adverse Event Monitoring System (SN/AEMS) indicated the importance of providing patient counselling on the use of herbal preparations.
2.3. *Curcuma longa*

**Introduction**

Turmeric (*Curcuma longa L.*) is a medicinal plant extensively used in Ayurveda, Unani and Siddha medicine as a traditional treatment of sprains and swellings caused by injury and as a home remedy for various diseases, as a dietary spice and a food preservative or colouring pigment (Shrikumar and Ravi, 2007; Chattopadhyay et al., 2004; Ammon and Wahl, 1991). The herb is also used in the textile and pharmaceutical industries and in Hindu religious ceremonies in one form or another in India (Srimal and Dhawan, 1973).

**Description of the plant**

*Curcuma longa* (CL), a perennial herb, is a member of the *Zingiberaceae* (ginger) family. The plant grows to a height of three to five feet, and is cultivated extensively in Asia, India, China, and other countries with a tropical climate. It has oblong, pointed leaves and bears funnel-shaped yellow flowers. The rhizome is the portion of the plant used medicinally (Leung, 1980). (Refer, figure 1.6. A herb plant and its rhizomes – *Curcuma longa*). Dried rhizome of CL is the source of the spice turmeric, the ingredient that gives curry powder its characteristic yellow colour. CL is used extensively in foods for both its flavour and colour. It has a long tradition of use in the Chinese and Ayurvedic systems of medicine, particularly as an anti-inflammatory agent, and for the treatment of flatulence, jaundice, menstrual difficulties, hematuria, haemorrhage, and colic. The current research is focused on CL’s antioxidant, hepatoprotective, anti-inflammatory, anticarcinogenic, and antimicrobial properties, in addition to its use in cardiovascular disease and gastrointestinal disorders (Strimpakos and Sharma, 2008).
Active constituents

The coloring principle of CL was isolated in the 19th century and was named curcumin, which was extracted from the rhizomes of CL, with yellow colour and is the major component of this plant, being responsible for the anti-inflammatory effects (Anderson et al., 2000). The chemical structure was determined in the year 1973 (Refer, figure 1.7. Chemical structures of the curcuminoids of *Curcuma longa*). Curcumin is soluble in ethanol, alkalis, ketone, acetic acid and chloroform; and is insoluble in water (Roughley and Whiting, 1973). It melts at 176-177°C and forms red-brown salts with alkalis.

![Chemical structure of curcumin](image)

Figure 1.7. Chemical structures of the curcuminoids of *Curcuma longa*

(Venkateswarlu et al., 2005).

1. Curcumin; R1=R2= OCH3
2. Demethoxycurcumin; R1= OCH3, R2=H
3. Bisdemethoxycurcumin; R1=R2=H

The other constituents of CL include flavonoids, volatile oils including tumerone, atlantone, and zingiberone, phellandreen, sabenene, cineol, borneoal, sesquiterpenes, curcuminoinds and essential oils (Chen, 1983). Apart from this, turmeric also contains proteins, carbohydrates, fats, minerals, fibres and vitamins (Bakhru, 1997).
From the major curcuminoids, curcumin, demethoxycurcumin and bisdemethoxycurcumin, curcumin is the best investigated component comprising 0.3 to 5.4% of raw turmeric, makes up 70 to 75% of the curcuminoids, demethoxycurcumin 15 to 20% and bisdemethoxycurcumin about 3% (Venkateswarlu, 2005). Many biological activities of curcumin have come in to light as the current research has progressed.

**Mechanism of actions based on preclinical studies**

**Antidepressant activity**

CL has been used for centuries in traditional Chinese medicine as a treatment for mental disorders including depression. Based on animal studies, CLE and curcumin have been analysed to explore antidepressant potential. One study, demonstrated the specific antidepressant effects of CLE *in vivo* which was postulated to be mediated in part through MAO A inhibition in mouse brain (Yu et al., 2002). The aqueous extracts, when administered orally to the mice from 140 to 560 mg/kg for 14 days, were able to elicit dose-dependent relation of immobility reduction in the tail suspension test (TST) and the forced swimming test (FST); whereas an oral administration of the extract only at a dose of 560 mg/kg produced observable MAO B inhibitory activity in mice brain. The effect of curcumin was also studied on depressive like behaviour of mice (Xia et al., 2005). The study showed that curcumin treatment at 5 and 10 mg/kg (p.o.) significantly reduced the duration of immobility in both the TST and FST but these doses that affected the immobile response did not affect locomotor activity. In addition, the neurochemical assays in the same study showed that curcumin produced a marked increase of serotonin and NA levels at 10 mg/kg in both the frontal cortex and hippocampus. DA levels were also reported to be increased in the frontal cortex and the striatum. These findings suggested the involvement of the central monoaminergic neurotransmitter systems for antidepressant-like effects of curcumin. The study was undertaken to determine the behavioural, neurochemical and neuroendocrine effects of the ethanolic extract from CL using the FST in male ICR strain of mice (Xia et al.,
2005). The putative antidepressant effect of chronic administrations of curcumin was also confirmed in the forced swimming test and bilateral olfactory bulbectomy (OB) models of depression in rats (Xu et al., 2005b). In this study, chronic treatment with curcumin (1.25, 2.5, 5 and 10 mg/kg, p.o.14 days) reduced the immobility time in the FST. Curcumin also reversed the OB-induced behavioural abnormalities such as hyperactivity in the open field, as well as deficits in step-down passive avoidance.

In addition, OB-induced low levels of serotonin (5-HT), noradrenaline (NA), high 5-hydroxyindoleacetic acid (5-HIAA) and 4-dihydroxyphenylacetic acid (DOPAC) in the hippocampus were observed, which were shown to be completely reversed by curcumin administration. A slight decrease in 5-HT, NA and DA levels was found in the frontal cortex of OB rats which was shown to be reversed by curcumin treatment in the study cited above. These results suggested that these antidepressant effects may be mediated by actions in the central monoaminergic neurotransmitter systems. All these investigations may add to an understanding of the mechanisms of the antidepressant effects of curcumin. The modified amine theory has suggested that the acute increase in the levels of the monoamines at the synapse may be only an early step in a potentially complex cascade of events that ultimately results in antidepressant activity (Pineyro and Blier, 1999).

**Antioxidant effects**

Previous studies have reported antioxidant potential of CL. Water- and fat-soluble extracts of turmeric and its curcumin component exhibited strong antioxidant activity, comparable to that of vitamins C and E (Toda et al., 1985). One study demonstrated that curcumin pre-treatment decreased ischemia-induced changes in the cat heart (Dikshit et al., 1995). One *in vitro* study measuring the effect of curcumin on endothelial heme *oxygenase-1*, an inducible stress protein, was conducted utilizing bovine aortic endothelial cells. Incubation (18 hours) with curcumin resulted in enhanced cellular resistance to oxidative damage (Mortellini et al., 2000).
Another *in vitro* study demonstrated that low concentrations of curcumin incubated with activated macrophages resulted in a decrease in mRNA levels and nitric oxide synthase activity, which demonstrates curcumin's antioxidant role in down-regulating nitric oxide formation, a key element in inflammation and possibly in the process of carcinogenesis (Brouet and Ohshima, 1995). Antioxidant activities of curcumin, demethoxycurcumin and bisdemethoxycurcumin by *in-vitro* model systems, such as the phosphomolybdenum and linoleic acid peroxidation methods were also reported (Ramirez-Tortosa et al., 1999).

**Biotransformation studies**

It was recognized that the therapeutic effectiveness of curcumin is limited due to its poor absorption from the GI tract. Oral doses result in only traces appearing in the blood, with most of the dose being excreted in the faeces (Chattopadhyay et al., 2004). A study in mice showed that curcumin was first biotransformed to dihydrocurcumin and tetrahydrocurcumin and that these compounds subsequently were converted to monoglucuronide conjugates curcumin-glucuronide, dihydro-curcumin-glucuronide, tetrahydrocurcumin-glucuronide, and tetrahydrocurcumin; the major metabolites (Lin et al., 2000). The researchers speculated that the reason curcumin could have an effect in the body despite its bioavailability “issues” may be due to the fact it largely consisted in conjugated forms such as glucuronide in human (and rodent) plasma and is therefore cloaked in the blood supply. Curcumin metabolism was explored in subcellular fractions of human and rat intestinal tissue as compared to hepatic fractions and intact intestinal sacs in rat (Ireson et al., 2002). Curcumin glucuronide was identified in intestinal and hepatic microsomes and curcumin sulfate, tetrahydrocurcumin and hexahydrocurcumin were found as metabolites in intestinal and hepatic cytosols from humans and rats. Curcumin was sulfated by human sulfotransferase isoenzymes. The extent of intestinal conjugation was found to be greater in intestinal fractions from humans than those from rats (Ireson et al., 2002).
As the curcumin has poor bioavailability due to its rapid metabolism in the liver and intestinal wall, the influence of piperine on the pharmacokinetics of curcumin in animals and humans was also studied (Shoba et al., 1998). In this study, the effect of combining piperine, a known inhibitor of hepatic and intestinal glucuronidation, was evaluated on the bioavailability of curcumin in rats and healthy human volunteers in which piperine was found to enhance the bioavailability of curcumin both in preclinical studies and in studies on human volunteers.

**Side effects and toxicity**

No significant toxicity has been reported following either acute or chronic administration of turmeric extracts at standard doses. Curcumin appeared to be relatively safe in preclinical studies when administered orally. In various animal studies, a dose range of 100-200 mg/kg body weight exhibited good anti-inflammatory activity and seemed to have negligible adverse effects on human systems. Oral LD$_{50}$ in mice was found to be more than 2.0 g/kg body weight (Kohli et al., 2005).

Subchronic studies (up to 90 days) in rats, dogs and monkeys generally showed limited adverse effects (Anonymous, 1996). In Chemoprevention branch-funded studies of commercial grade curcumin, minor changes in body weights in rats and hematological values in rats and dogs were not considered biologically significant (Anonymous, 1996). Consistent with prostaglandin synthesis inhibition, curcumin was shown to cause gastric ulcerations in mice, but only at a higher dose (Rasyid and Lelo, 1999; Anonymous, 1996; Ammon and Wahl, 1991). Increased incidences of clitoral gland adenomas in female rats, hepatocellular adenomas in female and male mice, and small intestinal carcinomas in male mice observed were not dose-related (Anonymous, 1996).

Oral curcumin has been associated with gall bladder contraction in human over a 2-year period after administration of a single 20 mg dose and therefore curcumin use is warned as imprudent in patients with cholelithiasis (Rasyid and Lelo, 1999).
Dosage

The typical canine dosage of curcumin is reported as 50-250 mg three times daily, depending on the size of the animal (Silver, 1997). For CL, the estimated average canine dosage is suggested as one-half teaspoon twice daily.

Feline dosages are in the range of 50-100 mg daily of curcumin and approximately one-quarter teaspoon daily if using whole turmeric. Equine dosages of curcumin are much higher due to the size of the animal, and range between 1,200 and 2,400 mg daily (Silver, 1997; Monograph, 2001). When CL is used as a medication in humans, doses are reported up to 5 g daily is reported to be non-toxic even at 100 times the usual intake (Ravindranath and Candrashekara, 1982).

Herbal drug interactions

Most herbs and supplements have not been thoroughly tested for interactions with other herbs, supplements, drugs, or foods. The interactions of curcumin mentioned in literature are mainly based on reports in scientific publications, laboratory studies on animals or traditional use (Brinker, 1998). It was postulated that CL might inhibit platelets in the blood and enhance the action of anti-platelet drugs increasing the risk of bleeding (Lee, 2005; Kosuge et al., 1985). Some examples include anti-platelet drugs such as aspirin, clopidogrel (Plavix®) and non-steroidal anti-inflammatory drugs such as ibuprofen (Motrin®, Advil®) or naproxen (Naprosyn®, Aleve®) and anticoagulants ("blood thinners") such as warfarin (Coumadin®) or heparin. Based on animal and human data, CL was postulated to lower blood sugar and low-density lipoprotein (LDL or "bad" cholesterol) and increase high-density lipoprotein (HDL or "good" cholesterol) and therefore having the additive effects with diabetes medications as well as cholesterol-lowering drugs such as lovastatin (Mevacor®) or atorvastatin (Lipitor®) as well as cholesterol-lowering herbs or supplements such as fish oil, garlic, guggul, or niacin (Tang et al., 2008; Baum et al., 2007; Babu and Shrinivasan, 1997).
In animals, CL was shown to protect against stomach ulcers caused by non-steroidal antiinflammatory drugs (NSAIDs) such as indomethacin (Indocin®) by increasing intestinal wall mucus (Rafatulla et al., 1990) and against heart damage caused by the chemotherapy drug doxorubicin (Adriamycin®) (Venkatesan, 1998). When taken with indomethacin or reserpine, CL was postulated to help reduce the number of stomach and intestinal ulcers normally caused by these drugs (Venkatesan, 1998).

However, it was suggested that, when taken in larger doses or when used for long periods of time, CL itself can cause ulcers. CL was claimed to lower blood pressure levels and was assumed to have an additive effect if taken with drugs that also lower blood pressure (Brinker, 1998). Some laboratory findings indicated that dietary turmeric may inhibit the anti-tumor action of chemotherapeutic agents such as cyclophosphamide in treating breast cancer and the cancer patients undergoing chemotherapy are advised to limit intake of turmeric and turmeric-containing foods, though more research is needed to support the claim (Kawamori et al., 1999). The herb was also found to inhibit camptothecin- mechlorethamine and doxorubicin induced apoptosis of breast cancer cell lines in vitro (Somasundaram et al., 2002).

It was implied, that CL may interfere with the way the body processes certain drugs using the liver's "cytochrome P450" enzyme system (Appiah-Opong et al., 2007). As a result, it was suggested that the levels of these drugs may increase in the blood and may increase the effects or potentially serious adverse reactions. So the patients using any medications are specifically advised to check the package insert and speak with a healthcare professional or pharmacist about possible interactions before using CLE (Anonymous, 2008a).

**Current findings and future prospects**

The ‘Turmeric effect’ created by the successful opposition of a US patent on Turmeric by CSIR, India, the herb has made its name known not only amongst the millions of common Indian people but also all over the world (Gupta and Balasubrahmanyam, 1998).
Previous studies have revealed the wide therapeutic potential of curcumin as anti-inflammatory, antiarthritis, antispasmodic, antimicrobial, anticancer, hepatoprotective, neuroprotective, anti-HIV and strong oxygen radical scavenging activity among others (Hatcher et al., 2008). The possible therapeutic and protective use of CL in the immune-associated depression is also need to be explored further in future. These justify further clinical research to evaluate more precisely the favourable effect of CL in order to explore its new areas of therapeutic applications.

The ongoing research continues to identify bioactive compounds in herb and their effectiveness in providing general wellness. However, increased interest and reports on efficacy of herbs have resulted in confusion among consumers. Moreover, the increase in use of herbal products in conjunction with prescription medications has resulted in previously unrecognized herbal drug interactions. So, in view of wide therapeutic application and potential use of curcumin, its potential of interaction with various drugs and herbs is also another area that needs urgent attention for the safe utilization of the proven therapeutic benefits of the herb. It is important for health care professionals including pharmacists, to communicate with their patients regarding the known benefits of this herbal remedy as well as consult on potential interactions and possible contraindications with medications.

Figure 1.8. depicts some preparations of three herbs commercially available in market, that of *Bacopa monniera*, *Centella asiatica* and *Curcuma longa*. (Refer, figure 1.8. Commercial preparations of the herbal extracts).
Figure 1.8. Commercial preparations of the herbal extracts.

Brahmi (Bacopa Monnieri) Supplements
Serving size: 1 Capsule
Servings per container: 60 Capsules
Himalaya group.

Bacopa monniera extract
Each tablet contains 225 mg of extract standardized for 20% of Bacosides (A+B).
Planetary formulas

Gotu Kola Herb, 250 mg,
100 Gotu Kola Tablets
Centella asiatica herb
Club Natural

Centella asiatica
Gotu Kola Herb by Nature's Way 100 Caps

Supplement Facts
serving size: 2 capsules

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Amount</th>
<th>%DV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gotu Kola (stem, leaf)</td>
<td>870 mg</td>
<td>0%</td>
</tr>
<tr>
<td>Sodium</td>
<td>5 mg</td>
<td>&lt;1%</td>
</tr>
<tr>
<td>gotu kola</td>
<td>950 mg</td>
<td>†</td>
</tr>
</tbody>
</table>

Turmeric Extract; 350 mg; 100 tablets
Source Naturals

Each Serving Contains %DV
Turmeric Extract Yielding 333 mg Curcumin 350 mg **
Bromelain (extracted from pineapple) (2,000 G.D.U. per gram*) 50 mg **
Pepper Fruit Extract (Bioperine®) 3.5 mg

“Cynara”: Standardized extract of turmeric- Curcuma longa.
Turmeric 30 One-A-Day tablets
Lichtwer Pharma, UK

Turmeric extracts
DaXingAnLing Gadol Sports Ingredient Co., Ltd
3. Antidepressant models in rodents

Introduction

Depressive disorder is a common illness that affects millions of people worldwide today. It is a leading cause of morbidity and mortality with a lifetime prevalence of about 15–20% and the patients often suffer from symptoms such as depressed mood, sleep disturbances and suicidal ideation (Nancy and Pang, 2008; Harro, 2004). Depressive episodes can be precipitated in some individuals by traumatic life events in childhood or adulthood and several animal models of depression have been generated accordingly. Existing animal models of human disease have proven of substantial value in elucidating basic pathophysiological mechanisms and in developing novel treatments. However, modelling human mental disorders in experimental animals is laden with complications as the animal models generally lack both clinical and scientific credibility and the same have thus, so far, failed to measure up to the complexity and heterogeneity of the clinical states labelled ‘depression’. Consequently, much of the neuroscience of animal modelling is framed around physiological and neurobiological phenomena that may be of relevance to only a minority of patients. Additionally, it was understood that inferring pathophysiology from apparent treatment responses overestimates the efficacy of existing treatments and tends to ignore reliable demonstrations of the ‘antidepressant effects’ of non-pharmacological interventions (Matthews et al., 2005). Previously, it has become clear that an approach based on individual differences is not only necessary in humans, but is also critical in animal models (Cornelius et al., 2008; Woodcock, 2007). As the Animals simply do not react uniformly to the same stimulus, it is a must to distinguish between individual subjects, and to select subgroups of animals in a given population that are particularly reactive and are thus readily transformed in different emotional states than animals that are less responsive to external stimuli. Also, the environment plays an important role in determining which behaviours a given species is predisposed to display based on lighting conditions and familiarity of experimental arena, social experience and
familiarity of partner(s) (Latane, 1969; Bolles and Woods, 1964), territorial advantage (Willner et al., 1989; Gentsch et al., 1988) and hierarchical position within a closed social group (Mitchell, 1993; Koolhaas et al., 1980), all the factors affect the social behaviour of rodents.

Due to all the variables, it is exceedingly difficult to envision an animal model that perfectly recapitulates the symptoms of depression in human patients. However, depression, as other mental disorders, constitutes of intermediate or so-called endophenotypes that can be reproduced independently and evaluated in animals, including physiological, endocrinological and neuroanatomical alterations as well as behavioural traits (Sarter and Bruno, 2002). The minimal requirements for a valid animal model of depression have been proposed previously (McKinney and Bunney, 1969), which suggested that the model should be (i) reasonably analogous to the human disorder in its symptomatology (face validity), (ii) Cause behavioural changes that can be monitored objectively, (iii) Produce behavioural changes that are reversed by the same treatment modalities that are effective in humans (predictive validity) and (iv) Should be reproducible between investigators. The aetiological validity of a model refers to the similarity between the trigger that is used to precipitate neurobehavioural abnormalities in animals and suspected aetiological factors of human depression. Face validity is used similarly, to describe superficial likenesses between symptoms of human depression and those induced in animals. Predictive validity refers to the ability of an animal model to predict the therapeutic efficacy of antidepressant treatments. On the other hand, the constructive validity refers to the internal mechanism or state that underlies the depression (Willner, 1997; Willner, 1984). Based on this, numerous behavioural paradigms have been established to elucidate face, predictive and construct validity of animal models of depression in various species (e.g. hamsters, voles, tree shrews, primates); but preclinical psychiatric research has clearly favoured the rat as animal model of choice until the advent and propagation of transgenic and gene targeting technologies in mice.
Increasing efforts have been dedicated to the development of appropriate and valid rodent models to mimic major depression in humans; however, the extrapolation of the results to humans obtained with such models was taken cautiously and the validity of the models was under close scrutiny (Yadid, 1998). So far, no depression-like syndrome that fully recapitulates the human syndrome has been established in rodents. Most investigators have relied instead on combinations of environmental triggers and neurobehavioural endpoints in laboratory animals to screen for antidepressant drugs or to model specific symptoms of depression. In addition, several minor variations have been applied to each model; each of them having advantages and disadvantages (Markou, 2005; Nestler et al., 2002).

Besides, all clinically useful antidepressant drugs (also called thymoleptics) potentiate, either directly or indirectly, the actions of NA, DA, and/or serotonin in the brain. This, along with other evidence, led to the biogenic amine theory which proposed that depression is due to a deficiency of monoamines such as norepinephrine and serotonin at certain key sites in the brain (Slattery et al., 2004). The amine theory of depression was probably overly simplistic, since it is now known that the antidepressant drugs, particularly the tricyclic antidepressants, affect many biological systems in addition to neurotransmitter uptake and it is not known which of these neurochemical systems is most responsible for the antidepressant activity; though the drugs with antidepressant properties in patients with severe depression also have various behavioural and neurochemical effects in animals. This has given rise to various animal models that have been suggested to be valid for research into the neurobiology of depression and the neurochemical mechanisms of the antidepressants (Jesberger and Richardson, 1985). The data is summarized in the table 3. below (Table 3. Summary of major behavioural paradigms used to assess certain depression-like phenotypes in rodents, their validity, advantages and disadvantage).
<table>
<thead>
<tr>
<th>Sr. No</th>
<th>Paradigm</th>
<th>Validity</th>
<th>Advantage</th>
<th>Disadvantage</th>
</tr>
</thead>
<tbody>
<tr>
<td>1a.</td>
<td>Forced swim test (FST). (Porsolt et al., 1977a; 1977b; 1978a; 1978b; 1979).</td>
<td>Predictive</td>
<td>-Sensitive to antidepressants.</td>
<td>-Sensitive to acute treatment only.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>-Easy to perform.</td>
<td>-Validity for non-monoamine Anti-depressants</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>uncertain.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>-High reproducibility.</td>
<td>-Risk of hypothermia.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>-Low cost.</td>
<td>-Not specific for SSRI.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>-High throughput.</td>
<td></td>
</tr>
<tr>
<td>1b.</td>
<td>Modified FST (Cryana et al., 2005a).</td>
<td>Predictive</td>
<td>-Sensitive to antidepressant</td>
<td>-Sensitive to acute treatment only.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>-Easy to perform</td>
<td>-Validity for non-monoamine antidepressants</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>uncertain.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>-Can detect SSRI</td>
<td>-Risk of hypothermia.</td>
</tr>
<tr>
<td>2a.</td>
<td>Tail suspension test (TST) (Steru et al., 1985).</td>
<td>Predictive</td>
<td>-Sensitive to antidepressant.</td>
<td>-Sensitive to acute treatment only.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>-Easy to perform</td>
<td>-Validity for non-monoamine antidepressants</td>
</tr>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>uncertain.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>-High reproducibility.</td>
<td>-Not applicable in rats.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>-Low cost.</td>
<td>-Applicable only in certain mouse strains.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>-High throughput.</td>
<td></td>
</tr>
<tr>
<td>2b.</td>
<td>Modified TST (Cryan et al., 2005b; Porsolt et al., 1987a).</td>
<td>Predictive</td>
<td>-Sensitive to antidepressant.</td>
<td>-Sensitive to some antidepressants.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>-Easy to perform</td>
<td>-Sensitive to acute treatment only and specificity is questionable.</td>
</tr>
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<td></td>
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<td></td>
<td>-Requires very strong stressors.</td>
</tr>
<tr>
<td></td>
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<td>-High reproducibility.</td>
<td>-Time consuming.</td>
</tr>
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<td></td>
<td></td>
<td></td>
<td>-Low cost.</td>
<td>-Ethical restrictions.</td>
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<td></td>
<td></td>
<td></td>
<td>-High throughput.</td>
<td></td>
</tr>
<tr>
<td>3.</td>
<td>Learned helplessness (Overmier et al., 196; Maier et al., 1976; Parmar et al., 2006; Harro, 2004).</td>
<td>Predictive</td>
<td>-Good predictive validity including alternation in HPA axis activity and REM sleep characteristic of depression.</td>
<td>-Sensitive to some antidepressants.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>-No false negatives.</td>
<td>-Sensitive to acute treatment only and specificity is questionable.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>-Requires very strong stressors.</td>
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<td>-Time consuming.</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>-Ethical restrictions.</td>
</tr>
<tr>
<td></td>
<td>Chronic mild stress (Muscat et al., 1992; Willner et al., 1997; Cheeta et al., 1994; Monroe et al., 1985).</td>
<td>Predictive</td>
<td>-Respond to wide variety of antidepressants.</td>
<td>-Poor reliability and reproducibility.</td>
</tr>
<tr>
<td></td>
<td>Chronic fatig syndrome (Kaur and Kulkarni, 2000).</td>
<td>Predictive</td>
<td>-Few false negatives and positives.</td>
<td>-Only some antidepressants are effective.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>For example, anticholinergics and antihistaminics.</td>
<td>-Labor intensive.</td>
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</table>
Animal models of depression: discussion

A widely used classic model for antidepressant activity is the ‘Forced Swim Test’ (FST) as described earlier (Porsolt et al., 1978a; Porsolt et al., 1977a), one of the despair-based models which were proposed to have relatively good predictive validity for monoamine-based antidepressants (Willner, 1984).

The model is based on the premise that, when rats or mice are forced to swim in narrow cylindrical apparatus from there is no escape, after an initial period of vigorous attempt to escape, they adapt a characteristic immobile posture in which they remain floating in water making only those movements necessary to keep their head above the water level. The immobility exhibited by rodents reflects the state of “behaviour despair” as they resign themselves to the experimental conditions, which is taken as an index of depression. In rats, experimental drugs are given in two to three doses between the initial drug-free swimming session and the second swim that follows 24 hours later. On the other hand, in mice, it is sufficient to study the effect of drugs compared to vehicle given before the single swimming session. In both the species, clinically active antidepressants reduced the time spent immobile (Porsolt et al., 1977a).

The FST model, besides being simple and inexpensive, is very reliable across laboratories, have high specificity in detecting novel drugs and selectively sensitive to antidepressant treatments. The model was found to reduce immobility in tricyclic antidepressants, monoamine oxidase inhibitors and atypical antidepressants as well as electroconvulsive shocks (Porsolt et al., 1977b). It was reported that psychostimulants reduce the immobility too, but in contrast to antidepressants, they caused marked motor stimulation. The false positives can be induced by opiates and antihistamines as (Porsolt et al., 1978b; 1977b).

Drugs which increase synaptic NA content were reported to reduce the immobility time in rats reflecting the activity of central catecholamine system (Porsolt et al., 1979); however, it was not clear which adrenocepter mediates this effect, since ß-stimulants were ineffective in the studies (Porsolt et al., 1979; Kitada et al., 1983).
As clonidine and yohimbine, agonist and antagonists of $\alpha_2$ adrenergic receptors were shown to reduced immobility time and similarly, the $\alpha_1$-adrenoceptor stimulant, phenylephrine, reduced immobility time but increases motor activity in the same studies (Clineschmidt et al., 1979); it was found difficult to ascertain whether the effect could be attributed to this property. Plus, neither $\alpha_1$ nor $\beta$ adrenergic antagonists were found to be active in the studies (Kitada et al., 1983; Borsini et al., 1981; Porsolt et al., 1979). The effects on serotonergic drugs were found to be uncertain. If, on one hand, clomipramine, femofexine, fluoxetine and quizapine reduced immobility time, the drugs like citalopram including others did not reduced it over 20 percent (Kitada et al., 1983; Borsini et al., 1981; Porsolt et al., 1979).

Also, drugs like 5-hydroxytryptophane and paroxetine were found to be ineffective, which were tested only after single dosing (Gorka et al., 1979; Gorka and Woitasik, 1980). Serotonergic antagonists had no effect in the studies on similar line (Porsolt et al., 1979; Borsini et al., 1981; Kitada et al., 1983).

Some experiments also have been carried out with drugs interfering with cholinergic transmission wherein it was found that anticholinergics reduced immobility time, and cholinomimetics increased it (Herman et al., 1981; Kitada 1983; Duncun et al., 1985). Also, Several GABA-ergic drugs like sodium valproate and musimol were found to reduce the immobility time (Borsini et al., 1986). All dopamine mimetic substances were found to reduce immobility time and dopamine antagonists were found to be either inactive or increase the immobility time in FST (Duncun et al., 1985; Borsini and Meli, 1988; Kitada et al., 1983; Porsolt et al., 1977a).

The extensive data collected on the modified rat FST suggested the role of serotonergic antidepressants, in inducing distinct behavioural profile in the FST compared to antidepressants that act through catecholamine mechanisms (Cryana et al., 2005a), though their ability to detect non-monoamine-based antidepressants has been scrutinized and questioned (Borsini and Meli, 1988).
Also, the method needed modification to be able to detect drugs that have their primary molecular mechanism of action selectively on the serotonergic neurons. Recently, some procedural modifications introduced in FST have enabled SSRI-induced behavioural responses to be measured (Cryana et al., 2005a). The modified rat FST changed several of the testing conditions and scoring procedures used by the original FST procedure. A clear round cylinder of at least 20 cm diameter has been used with the modified FST and the water depth was increased from traditional depths of 15–18 to 30 cm. The modification of the FST also involved the development of a more complete description of the active behaviour of rats in the cylinder consisting of swimming and climbing or struggling; a multiple targeted behaviours which can be scored from videotapes instead of real-time scoring.

Selective serotonin reuptake inhibitors (SSRIs), currently the most widely prescribed antidepressants, were not reliably found to reduce immobility (Harro, 2004). It has been established in many laboratories that drugs acting primarily on noradrenergic neurons significantly reduced immobility and increased climbing/struggling, whereas SSRIs, as measured either by time-sampling of behaviour or separate real-time measurements of recorded behaviours, exerted, in statistical terms, their most significant effect on swimming (Harro, 2004). FST provides a tool to probe the role of various neurochemical circuits and receptor subtypes involved in the effects of antidepressants using a model relevant to stress-evoked depressive behaviour (Cryana et al., 2005).

Another despair based model is ‘Tail suspension test’ (TST), described as a dry-land version of the FST (Steru et al., 1985). The test is based on the fact that animals subjected to the short-term, inescapable stress of being suspended by their tail, will develop an immobile posture.

In the test described, male, mice (Balb-cJ) were hung upside down by a wire, thread or by an adhesive tap (20mm form the tip of the tail) 50 cm above the ground.

After an initial vigorous movement to escape the awkward situation, it assumed an immobile posture and the period of immobility during last 4 minutes of total 6 minutes
duration was noted. Mice provided better results than rats and the test is simple, reliable and rapid for screening antidepressants and is able to separate locomotor stimulant dose from antidepressant dose. Another advantage is that, this test does not induce hypothermia that results from immersion of animal in to water in forced swimming test. Plus, it provides precise objective measurement of duration of immobility and is more sensitive to lower doses of drugs with clear dose effect relationships. Variety of antidepressants were found to reduces the duration of immobility and so were the psycho stimulants and atropine; in contrast to which diazepam was found to increases the same (Cryana et al., 2005b). An obvious advantage of this test is its ability to detect a broad spectrum of antidepressants irrespective of their underlying mechanism. Also, the method is inexpensive and unsophisticated, methodologically and easily amenable to automation. Automation enables the easy and precise assessment of additional parameters such as power of movements (Cryana et al., 2005b; Porsolt et al., 1987).

Another form of modified behaviour despair test is chronic fatigue test, in which FST is done continuously in rats or mice where they are forced to swim individually in glass jar for 7 days which produce depression and fatigue resembling chronic fatigue syndrome (CFS). CFS is a heterogeneous disorder of unknown etiology characterized by fatigue, neuropsychiatry symptoms and related somatic complaints (Kaur and Kulkarni, 2000).

Chronic stress is a risk factor for development of many psychopharmacological conditions in humans, including major depression and anxiety disorders. It does play a role in the aetiology of melancholia and continual presence of it during antidepressant therapy would usually be the norm. The reversal of an established behavioural deficit during the continued presence of the stressor is an important feature of this model.

To understand the neurobiological mechanism underlying depression, cognitive and emotional consequences chronic stress, it is very necessary to employ an animal model that exhibit similar effects. Repeated presentation of the same stressor usually leads to
adaptation. However, adaptation can be prevented by presenting a variety of stressors in an unpredictable sequence which can produce a behavioural state characteristic of depression in rodents (Katz, 1981a). Based on this, previous studies have shown that three weeks of exposure to electric shocks, immersion in cold water, immobilization, reversal of the light/dark cycle followed by session of exposure to loud noises, bright lights and variety of other stressors when followed immediately by an open field test, showed increase in open field activity and the effect, which was not observed in chronically stressed animals (Katz, 1982; 1981a; 1981b; Katz et al., 1981). The model has a great many positive features and is probably the most valid animal model of depression currently available.

One study has investigated the behavioural parallels between adaptation to stress and antidepressant treatment using the FST (Platt and Stone, 1982). Restraint stress given repeatedly for 11 days significantly reduced immobility on this test while, a single application of stress had no effect. The reduction in immobility produced by repeated restraint was quantitatively similar to that produced by repeated administration of desmethylimipramine. These results confirmed previous findings of similarities in the behavioural and neurochemical response to chronic stress and chronic antidepressant treatment, where it was found that, antidepressants and stress when administered chronically; they reduce the density of beta adrenoceptors in various region of rat brain (Platt and Stone, 1982), which was accompanied by decrease in NA-sensitive adenylate cyclase activity, although the relationship between stress and depression remained incompletely understood.

The learned helplessness paradigm is another model based on the observation that animals exposed to uncontrollable stress (usually electric shocks) in one situation, subsequently fail to escape shock in a different situation when escape is possible (Maier and Seligman, 1976; Overmier and Seligman, 1967). This model is reported to have a good predictive validity including alterations in hypophyseal-pituitary axis (HPA) activity and rapid eye movement (REM) sleep characteristic of depression and can be used as
an additional screening procedure but the model is time consuming and its specificity is questionable. Other problems with this model are that the changes persisted for only a couple of days, reducing ease of method use and the need to repeatedly administer shocks that has contributed to its unfavourable image, even unacceptable by ethics committees in some countries.

A variety of antidepressant drugs were found to prevent the effect of chronic stress excepting MAOI tranylcypromine. In contrast, some psychotropic drugs lacking antidepressant activity failed to prevent the effect of stress (Soblosky and Thurmond, 1986). In addition to causing changes in open field activity, chronic stress was also shown to increase plasma corticosteroid levels. This effect showed the same spectrum of pharmacological sensitivity, with the exception that an anticholinergic was also effective. The chronic stress model has been utilized little because of the levels of severity employed raise serious ethical problems. Also, a major drawback is that the model has proved extremely difficult to implement reliably and reproducibly in laboratories.

In contrast to the extensive array of drugs correctly classified in chronic mild stress experiments, very few false positives or false negatives have been reported for reward based models (Cheeta et al., 1994; Monroe et al., 1985). In the studies mentioned, antidepressant treatment were found to have no effect on sucrose consumption or intracranial self stimulation (ICSS) threshold in non stressed animals but following the reduction of sucrose intake by stress, normal behaviour was gradually restored by chronic treatment (two to five weeks) with a wide variety of antidepressants, including tricyclic antidepressants (TCAs), SSRIs, a specific NA reuptake inhibitor (maprotiline), *monoamine oxidase*-A inhibitors (MAO-A inhibitors), atypical antidepressants such as mianserin, buspirone, sulpride and also some agents of uncertain antidepressant status, such as antihistaminic and anticholinergic drugs. On the other hand, the ineffective drugs include the anxiolytic chlordiazepoxide, various neuroleptics, amphetamine, and morphine (Muscat et al., 1992).
Fluoxetine, maprotiline and mianserin (but not chlordiazepoxides) were also found to restore the rewarding properties of food, as assessed in above paradigms.

Stress models appear to have greater aetiological validity compared with those that rely on brain lesions, immune stimulations or monoaminergic depletion, which are not common aetiological factors in human depression. Although some equate aetiological validity with construct validity. For example, evidence of reduced reward in animal models might be related in terms of underlying mechanisms to symptoms of anhedonia in humans with depression. Studies of the neural basis of the chronic mild stress-induced anhedonia have focused primarily on the mesolimbic DA system (Dziedzicka-Wasylewska et al., 1997). The behavioural changes in animals subjected to this model are accompanied by a decrease in D2/D3-receptor binding and D2-mRNA expression in the nucleus accumbens, and a pronounced functional subsensitivity to the rewarding and locomotor stimulant effects of the D2/D3 receptor agonist quinpirole, administered systemically or within the nucleus accumbens. All of these effects were also found to be reversed by chronic antidepressant treatment (De Montis et al., 1995).

The earliest pharmacological models of antidepressant-like activity had significant impact on establishing the monoamine theory of depression, which assumed that an elevation of serotonin and NA levels will improve depressive symptomatology. The reserpine model is based on the capability of antidepressants to reverse the inhibitory effects of reserpine on motility in rats and mice (Leith and Barrett, 1980), as reserpine, an antihypertensive and antipsychotic drug, is capable of non-selectively depleting brain monoamines and thereby induces a syndrome of locomotor hypomotility and reduced body temperature in rodents. A similar approach is underlying the 5-hydroxytryptophan (5-HTP)-induced behavioural syndrome.

To identify compounds that enhance synaptic concentration of serotonin, the test detects elevated levels of serotonin by measuring the potency to further increase the behavioural syndrome induced by administration of 5-HTP, which is the metabolic precursor of 5-HT. The test provides a rapid and accurate index of selective serotonin
reuptake inhibitor (SSRI) potency in vivo (Cassens et al., 1981). These models offer good predictive validity in terms of monoamine-based antidepressant activity; albeit they do not model core symptoms of depression.

Concurrent with these studies, other groups have focused on neurochemical and physiological alterations that might account for the antidepressant sensitive behavioural alterations. Much interest has been placed on the serotonergic system with a 5-HT hyperinnervation of the frontal cortex (Bissette, 2001) and stressor-induced alterations in 5-HT-mediated activity observed subsequent to bulbectomy (Holmes et al., 1998). Furthermore, increased striatal glutamate release during novelty exposure-induced hyperactivity in the test has postulated a modulatory role of glutamate on the antidepressant-sensitive response (Wrynn et al., 2000).

Increases in the concentrations of the neuropeptides (or their encoding genes) corticotropin-releasing factor, thyrotrophin-releasing factor, somatostatin (Slotkin et al., 1999) and neuropeptide Y (Nutt, 2006), which might play a role in mediating the antidepressant-sensitive behaviours, have also been established (Slotkin et al., 1999; Nutt, 2006). Imaging studies demonstrated alterations in signal intensities in cortical, hippocampal, caudate and amygdaloid regions in olfactory bulbectomized animals compared with sham-operated controls (O’Neil and Moore, 2003).

In addition, from the ventricular enlargement evident in bulbectomized animals, it has been suggested that these structural changes correlate somewhat with those seen in depressed patients. Over the years, several modifications of the existing models and new neurochemical rodent models have been developed to explain depressive disorders. Of late, the power of genetic approaches has led to a significant shift in the concept of genetic models of depression. There are several genetic and non-genetic aetiological sources of early origin of individuality which were used to identify factors underlying predisposition to depression have been summarised previously (Lathe, 2004). The more traditional approach of selective breeding to provide different behavioural phenotypes has been supplemented by genetically modified mice.
As the first genetic model of depression Flinders Sensitive line (FSL) and Flinders Resistant Line (FRL) were selected for their differential hypothermic responses to an anticholinesterase agent (Overstreet et al., 2005). Animals selectively bred for their differential susceptibilities to stress-induced changes in swim-test activity (Rezvani et al., 2002) and strains with a high immobility in the swim-test such as the Fawn-Hooded rat and Wistar-Kyoto rats as genetic animal models for depression are described (Scott et al., 1996; Will et al., 2003). The FSL rats exhibited a high predictive validity and displayed changes in brain that were found to be consistent with the cholinergic, serotonergic, dopaminergic, NPY, and circadian rhythm models but not the noradrenergic, HPA axis or GABA-ergic models of depression (Overstreet et al., 2005). Refined molecular technologies and the creation of genetically engineered mice have allowed to specifically target individual genes involved in regulation of corticotropin releasing factor (CRF) system elements (e.g. CRF and CRF-related peptides, their receptors, binding protein), as described earlier (Martin et al., 2005). Transgenic mice overexpressing CRF (genetic C57/B6!SJL) exhibited prominent endocrine abnormalities involving the HPA system, such as high plasma levels of ACTH and corticosterone and displayed physical changes similar to the stigmata seen in patients with cushing’s syndrome, such as excess fat accumulation, muscle atrophy, thin skin, and hair loss (Stenzel-Poore et al., 1992). Rats have been selectively bred for susceptible to learned helplessness (Kohen et al., 2003; Edwards et al., 2000). Apart from high and low level of immobility in mice in the FST (Scott, 1996), other genetic models are based on an underlying alteration in the function of both cholinergic and serotonergic neurotransmitter systems (Overstreet, 2002; Knapp et al., 2000). A mouse model has also been derived from animals bred for spontaneous high or low immobility scores in the TST (H/Rouen mice ‘depressed’ or ‘helpless mice’) (Vaugeois et al., 1996). Many such stress models of depression by forming genetically vulnerable strains have been described based on the plausible interaction between stress and genetic vulnerability (Henn and Vollmayr, 2005; Maier and Watkins, 2005).
Conclusion and future prospects

From discussion above, it is apparent that there is a significant overlap between the functional abnormalities in rodent models and those changes that have been reported to occur in the patient with major depression. Several minor variations have been applied to each model. All models consist of a manipulation and at least one dependent variable, the choice of which is based on the aspect of depression that one wishes to model. The choice and design of a dependent measure is not always easy due to the fact that our knowledge of the etiology of depression is still limited and based largely on inferences drawn from the presumed modes of action of clinically available antidepressant treatments. In addition, despite every possible attempt to standardize experimental conditions at different laboratories; important inter-laboratory differences, including significant strain-effect interactions, still emerge and it seems advisable to ensure that the animals belong to the specific species and are inbred to avoid the variability of the results (Pawlak et al., 2008). Many avenues are opening up for researching animal models of depression. New research is progressing on the role of nitric oxide (NO) -mediated neurotransmission in the dorsal raphe nucleus in producing significant and complex motor and emotional effects in animal models of anxiety and depression (Spiacci et al., 2008) and on the effect of A history of caloric restriction induces neurochemical and behavioural changes in rats consistent with depression (Chandler-Laney et al., 2007). Knockout strategies have opened up an entire new avenue for selection of drug targets in depression.

As the current models are refined continuously to reveal the therapeutic potential of a broad range of compounds, the old rule of thumb is to ensure the use of same bred animals in the experiments for uniformity of the results, use of oral graded dose (Intraperitoneal, in case of new drugs), subchronic administration (3-5 days) in case of plant extracts and avoiding false negatives or false positives at the same time using a battery of tests in screening antidepressants in laboratories which need to be complemented by the corroborative biochemical paradigms (Bhattacharya et al.,}
(1999b). Also, for nocturnal animals like rats and mice, determination of the effect of psychotropic drugs on natural action patterns of behaviour needed to employ observations during the dark phase of the light-dark cycle.

Despite the fact that none of the presently available animal models is able to model all aspects of depression and most likely never will; existing paradigms have proven extremely useful not only in the identification and improvement of antidepressant substances, but also in the validation of neurobiological concepts. To strengthen paradigms modelling disease etiology to improve their reliability and to develop novel tests that will allow to pick up classes of antidepressants beyond monoamines pose major challenges for the future. To accomplish these goals, it seem necessary to implement recent advances emerging from psychiatric genetics, non-invasive neuroimaging research and biomarker identification. As important an issue is the full incorporation of ethical 3R (Replacement, Reduction and Refinement) principle for reducing suffering and distress and maintaining optimum welfare in animals to ensure ethical and scientific validity to animal experimentation (Manciocco et al., 2009), which might eventually result in improved methodologies over time. 